Spider colour polymorphism is shaped by precipitation, not ambient temperature

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ABSTRACT

Colour polymorphism, the presence of multiple colour variants within a population, is a common example of intraspecific phenotypic variation and has served as a model for studying drivers of diversity. Climatic factors can influence the distribution and abundance of colour variants, yet research often focuses on lineages where sexual selection covaries with the climate-colouration associations. Research has also focussed disproportionately on vertebrates and a few insects, neglecting other taxa where polymorphism is widespread, like arachnids. Here, we investigated climatic factors influencing colour variation in a widely distributed colour polymorphic spider using a combination of controlled experiments, field measurements and tests for macroecological associations. We showed in the lab and field that dark colouration is unlikely to provide biologically relevant thermal benefits; yet different colour morphs occupy distinct climatic niches across Australia. Precipitation, rather than temperature, appears to be the strongest driver of colour morph frequencies: there were higher frequencies of dark individuals in regions with higher precipitation while orange individuals were more common in drier areas. We discuss potential mechanisms related to background matching or pathogen protection in humid environments. Our findings highlight the role of climatic factors in shaping colour variation, and demonstrate the value of combining macroecological, field, and laboratory approaches.

KEYWORDS

Colour variation, Gloger´s rule, humidity, geographic variation, thermal melanism hypothesis

INTRODUCTION

Intraspecific variation has extensive evolutionary and ecological implications and is the raw material for adaptation (Des Roches et al., 2018). For instance, populations with greater variation generally have increased resilience to perturbations and enhanced ability to adapt to rapidly changing conditions (González-Suárez & Revilla, 2013; Moran et al., 2016; Palacio & Clark, 2023). Identifying the drivers that produce and maintain variation is essential to understand eco-evolutionary processes, including patterns of species diversity, resilience, and responses to environmental change (Bolnick et al., 2011). Environmental factors can shape variation within and between populations; but these are commonly studied in isolation. For example, many studies have examined large-scale correlations between phenotypic traits and climate variables among populations. Conversely, many studies consider adaptive variation within populations (Mclean & Stuart-Fox, 2014; Svensson, 2017). However, to understand how environmental factors shape intraspecific variation, it is important to integrate both macroecological patterns and within-population dynamics.

Species with discrete within-population phenotypic variation (i.e., polymorphism) provide an ideal model to understand how environment factors shape intraspecific variation (Ford, 1945; Gray & McKinnon, 2007; Jamie & Meier, 2020). Specifically, environmental factors can determine the presence or frequency of discrete phenotypes within and between populations (Endler, 1977; Kassen, 2002; Dreiss et al., 2012). For instance, in some colour polymorphic species, morph frequencies — and even the presence of polymorphism — can vary strongly across environmental gradients (Mullen & Hoekstra, 2008; Wittkopp et al., 2011; Roulin et al., 2011; Dreiss et al., 2012a; Pérez i de Lanuza et al., 2018). In the black sparrowhawk, *Accipiter melanoleucus*, for example, the frequency of dark and white colour morphs varies clinally with light environment due to differential crypsis (Tate et al., 2016), while in oldfield mice, pigmentation varies along a vegetation gradient (Mullen & Hoekstra, 2008). It is no surprise that these and many other examples involve colour polymorphic species because colour is easily scored and can be functionally associated with different environmental variables.

Two of the most important climatic gradients associated with colour variation are temperature and humidity. Correlations between temperature or humidity have been observed in a wide range of taxa and are known as Bogert’s rule (or the thermal melanism hypothesis) and Gloger’s rule respectively (Goldenberg et al., 2022). According to the thermal melanism hypothesis (Clusella Trullas et al., 2007; Clusella-Trullas et al., 2008) darker individuals should be more abundant in environments with low ambient temperatures, given the thermoregulatory benefits of dark colours, especially for ectotherms (Watt, 1968; Meredith & Sarna, 2006; Castella et al., 2013). Even though this pattern has been observed in multiple species (Clusella-Trullas et al., 2008; Broennimann et al., 2014; Azócar et al., 2016; Bishop et al., 2016; Pinkert et al., 2017; Heidrich et al., 2018; Dalrymple et al., 2018; Hantak et al., 2022), there are cases were dark colouration is associated with other functions or climatic factors (Roulin, 2016; Smith et al., 2016; Delhey et al., 2019; Lopez et al., 2021; Pepi et al., 2022; Passarotto et al., 2022; Hastings et al., 2023). Additionally, in many cases, we ignore how ambient temperature is mechanistically associated with the presence of dark colouration and whether the degree of colour variation in the wild can lead to biologically relevant differences in temperature regulation across morphs. In other words, large-scale correlations are often not supported by experimental evidence.

Similarly to temperature, humidity shapes the geographic distribution of colouration in many taxa (Friedman & Remeš, 2017; Delhey, 2019; Cerezer et al., 2020; Lopez et al., 2021; Marcondes et al., 2021; Kang et al., 2021). Darker individuals appear to be more abundant in places with higher humidity or precipitation than in drier places (Delhey, 2019). This pattern does not have a clear causal explanation; it is possible that it emerges as a consequence of improved background matching (Stevens & Merilaita, 2008; Cheng et al., 2018), pathogen protection (Mackintosh, 2001; Nosanchuk & Casadevall, 2003) or a consequence of pleiotropic effects (Ducrest et al., 2008). In addition to the lack of clarity on the mechanisms behind Gloger's rule, there are some taxa, such as amphibians and arachnids, that are notably underrepresented in the available empirical evidence supporting these macroecological patterns (reviewed in Delhey, 2019).

Most of our understanding of how climatic variables shape colour variation comes from vertebrate taxa where sexual selection can strongly affect the presence and maintenance of colour polymorphism (Roulin & Bize, 2007; Wellenreuther et al., 2014). This means that the interpretation of the association between climate and coloration needs to account for the confounding effect of sexual selection (Lancaster et al., 2017; Pérez i de Lanuza et al., 2018). Web-building spiders provide an opportunity to explore climatic drivers of phenotypic diversity interpedently of sexual selection. They are a diverse group (> 6,000 species in the families Theridiidae and Araneidae alone) with at least 140 colour polymorphic species (Salgado-Roa et al., 2024), yet they have limited visual capabilities. Most web-building spiders examined to date have only one photoreceptor (Yamashita, 1985; Tiedemann et al., 1986), suggesting they may lack colour vision. This visual limitation implies that colour signals might not play a significant role in sexual selection for these species. As a result, their coloration may be shaped entirely by environmental factors rather than intraspecific interactions.

Here, we combined controlled experiments, field measurements and macroecological analyses to examine the climatic drivers that influence the composition and abundance of different colour variants across multiple populations in a widespread colour polymorphic spider (*Austracantha minax*) with three different morphs (Figure 1). We first explored the thermal benefits of dark colouration by comparing heating rates of light and dark morphs under a solar simulator in the laboratory and comparing body temperatures of the two morphs in the field. At a broader scale, we investigated which climatic variables could explain colour variation in the Christmas spider within and across populations. We compared the climatic niche of the colour morphs using over 1254 field observations and public records from databases and museum specimens. Considering that the frequency of the morphs can change throughout the species' distribution, we also quantified morph frequency across 49 populations in Australia. Our study reconciles experiments conducted under controlled conditions with field observations and macroecological associations to understand the drivers of phenotypic diversity at the intraspecific level in an unexplored group.

MATERIALS AND METHODS

Study species

Austracantha minax, the Christmas spider, is a colour polymorphic species widely distributed in Australia. Females exhibit two main colour morphs: one is completely dark on both its dorsal and ventral sides; and the other presents black, white, and yellow on the dorsal side, and a mosaic yellow pattern ventrally (Waldock, 1991). A third colour morph has been described for this species; however the reports are limited to only a few places in Western Australia (Hogg, 1914; Waldock, 1991). This colour variant appears to be orange and white on its dorsal side and completely orange on its ventral side (Waldock, 1991). Based on previous museum and field observations, as well as early studies (Waldock, 1991; Elgar & Bathgate, 1996; Mascord, 1996; Lloyd & Elgar, 1997), females of the Christmas spider tend to be bigger, more abundant, live longer, and exhibit clearer phenotypic variation than males. These observations prompted us to explore the effect of climatic variables on the colour polymorphism of *A. minax*, focusing exclusively on females.

Heating rates experiment

Correlations between colour variation and climate can be driven by several factors, one of the most important being thermal needs. To investigate potential functional explanations for ecogeographical patterns, we quantified heating rates in the two most common morphs — the black morph and the black-and-white morph — under controlled conditions. The equipment and protocol for this experiment are based on and fully described in Wang et al. (2021) and Ospina-Rozo et al. (2022). For this experiment, we used a solar simulator (Sciencetech UHE-NS) emitting an energy intensity of 1,000 W/m², equivalent to the solar radiation typical at an equatorial site during a clear day around solar noon. The light source mimics the power and irradiance spectrum of the sun, spanning 300 nm to 1700 nm (AM1.5G). To measure heating rates, we enclosed the samples within a sealed glass thermal chamber, illuminated from above by the light source (Supplementary figure 1). This chamber was linked to a cooling system (thermofisher accel 250 lc) to minimise conduction or convection effects during experimentation by maintaining air temperature at 20 ± 0.5°C (Supplementary figure 1).

To measure the change in spider temperatures, we sacrificed the spiders immediately before the experiment using a CO_2 chamber, then inserted a thermocouple (approximately 5 mm TP-K01, K type, Center Technology Corp., Taiwan) into the spinnerets of the spider. Another thermocouple was positioned at the same level as the sample to monitor the air temperature inside the chamber. Both thermocouples were connected to a thermometer (Center 521, Center Technology Corp., Taiwan), recording temperatures every 10 seconds for 5 minutes.

We measured a total of 20 black individuals and 20 black-and-white individuals on both their ventral and dorsal sides. To compare heating rates between the colour morphs on each side, we employed two methods. One method involved calculating the total temperature differences between the initial and final measurements after 5 minutes (ΔT), while the other measured the maximum change in temperature in 20-second intervals (maxHR = ΔT/sec). Additionally, considering that larger objects are expected to heat up more slowly, we recorded the weight of each sample before the start of each trial. To test for differences in hearing rates between morphs, we built linear models per side (dorsal/ventral) in R v4.2.0 (R Core Team, 2021) using either ΔT or maxHR as response variables and colour morph (black or black-and-white) and weight as predictors. The assumptions of the linear model were verified using diagnostic plots.

Field thermal measurements

To explore if our measurements of heating rates under controlled conditions were reflected in the field and biologically relevant, we compared the body temperature of the black and black-and-white individuals in the field. We measured the body temperature of 15 black females and 15 black-and-white females in a population near Ballarat, Victoria, Australia (latitude: -37.8215, longitude:143.7585). To ensure we measured all individuals under the most similar conditions possible, we conducted measurements on a cloudless day at noon along a transect of less than 50 meters.

We measured the body temperature of the individuals on their dorsal and ventral side using an infrared thermal camera (FLIR t540). Raw images were processed with the manufacturer software (FLIR Tools v6.4) to extract average temperatures per side. Simultaneously, we recorded wind speed with an Anemometer (BTMETER BT-100), air temperature with a thermometer (Center 521, Center Technology Corp., Taiwan) and the luminosity level (measured in lux) using a digital illuminance device (Model: LX1330B-Dr. meter; spectral sensitivity ranging from 400 to 700 nm). These devices were positioned parallel to the individuals. We also measured the height of the web of every spider.

We analysed each body side (dorsal and ventral) independently by building linear models in R v4.2.0 (R Core Team, 2021). The models included body temperature as response variable and colour morph (black or black-and-white), ambient temperature, wind speed, luminosity, and web height as predictors. We checked for collinearity between the predictors using the function *check_collinearity* from the R package *performance v0.10* (Lüdecke et al., 2021) and calculating their Pearson’s correlation. Diagnostic plots were used to confirm the assumptions of the linear model.

Geographical records data collection

To test whether and which climatic variables can explain colour variation in *A. minax*, we obtained geographical records from three different sources. First, we visited four of the main arachnological collections in Australia: Western Australian Museum, Queensland Museum, Australian Museum, and Museums Victoria, and extracted coordinates for a total of 357 specimen records. Second, we collated all the research-grade records of the species on iNaturalist on the 11th of November 2023. All these records had images associated and consensus about their identification. We removed records with poor-quality pictures and a coordinate uncertainty > 1 km. This resulted in a total of 1829 records with photographs from iNaturalist. Finally, we also added 47 new geographical records from our own surveys of locations across southeast and southwest Australia that had suitable environments for the spider. Our final dataset consisted of 1,254 records, 1097 records for the black-and-white colour morph, 123 records for the black morph, and 34 records of the orange morph (Figure 1).

Climatic niche variation among colour morphs

We tested if the three colour morphs of the Christmas spider differ in their realised climatic niche (Peterson et al., 2011). To extract climatic data that represents the conditions in which adults are found in the field, we focused on the months when the species shows the highest activity levels. Based on all the records with associated collection dates, we defined the months of peak activity by excluding those months that had fewer than 5% of the reports. We found that 95% of the records were form October to May, so we used those months to extract climatic data.

Using the months of activity we downloaded climatic data from CHELSA v2.1 (Karger et al., 2017). This resource offers monthly climatic information from 1979 to 2019, derived by downscaling ERA-Interim climatic reanalysis to a resolution of 1 km. We selected this global dataset over others because its spatial resolution is higher (e.g. Era5 has a spatial resolution of approximately 31 km). From this dataset, we downloaded six climatic variables that we hypothesised could be linked to morph variation: mean daily maximum air temperature (extracted automatically at 2 meters), mean daily minimum air temperature (extracted automatically at 2 meters), precipitation, surface downwelling shortwave flux in air (i.e. solar radiation), potential evapotranspiration, and climatic moisture index. To account for temporal variation or biases due to extreme conditions, we generated raster layers for Australia for each variable by averaging the values for the months of peak activity of the species (October to May) over the last decade of available data (2009-2019). The values for each record were extracted using the function *extract* from the R package *terra* v1.7 (Hijmans, 2023).

Using the climatic data, we implemented univariate and multivariate comparisons between the colour morphs of the Christmas spider. For the univariate comparisons, we computed the density profiles per colour morph of every climatic variable and Tukey honest significant difference (Tukey HSD) tests to determine which of the colour morphs were significantly different from one another across the six climatic variables. For the multivariate analysis, we implemented a COUE (Centroid shift, Overlap, Unfilling and Expansion) scheme (Guisan et al., 2014). This framework is commonly used to test differences in the climatic niche of different populations of the same species (Collart et al., 2021; Farquhar et al., 2022; Y. Wang et al., 2023); for example, to compare invasive and native ranges of invasive species (Bates & Bertelsmeier, 2021).

For the COUE scheme, we first defined the geographical range of each colour morph by adding a buffer of 100 km radius to every geographical record of each morph (Following, Pittiglio et al., 2018; Farquhar et al., 2022; Whitford et al., 2024). Using the geographical range of each colour morph, we defined the spatial extent of their environmental background by intersecting the range of each morph with a layer of the terrestrial ecoregions of the world, which were obtained from Dinerstein et al. (2017; https://ecoregions.appspot.com/). The black-and-white morph occupied a greater number of ecoregions (34 ecoregions), followed by the black morph (21 ecoregions) and orange morph (17 ecoregions). Then, we randomly sampled 10,000 background points within all the ecoregions occupied by each colour morph (30,000 background points). These points represent the environmental background, which is the available environment that might be suitable for each colour morph, but that is not in our sampling (Barbet-Massin et al., 2012). These steps were done using functions from the R package sf (Pebesma, 2018). We repeated this analysis adding a buffer of 50 km radius to every geographical location, instead of 100 km, obtaining equivalent qualitative results.

After sampling the background random points, we extracted the climatic values for each of these background records from the CHELSA v2.1 layers, and combined them with the climatic values of the geographical records of the colour morphs (i.e. presence records). We summarized climatic variation from all (presence and background) records in two principal components (PCs) which explained 95.4% of the climatic variation and represented the environmental space of the whole species (i.e including both observations and possible suitable areas for all the morphs; Supplementary figure 2). This information was used as an input for the R package *ecospat* (Di Cola et al., 2017), where we implemented the COUE scheme to explore the similarity of the climatic niche of the colour morphs. First, we created grids of 100 x 100 cells per colour morph that represented the occurrence density of each morph over the environmental space of the whole species (summarized in the two principal components). Then, using the grid topology of all the colour morphs, we compared their climatic niche estimating the D index (Schoener, 1968) and the similarity statistic I (Warren et al., 2008) between all the colour morphs with the R package *ecospat* (Di Cola et al., 2017). The values of D and I range between 0 and 1, where 0 indicates no niche overlap between the niche occupied by colour morphs, and 1 represents complete overlap.

To evaluate the statistical significance of *D* and *I*, we performed tests of niche equivalency and similarity using 10,000 permutations (pseudo-replicates) to create a null distribution of similarity values based on the background environment of each morph. This allowed us to compare the observed values of *D* and *I* against a null distribution to implement a one-tailed hypothesis test. We tested this for niche conservatism (niche overlap is more equivalent/similar than random expectations) and for niche divergence (niche overlap is less equivalent/similar than random expectations).

Climatic factors shaping colour morph frequency

The analysis of geographical distribution of the colour morphs described above provides a broad scale comparison between the environments where morphs are found, but multiple morphs can be found in some locations. To gain insights into the drivers of abundance for each colour morph, we measured their frequency in the field. We sampled 49 locations encompassing 36 degrees of longitude and 6 degrees of latitude in south-eastern and south-western Australia (Figure 2). Taking advantage of the semi-social behaviour of the species, we searched for aggregations of individuals of *A. minax* at these locations. When these aggregations were found, we conducted an exhaustive search aiming for a minimum of 50 females within a radius of 1km. We categorised the individuals found at each location as black, black-and-white, or orange (Figure 1). In total, we counted 15,214 spiders, with a mean of 310.49 spiders per location, a minimum of 85 individuals and a maximum of 817.

We used the colour morph frequency information from the field to test whether the variation in morph frequency was associated with climatic factors. The climatic information in this case was obtained from the Australian Gridded Climate Data / Australian Water Availability Project dataset (AGCD/AWAP; Jones et al., 2009), rather than from CHELSA v2.1. We decided to use this dataset instead of the global dataset (CHELSA v2.1), because AGCD/AWAP offers higher regional specificity and temporal specificity (daily data per hour), which is ideal for this finer-scale analysis. We were unable to use this dataset for the continental climatic analyses (climatic niche variation among colour morphs) because extracting data from this dataset for such a high number of records (~32,000) was extremely computationally intensive. In the supplementary material we show that both CHELSA and AWAP datasets are highly correlated and lead to similar conclusions.

Form the AGCD/AWAP, we downloaded the daily values of the following five climatic variables: vapor pressure, minimum temperature, maximum temperature, precipitation, and solar radiation. We also accounted for temporal bias by calculating the average of each location for the months of peak activity of the species (October to May) over the last decade of available data (2011-2021). Before the analyses, we assessed potential collinearity between the climatic variables by exploring their Pearson correlation (Supplementary figure 3). Following that, we built a dissimilarity matrix with the absolute values of the correlation (1-correlation values) to perform a hierarchical clustering analysis with the function *hclust* in R v4.2.0 (R Core Team, 2021). We considered a pair of climatic variables as independent if they had a pairwise distance > 0.5 (Supplementary figure 4).

We constructed multiple matrix regression (MMR) models using non-collinear variables with the R package ecodist v2.1 (Goslee & Urban, 2007). The predictors comprised distance matrices for each climatic variable, calculated as the Euclidean distance between pairs of locations, along with a matrix of geographic distances between sampling locations. The latter calculation was performed using the R package geosphere v1.5 (Hijmans, 2016). The response variable was the Euclidean distance in colour morph frequencies between pairs of locations (e.g., between locations A and B), calculated as follows:

$$dist(A,B) = \sum_{i=1}^{n} \sqrt{(fA_i - fB_i)^2}$$

Here, fA_i and fB_i represent the relative frequency of morph i in locations A and B, respectively. Predictors were log-transformed if their distribution significantly deviated from normality. All matrices were scaled and centered before analysis to ensure comparable linear coefficients (Wang, 2013).

We conducted two MMR models considering the three colour variants of the Christmas spider to avoid including highly correlated predictors in the same model. We found that vapour pressure was highly correlated with minimum temperature, and maximum temperature correlated with solar radiation, while precipitation was not strongly correlated with the other variables. Hence, the first model included precipitation, minimum temperature, maximum temperature, and geographical distance as predictors. For the second model, we used precipitation, vapor pressure, solar radiation, and geographical distance as predictors. If either of these models contained a statistically significant predictor, we evaluated the same model using pair-wise comparisons between morphs (instead of the three morphs at once) to examine if certain variables strongly influenced frequency changes in specific morphs. Therefore, in this case the estimation of the Euclidean distance in colour morph frequencies was done the same way as explained earlier but excluding one morph from the calculation.

Given that measures of distance can reduce the statistical power to test associations between variables (Legendre & Fortin, 2010), we also built linear models for each colour morph using morph frequency as the response and the climatic variables that had a significant effect in the MMR models as predictors. We applied the logit transformation to the relative frequency of the colour morph in each location as the response variable. We examined whether the model assumptions were met by plotting the normality of residuals and assessing heteroscedasticity. The same methodology described in this section was repeated using the CHELSA v2.1 dataset to validate the results across climatic datasets.

RESULTS

Thermal differences between black and black-and-white morph

We found that under controlled conditions, the black morph heated faster than the black-and-white morph on both the dorsal ($F_{1.97}$ = 6.44, P-value = 0.01, R2 = 0.18) and ventral sides (F_3 = 7.24, P-value = 0.01, R2 = 0.1). The same pattern was supported by the two methods of calculating heating rate (Supplementary Table 1-2 and Figure 3). The difference in heating rate was small, however, varying in less than 1 degree after 5 minutes of solar exposure on both ventral and dorsal sides. Our field measurements revealed no temperature difference between the black and black-and-white morphs on either the dorsal (Figure 3) or ventral sides (Figure 3). Ambient temperature, wind speed and light intensity appear to be the most important factors explaining the variation in body temperature in our field data (Supplementary Table 3).

Climatic niche variation among colour morphs

In the different sources of geographical records that we accessed (public records, museum collections, and field observations), the black-and-white morph was the most prevalent colour morph (87.5%), followed by the black morph (9.8%). The orange morph was rare, with a prevalence of only 2.7% (Figure 1).

Visual examination of the climatic niche of all colour morphs in a density space per climatic variable, along with the pairwise significance test (Tukey HSD), suggests that the black and orange morphs exhibit differences across all variables except for mean minimum temperature (Figure 4, Supplementary Table 4, Supplementary Figures 5 & 6). We also observed a similar pattern when comparing the black-and-white with the orange morph; however, they showed similar values of mean maximum temperature. (Supplementary Table 4). Notably, the black and black-and-white morphs differ in potential evapotranspiration, precipitation, solar radiation and air temperature, but not in climatic moisture index (Supplementary Table 4).

We found that the climatic niche of the orange morph was the most dissimilar, presenting low to medium overlap values when compared with the black (D=0.25, I=0.45) and black-and-white (D=0.21, I=0.39) morphs (Supplementary Table 5). In contrast, the climatic niches of the black and the black-and-white morphs were more similar, with high to medium overlap values (D=0.64, I=0.82). These results are consistent with a visual inspection of the density of occurrences in the environmental space (reduced multivariate dimensions to two principal components) where the black-and-white morph has a higher overlap with the black morph than with the orange morph (Figure 2). When comparing the environmental densities of the black and orange morphs, the segregation is even more clear (Figure 2); the black morph is more frequent in areas with higher precipitation and climatic moisture index, and the orange morph has a higher density of points in areas with more solar radiation and higher temperature (Figure 2).

Our test for niche conservatism and niche divergence supports the idea that the black-and-white and black morph share more similar niches than expected by chance (D=0.64, I=0.82, P-value< 0.001; Supplementary Table 5). Conversely, we found that the orange morph's climatic niche is less equivalent than expected by chance when compared to the black morph (D=0.25, I=0.45, P-value= 0.003; Supplementary Table 5) and the black-and-white morph (D=0.21, I=0.39, P-value= 0.003; Supplementary Table 5).

Climatic factors shaping colour morph frequency

In the field, we observed that the black-and-white morph was the most abundant colour variant in most locations, exhibiting a mean relative frequency of 88% (min= 43.5% to max= 100%). The black morph ranked as the second most abundant morph in our sampling, showing a mean relative frequency of 11.8% (0.3% to 48.8%). Lastly, the orange morph appeared as the least frequent, with a mean relative frequency of 9% (0.18% to 56%). (Figure 2).

Our MMR models indicate that precipitation is the strongest predictor of morph frequency change across our sampling, and there was little effect of geographical distance and temperature (Figure 4; supplementary table 6). Pairwise models suggest that the effect of precipitation is reduced ($r_{partial}$=0.087, P-value=0.051) when only comparing black and black-and-white morphs. However, the effect of precipitation is stronger when comparing only the orange morph and black morph ($r_{partial}$=0.37, P-value<0.001; supplementary table 7) or the orange morph and the black-and-white morph ($r_{partial}$=0.16, P-value<0.001; supplementary table 7). Similar results were obtained with the CHELSA dataset (supplementary table 7).

Given that precipitation had the strongest effect in the analysis above, we explored the effect of precipitation on the frequency of each colour variant using linear models. Precipitation did not predict the frequency of the black-and-white morph ($F_{5.49}$=2.12, P-value = 0.15, R^2=0.02;

Figure 2), but had a significant positive effect on the frequency of the black morph ($F_{17.15}$=14.77, P-value < 0.001, R^2=0.30), which was more abundant in places with higher precipitation (Figure 2). Conversely, the orange morph seems to follow the opposite pattern, with higher relative frequency in places with lower precipitation (Figure 2), but there is only marginal statistical support, possibly due to lower sample size ($F_{6.44}$=4.64, P-value = 0.06, R^2=0.31, morph present in 13/49 populations). We obtained similar results with CHELSA dataset for all the colour morphs, except the orange morph ($F_{0.27}$=0.11, P-value = 0.74, R^2=0.01).

Discussion

Integrating macroecological patterns, within-population trends, experiments under controlled conditions and field observations, we revealed the climatic drivers shaping intraspecific colour variation of a widespread colour-polymorphic spider. We found little evidence for thermal benefits of dark colouration, or that temperature drives variation in morph frequency or presence. Instead, differences in the realised climatic niche of colour morphs, and their frequency and presence, were strongly linked to precipitation. Specifically, there is a higher frequency of dark individuals in wetter areas and a higher frequency of orange individuals in drier areas. Our results uncover the crucial role of environmental factors in shaping the presence and frequency of discrete phenotypes within and between populations.

Under controlled laboratory conditions the black morph had a slightly higher heating rate compared to the black-and-white morph. However, we detected no difference in body temperature of the two colour morphs under typical field conditions (average air temperature 30.32°C). Although our field data represent a single snapshot of environmental conditions, our results are consistent with previous evidence that discounted, under field or laboratory conditions, any thermal differences between bright and dark colour variants in other arthropods (Umbers et al., 2013). The small differences in heating rate under controlled conditions may only correspond to differences in heating rate or body temperature in variable field conditions under very limited circumstances. For example, differences in radiative heat gain are expected to be highest in cool, sunny conditions (Kingsolver & Wiernasz, 1991; Zverev et al., 2018; Britton & Davidowitz, 2023). Colour may also have minimal effect on body temperature for very small animals because their body temperature is coupled to convection rather than radiation (Parry, 1951; Stevenson, 1985; Shine & Kearney, 2001; Umbers et al., 2013). In such cases, other factors such as convective and evaporative heat loss can have a greater impact on body temperature than body colouration (Turner & Lombard, 1990; Umbers et al., 2013). This is supported by our field measurements of body temperature, where variation is mostly explained by ambient temperature and wind speed (Supplementary Table 3).

The lack of effect of temperature in our field and laboratory approaches aligns well with our realised climatic niche comparison, which shows that colour morphs differ in almost all climatic variables except for minimum temperature. The black and black-and-white morphs are the most similar in their climatic niche, while the orange and black morphs are the most climatically distinct replacing each other throughout our sampling areas (9 of 38 populations with black individuals also have orange individuals). Our colour frequency measurements across multiple populations also indicated that precipitation is the most important climatic factor affecting the distribution of colour morphs in the Christmas spider. Specifically, higher precipitation favours a greater abundance of black individuals while limiting the presence of the orange morph in the same areas. Higher abundance of black individuals in wetter areas has been reported in other lineages (Friedman & Remeš, 2017; Delhey, 2019; Cerezer et al., 2020; Lopez et al., 2021; Marcondes et al., 2021; Kang et al., 2021). This pattern may arise as a consequence of the role of melanin in antimicrobial protection (Mackintosh, 2001; Wilson et al., 2001; Nosanchuk & Casadevall, 2003), assuming higher pathogen density in high-humidity environments. However, in spiders black and browns are usually produced by ommochrome pigments (a combination of ommatins and ommins) (Seligy, 1972); in fact, melanin was only recently reported in spiders for the first time (Hsiung et al., 2015).

An alternative explanation for the frequency variation of the black and orange morphs of the Christmas spider across a precipitation gradient is that could arise, at least partly, as a consequence of local adaptation associated with background matching (Stevens & Merilaita, 2008; Cheng et al., 2018). Background matching in web-building spiders is crucial to reduce predation (Pekár, 2014; Robledo-Ospina & Rao, 2022) and increase prey capture (Nakata &

Shigemiya, 2015; Nakata, 2021) because they are restricted to their webs. A post-hoc exploration of the association between soil brightness and colour variation suggests that the black morph is more prevalent in forested locations with darker soils, while the orange morph is more abundant in brighter soils characteristic of the most arid regions of Australia (Supplementary figure 7). Available data on background colour are very coarse, which hinders a formal analysis, and there are inescapable links between soil colour and climatic variables, but background matching could be behind the association between precipitation and coloration in this system. In fact, background matching to reduce predation or enhance prey capture can promote colour variation both within and between populations across multiple lineages and types of environments (Bond, 2007; Tate et al., 2016; Matthews et al., 2018; Nosil et al., 2018; Fark et al., 2022; Villoutreix et al., 2023). However, this hypothesis needs to be formally tested by collecting more detailed data on background colours in the field and quantifying background matching. We also note that the sources of selection could be different between the alternative morphs: pathogen protection could drive prevalence of black individuals and camouflage could favour orange individuals in each environment.

Our data also showed that the black-and-white morph is the most successful colour variant. This morph was the most abundant in all our sources of collection records, exhibited the highest frequency in nearly all sampled locations (minimum 43.5%), and was the only colour variant found in isolation. This geographical pattern, coupled with the spatial replacement of black and orange colour variants correlated with precipitation, suggests that colour polymorphism in this species may be maintained through the interplay of multiple selective processes. Colour polymorphism can arise and be maintained when diverse selective pressures act in opposing directions, favouring different colour variants (Endler, 1983; Nokelainen et al., 2011; Poloni et al., 2024). This phenomenon has been documented in various arthropods, where positive frequency-dependent selection favouring one morph is counterbalanced by negative frequency-dependent selection favouring another phenotype

(Takahashi et al., 2011; Chouteau et al., 2017; Poloni et al., 2024). Another possibility is that the coexistence of multiple colour morphs is a consequence of highly heterogeneous environments that impose different forms of spatially and temporally varying selection at a small geographical scale (Nosil et al., 2024). To test these hypotheses, further research is needed to identify the functions of body coloration in *Austracantha minax,* given that we currently do not know whether colour in this species acts as a warning signal or improves prey capture.

Taken together, our work reveals a strong link between precipitation and intraspecific colour variation in the Christmas spider and rules out any important effect of ambient temperature driving colour variation in this species. This level of understanding was possible only through the combination of broad scale, field, and laboratory approaches, providing important evidence for the role of the environment in driving the evolution of colour morphs in spiders. The patterns described here should be explored in depth to elucidate the mechanisms maintaining both within and between colour variation in this species. Given the lack of studies on most invertebrate taxa, it is also important to test the prevalence of the links found here in other polymorphic species, especially in those species with dark or melanic morphs.

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Conflict of Interest

We declare that there are no competing interests in the publication of this work.

Author contributions

F.C.S.-R conceptualisation, funding acquisition, methodology, investigation, data curation, formal analysis, visualization, writing—original draft; D.S.-F. conceptualisation, funding acquisition, supervision, writing—review and editing; I.M. conceptualisation, funding acquisition, supervision, writing—review and editing.

Data availability

All the scripts and information about the data are available here: https://github.com/fcsalgado/christmas_spider_climate

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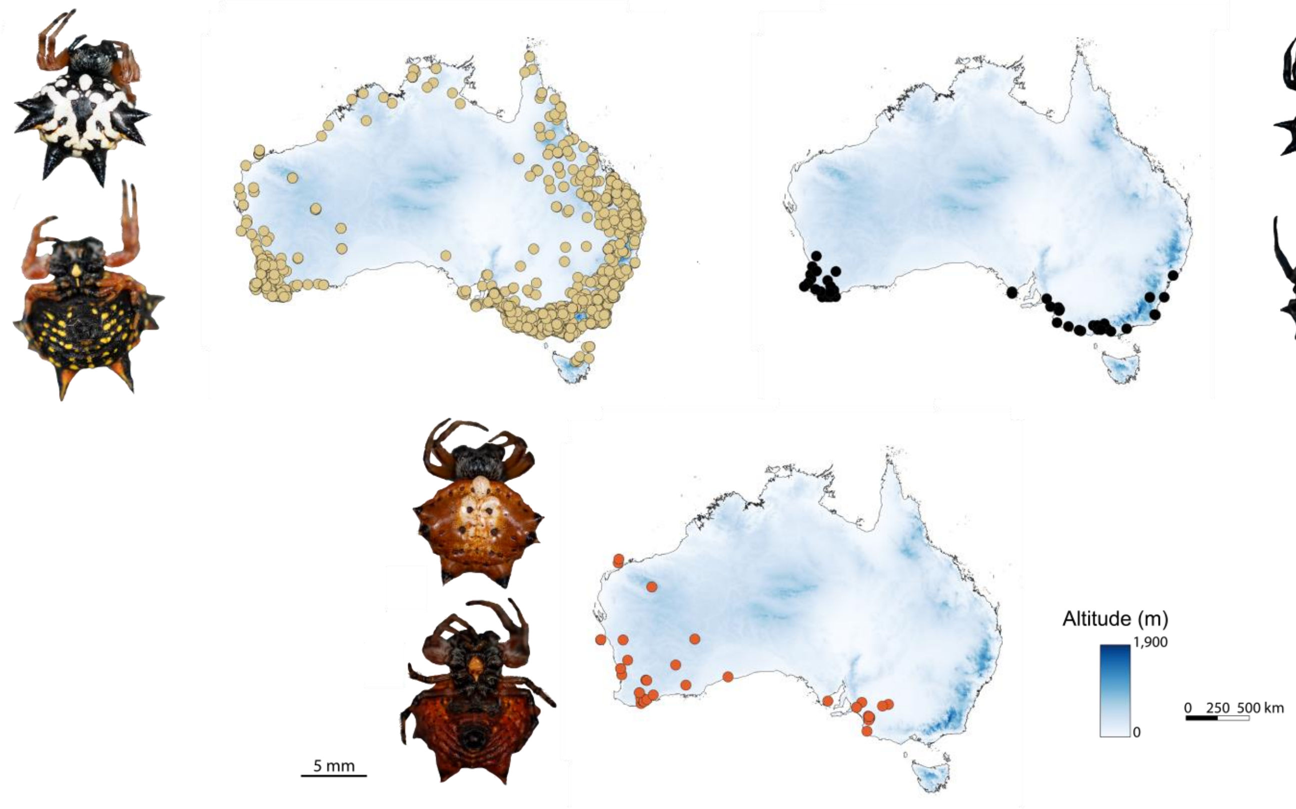

Figure 1. *Austracantha minax* colour variation and morph distribution. Each map represents the geographical records collated for each female colour variant plotted on an altitude background. The top-left panel corresponds to the black-and-white morph (1097 records), the top-right to the black morph (123 records), and the bottom panel to the orange morph (34 records).

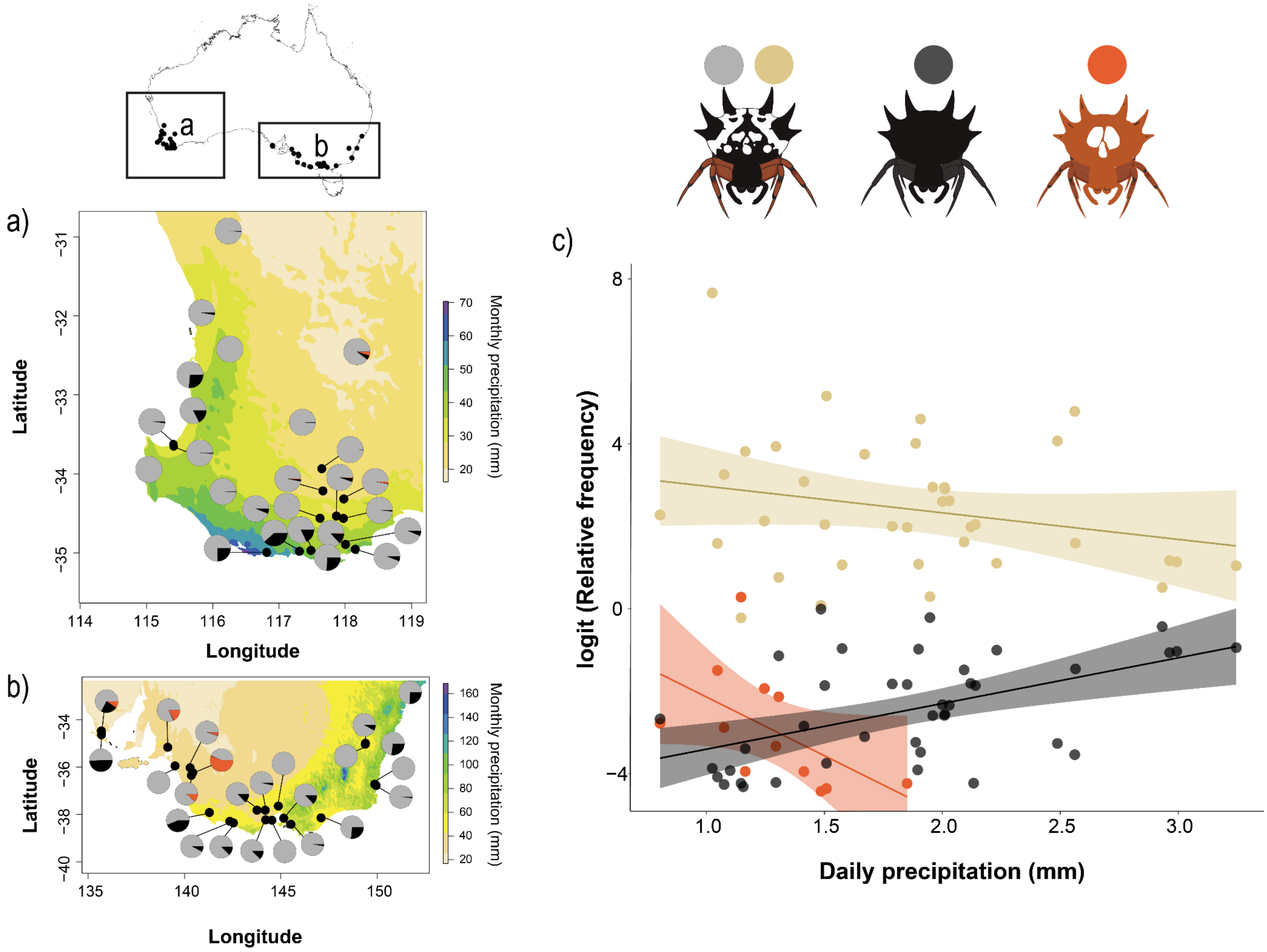

Figure 2. Variation in relative frequency for each colour morph and its association with precipitation. The maps show the relative frequency of the black-and-white (grey colour), black, and orange morphs across 49 locations in a) south-western and b) south-eastern Australia. c) Linear model association between the relative frequency of each colour morph and daily precipitation. Line represents estimate from the model and shaded area represents the confidence interval of the regression.

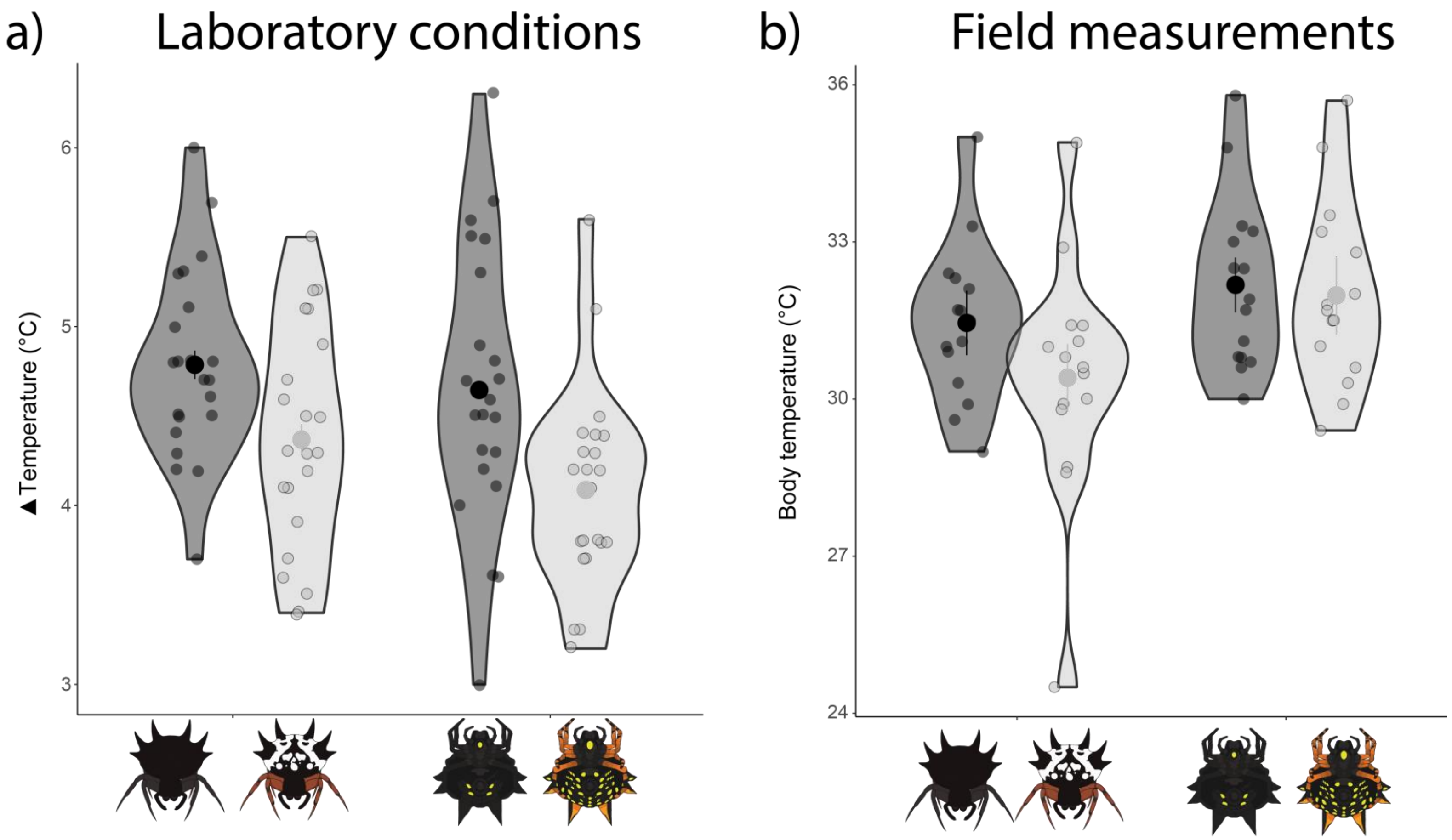

Figure 3. Differences in temperature between the black-and-white and black colour morphs on their ventral and dorsal sides. a) Heating rates experiment under controlled laboratory conditions. b) Field temperature measurements. The dots represent measured values per individual per morph, while the big circles and bars represent the mean ± standard error of the predicted values from the linear models.

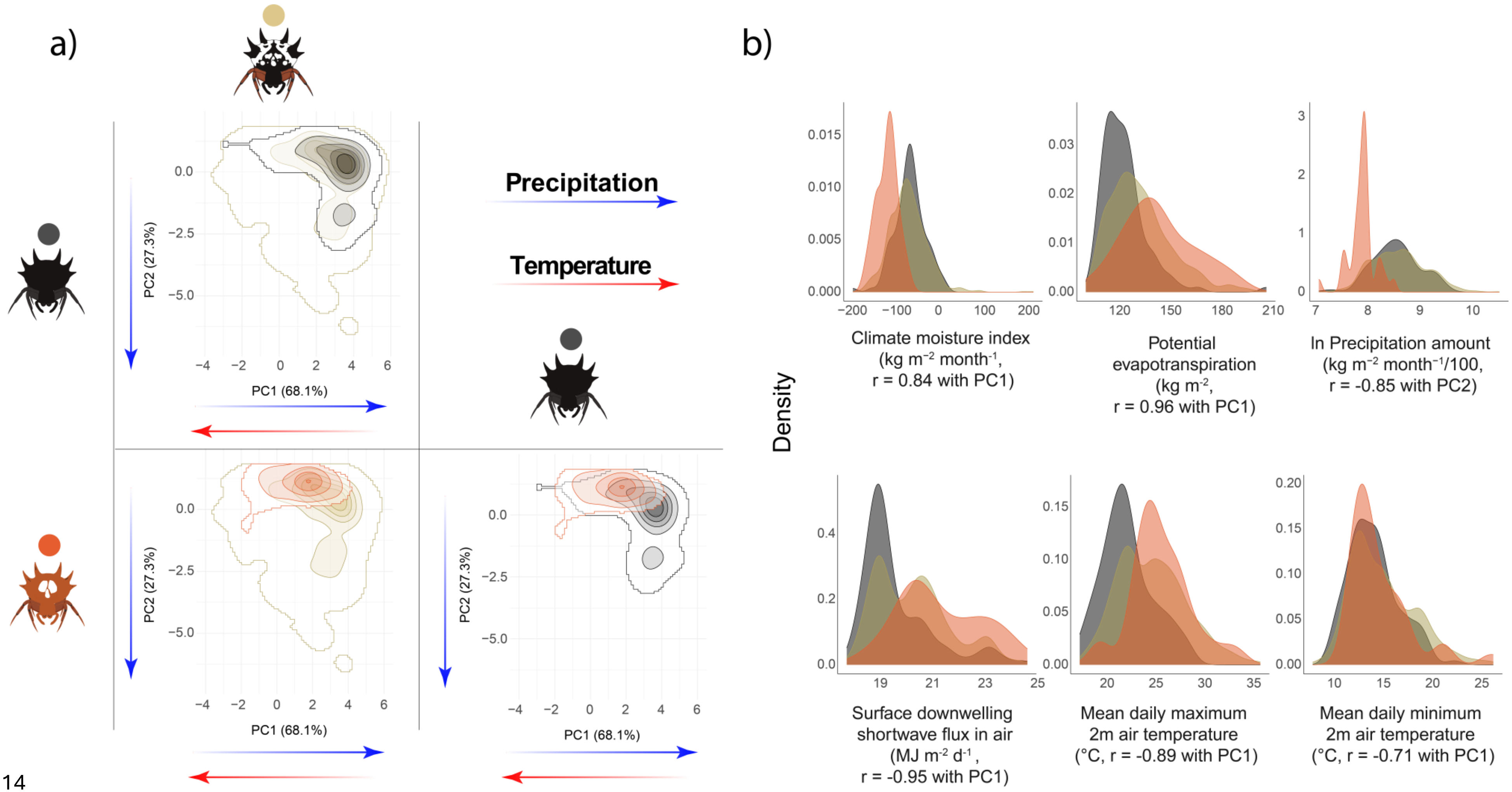

Figure 4. Realized climatic niche of the colour morphs of the Christmas spider. A) Multivariate pairwise comparison of the climatic environment of the black-and-white (yellow), black (black), and orange (orange) morphs. Darker colours represent a higher density of records. Blue arrows represent the

direction of increase of precipitation, climatic moisture index and potential evapotranspiration values in both PC1 and PC2. Red arrows symbolize the increase of solar radiation and temperature values on PC1. B) Density distribution of the records of each climatic variable and their correlation with the principal components in plot A. All density plots per variable are available in supplementary material (Supplementary Figures 5 & 6).