

# Temperature differently affects body pigmentation of the paper wasp *Polistes dominula* along an urban and a wider geographical gradient

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## ABSTRACT

In insects, different pigments, such as melanins and pterins, are involved in thermoregulation. The degree of melanisation often varies along geographical gradients, according to the so-called thermal melanism hypothesis, i.e. darker forms are found in colder places because they can warm up more quickly. Similarly, pterins work as heat sinks and thus are expected to be more abundant in colder sites. Cities, which are warmer than surrounding areas (Urban Heat Island (UHI) effect), might also be expected to influence pigmentation, although studies are lacking. Here, we sampled workers of the social paper wasp *Polistes dominula* (Christ, 1791) (Vespidae) across an urbanisation gradient in an Italian metropolis and used iNaturalist pictures of this species across Italy to study pigmentation patterns at both urban and larger geographical scales. We found a lower yellow intensity of abdominal spots at warmer locations. Scanning Electron Microscopy strongly suggested that yellow colouration is due xanthopterin, known to be the heat sink molecule in other social vespids. Thus, wasps from warmer (i.e., urban) environments are likely to have fewer xanthopterin granules, in line with the lack of need for heat storage due to the local thermal gradient (UHI effect). At the country level, we found that wasps at higher latitudes had smaller yellow spots on the thorax and only two spots instead of four at higher altitudes, in full accordance with the thermal melanism hypothesis. In conclusion, climatic conditions seem to affect insect colour patterns both along urban and wider geographical gradients, although colour changes may affect different body parts and pigments likely according to different needs.

## 1. Introduction

Colour in animals is an important trait involved in both behavioural responses (e.g., Cyriac and Kodandaramaiah, 2019; Duarte et al., 2017) and physiological adaptations (e.g., Gourgoulianni et al., 2023; Lopez et al., 2021). In insects, colour is produced by the absorption or reflection of sunlight either through pigments or through physical properties of cuticular structures (Chapman, 1998). Pigments can be divided into three main categories. Melanins are mainly responsible for black (eumelanin), brown, or red (pheomelanin) (e.g., Polidori et al., 2017; Popadić and Tsitlakidou, 2021), pterins (such as xanthopterin), ommochromes and carotenoids are responsible for shades of red, orange and yellow (Andrade and Carneiro, 2021; Badejo et al., 2020b), while bile pigments are responsible for green to blue colourations (Rothschild and Mummery, 1985). Changes in the relative proportions of these pigments essentially produce all the shades of chemically derived colour found in insects.

Melanins are the most widely abundant pigments in animals

(San-Jose and Roulin, 2018) and, in insects, at least one form, eumelanin, is involved in several physiological functions such as immunocompetence, protection from ultraviolet radiation, protection from desiccation and/or thermoregulation (Lopez et al., 2021). Given its physiological importance and phenotypic plasticity, the degree of melanisation can be observed to vary with changes in the environment. The thermal melanism hypothesis (e.g., Lusia, 1961; Clusella-Trullas et al., 2008) states that colder habitats should filter for more melanic individuals because they can heat up faster than lighter individuals due to lower surface reflectance (Trullas et al., 2007). This may give darker individuals an advantage over lighter individuals in colder environments (Forsman, 2011). This hypothesis has been widely tested and accepted, particularly at intraspecific level, as demonstrated by colour variation in insects along altitudinal (Bishop et al., 2016) and/or latitudinal (Harris et al., 2012) gradients, as well as along seasonal temperature variation (Novella-Fernandez et al., 2023).

Xanthoptern-based yellow segments protect the cuticle of some insects from potentially harmful solar UV radiation (similar to melanin)

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but also serve as a battery that stores excess heat absorbed by the dark cuticle (Ishay and Pertsis, 2002). This suggests that a greater abundance of this pigment should be favoured in colder climates – similarly to melanin – where it is necessary to store efficiently the heat (Badejo et al., 2020a). However, in contrast to eumelanin, patterns of xanthopterin abundance in relation with climatic variation were not investigated yet. Indeed, while the size and number of yellow spots were tested against temperature across wide (continental) and local (rural-urban) gradients in few studies (Badejo et al., 2020a; De Souza et al., 2017, 2020), yellow intensity, that could be considered as a proxy of xanthopterin abundance, was never considered.

Currently, changes in temperature regimes are occurring at both macroscale (Hansen et al., 2006) and microscale (Gago et al., 2013). At large scales, thermal gradients can occur along both latitudinal and altitudinal gradients (Cuesta et al., 2017). However, thermal gradients can also occur at small scales, for example in cities. Urbanisation is the process that leads to the expansion of cities at the expense of natural habitats (Cheela et al., 2021), and is considered one of the main current causes of environmental change (Kalnay and Cai, 2003). Highly impervious (i.e., cemented) surfaces act as heat sinks, leading to the so-called Urban Heat Island (hereafter, UHI) effect (Liu et al., 2020). Due to the UHI effect, urbanised areas tend to be hotter than neighbouring rural or semi-natural habitats, creating a local thermal gradient. Hence, at both macro- and micro-scale, including urban scale (e.g., Leveau, 2021; Polidori et al., 2023), change in pigmentation patterns could be expected for insects.

Insects have been extensively studied in cities, particularly in the light of the ecosystem services they provide (Bolund and Hunhammar, 1999). Aculeate wasps (Hymenoptera) provide important ecosystem services as pest controls, biological indicators, or seed disperser (Brock et al., 2021). In some well-studied social vespids (*Vespula* and *Vespa*), black is due to eumelanin dispersed from the outermost layer of the exocuticle down to the hypocuticle, while yellow is due to a layer of xanthopterin granules within a thin layer of hypocuticle (Ishay and Pertsis 2002; Plotkin et al., 2009). In urban environments, little is known about pigmentation patterns of social wasps. One study reported that workers of *Vespula vulgaris* (Linnaeus, 1758) (Vespidae: Vespinae) had differences in the frequency of colour morphs between urban and rural zones in Helsinki (Finland), though not in other (smaller) cities (Badejo et al., 2020a). However, the observed variations in that study were not explicitly tested against temperature. On the other hand, several studies investigated the role of pigmentation in social wasps as response to changing environmental conditions on a larger scale. For example, De Souza et al. (2017) found darker *Polistes* wasps in colder environments compared to warmer habitats across large geographic ranges. This was also shown across altitudinal ranges, as De Souza et al. (2020) found *Agelaia* wasps with darker thoraxes in high altitude environments. Both findings are consistent with the thermal melanism hypothesis and highlight that melanisation may indeed play a key role for thermoregulation.

Here, we investigated pigmentation variation in the social paper wasp *Polistes dominula* (Christ, 1791) (Vespidae: Polistinae) across two different environmental gradients: at a small scale by sampling wasps along a temperature/urbanisation gradient in the metropolitan city of Milan; and at a larger scale by using *P. dominula* public records on iNaturalist for the Italian peninsula. *Polistes* species are found in a wide range of climatic conditions, which underpins their strong adaptive capacity (Sheehan et al., 2015; Turillazzi and West-Eberhard 1996). These wasps are commonly found in both anthropised and semi-natural habitats (Höcherl and Tautz, 2015), with urban habitats being heavily occupied as they prefer to build their nests, made of chewed wood fibres (Bagriacik, 2012), in sheltered locations associated with man-made structures (Reed et al. 1979; Pérez-Bote and Mora-Rubio 2020). Furthermore, *Polistes* species show a variety of yellow spots on their otherwise black bodies (Turillazzi and West-Eberhard 1996), with colour patterns also involved in intra-specific visual communication

(Tibbetts and Dale, 2004; Cervo et al., 2015). They therefore provide a good model for testing temperature-dependent variations in pigmentation in cities. Previous studies reveal that *P. dominula* is prone to show intra-specific variations across urbanisation gradients, hence acting as a “sentinel” to urbanisation pressure. For example, both body size and wing loading are reduced (Ferrari et al., 2024), gut histological damages are more abundant (Polidori et al., 2018) and accumulation of pollutants is greater (Urbini et al., 2006; Polidori et al., 2018) in strongly urbanised areas. However, variation of pigmentation in response to the UHI effect has been neglected to date in this species.

Specifically, by measuring the size of yellow and black spots (at both urban and country scale) and quantifying the colour intensity of both yellow and black areas (only at urban scale), we tested the following two hypotheses based on the known role of eumelanin (black) and xanthopterin (yellow) in vespid wasps: 1) following the thermal melanism hypothesis, we expect warmer sites to select for lighter individuals (either through a reduction in black intensity or areas); at the small scale, warmer sites are associated with the UHI effect, and 2) following the heat storage need hypothesis, we expect warmer locations (UHI effect) to filter for reduced yellow intensity or areas, as there is no need for heat storage due to the urban local thermal gradient.

## 2. Materials and methods

### 2.1. Sampling activity and landscape characterisation

The study area is the metropolitan city of Milan (45°28'01"N 9°11'24"E) situated in Lombardy, northern Italy. The site selection was made according to a commonly used proxy for urbanisation, i.e. the proportion of green and impervious surfaces (Fenoglio et al., 2021). Such proportions were retrieved from land-use data available in DUSAF6.0 (<https://www.dati.lombardia.it/Territorio/Dusaf-6-0-Uso-del-suolo-2018/7rae-fng6>), that exploits aerial photogrammetry, with a resolution of 20 m. The shapefile in DUSAF6.0 was imported in QGIS v3.16.15 (QGIS.org, 2022. QGIS Geographic Information System. QGIS Association. <http://www.qgis.org>) and buffers with a radius of 500 m centred on the geographical coordinates of each sampling site ( $N = 11$ ) were created. From this circular cut-out, we extracted the proportion of impervious surface (Grüter and Hayes, 2022). Each site was separated by at least 1 km between each of them to ensure spatial independence (Greenleaf et al., 2007).

The sampling activity took place in April and May 2022. We collected 5 to 15 wasps (all workers) *per* site with an entomological net mainly around nests. Specimens were first stored in icebags on field and then preserved in the laboratory at  $-20^{\circ}\text{C}$ . For each specimen, we assigned a value of mean temperature calculated across the rough period in which they developed as larvae (March–May 2022), following Ferrari et al. (2024). We used land surface temperature estimated using the product MOD11A2 (<https://modis.gsfc.nasa.gov/data/dataproduct/mod11.php>) downscaled from the original resolution of 1 km to a finer resolution of 100 m through bilinear interpolation, and then we calculated mean land surface temperatures for each sampling site within a 500 m radius buffer (as in Tommasi et al., 2022). We checked for possible spatial autocorrelation of these temperatures with Moran's test (Mathur, 2015) excluding possible biases due to autocorrelation ( $I = -0.063$ ,  $P = 0.570$ ). In addition, we observed that temperature was associated with the proportion of impervious surfaces (Spearman's  $\rho = 0.491$ ) and that more natural sites (impervious areas with mean  $\pm$  standard error of  $22.757 \pm 6.229$ ) had a lower mean temperature ( $28.31^{\circ}\text{C} \pm 0.867$ ) than more urbanised sites (impervious areas:  $73.524 \pm 7.156$ ; temperature:  $30.679^{\circ}\text{C} \pm 0.870$ ). Urban sites have significantly more impervious areas (Mann-Whitney test,  $z = 2.647$ ,  $P = 0.008$ ) and a higher temperature but not statistically significant (Mann-Whitney test,  $z = 1.369$ ,  $P = 0.171$ ), likely due to the low sample size. Overall, 97 individuals from 11 sites were analysed in this study (Fig. 1A). In these sites, the proportion of impervious surface ranges

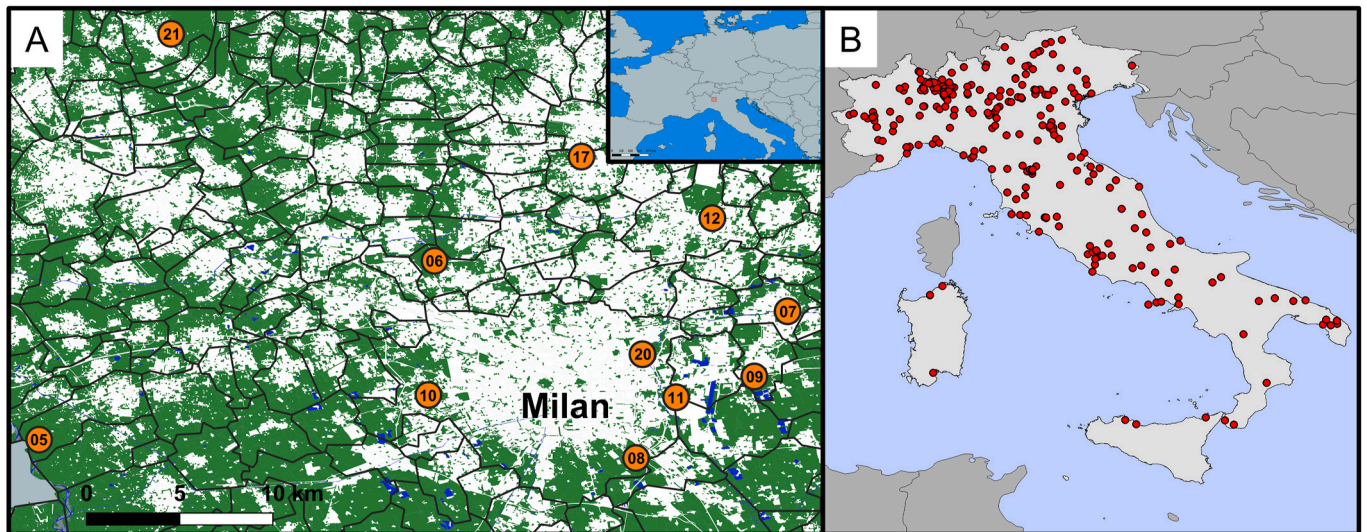


Fig. 1. A) The orange dots, with numeric ID inside, represent the sampling sites in the Metropolitan City of Milan. The map also shows in white impervious surfaces, in green vegetated areas and in blue water. On the right-upper part, a map of Europe showing the location of the sampling activities. B) Map of Italy showing the records from iNaturalist used in this study.

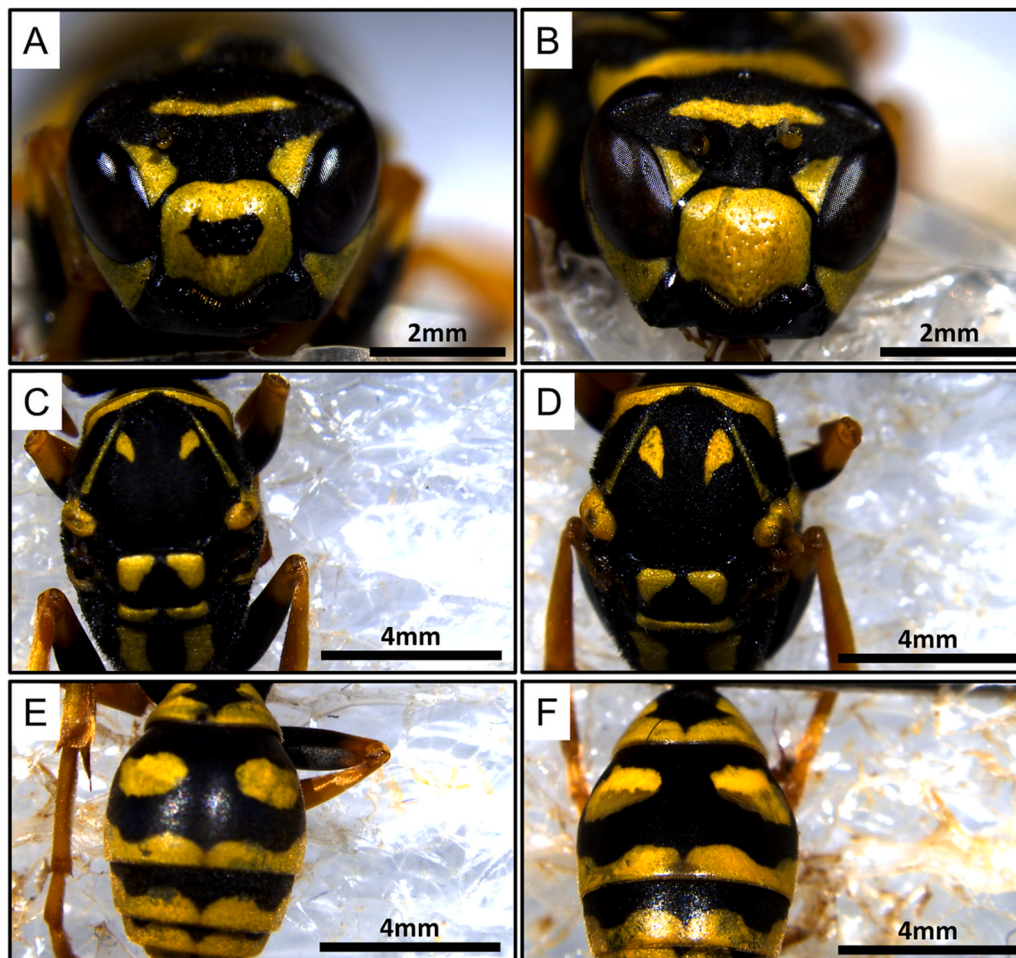


Fig. 2. Examples of the variations in melanisation patterns in *P. dominula* sampled across the urbanisation gradient. A-B) show examples of the variations in the size of the black spot on the clypeus. C-D) show examples of the variations in the size and colour of the yellow spots on thorax and E-F) on the 2<sup>nd</sup> metasomal tergite.

between 1 and 88% and temperature between 25.8 and 32.5 °C.

## 2.2. Melanisation metrics and colour quantification

Before the analysis, we measured the intertegular distance (ITD), the minimum linear distance between the tegulae, as proxy for body size (Ferrari et al., 2024). All ITD measures were taken with LEICA MZ75 stereomicroscope mounted with a LEICA flexacam C3 camera (accuracy 0.001 mm). We then took a frontal photo of the clypeus and a dorsal photo of the entire wasp (Fig. 2). To ensure the maximum reproducibility among all the pictures, all the photos were taken with the same low light intensity, all in the same day and in a dark, closed room.

Using the software ImageJ (Schneider et al., 2012), we measured different parameters related to melanisation degree. Due to the curvature of the body and overlapping segments we could not measure the same parameters (e.g., the proportion between black and yellow areas) on all the body parts. On the clypeus, we measured the *proportion of black area* as the ratio between the yellow area and the area of the black spot in the middle (Fig. 2A and B). On the thorax, we measured the total *area of the four yellow spots* (with larger spots meaning a reduced black surface, for the same body size) that can be found on the mesonotum between the tegulae (Fig. 2C and D). Then, on the 2<sup>nd</sup> metasomal tergite (morphologically the 3<sup>rd</sup> abdominal tergite), we measured the total *area of the two yellow spots* (with larger spots meaning a reduced black surface, for the same body size) (Fig. 2C and D). On this region we also measured the *length of the melanised area* (see Badejo et al., 2020a, 2021) as the linear width between the upper border and the bottom yellow pattern of the tergite (Fig. 2C and D). For each of these body parts (clypeus, thorax, tergite) we also measured the *brightness of black* (i.e., the luminance value which is a measure of black intensity) in 20 random selected pixels on the melanised portion of the cuticle (following De Souza et al., 2017) and averaged these values for each specimen. Brightness spans from 0 (completely black) to 255 (completely white). Finally, we quantified the mean *Red, Blue, Green* and *RGB value of the yellow spots* of the cuticle on both the clypeus and the 2<sup>nd</sup> metasomal tergite. To do so, we selected three non-overlapping Region of Interests (ROIs) of 0.2 mm<sup>2</sup> from which we extracted the mean RGB value (similarly to Byers, 2006; Makled and Tahoun, 2015; Lehnert et al., 2011). For yellow spots, we assumed that the closer the measured value is to the average RGB of pure yellow, FF0000, RGB: 255, 255, 0, the “more yellow” the cuticle is (Byers, 2006). Reduction in Red and Green components mean that the yellow is darker and less plain. This method provides a rough and easy to interpret measure of the intensity of yellow. We excluded the thorax from the latter measurement because the yellow spots are too small to create a ROI that covers an adequate proportion of the cuticle. All the parameters are summarised in Table S1.

## 2.3. Records selection and metrics from iNaturalist

To analyse comparable melanisation patterns in *P. dominula* along a wider environmental gradient, we used records and pictures uploaded to iNaturalist (<https://www.inaturalist.org/>), as in Davis et al. (2022). iNaturalist is a popular website and app that is a joint initiative of the California Academy of Sciences and the National Geographic Society. On this website, people can upload geo-referenced observations of organisms, and the community can taxonomically identify these observations. We selected only those photos related to female wasps, with a dorsal view of the insect, within the political boundaries of Italy, and taken between January 2018 and December 2022. We decided to use the year 2018 as a threshold because the records before this year were so scarce that they could cause imbalances and a possible misleading interpretation of the statistical analysis.

We assigned latitude, longitude, and altitude to each record. We also assigned the average annual temperature of the place where the photo was taken. To do so, we retrieved temperature data from the product MOD11A2 (<https://modis.gsfc.nasa.gov/data/dataproduct/mod11.php>)

with a scale of 1 km. Overall, we found useful records and pictures along the whole Italian peninsula (Fig. 1B), hence covering wide gradients of the selected climatic variables (Fig. S1). Observations included in iNaturalist were published under one of the following licenses or waivers: 1) <http://creativecommons.org/publicdomain/zero/1.0/>, 2) <http://creativecommons.org/licenses/by/4.0/>, 3) <http://creativecommons.org/licenses/by-nc/4.0/>.

For each selected record, we measured the head width as proxy for the insect body size (Ortolani and Cervo, 2010), the *area and number of yellow spots on the thorax* (with smaller areas of yellow or less spots corresponding to larger areas of black pigmentation, for the same body size) and the *distance between the two yellow spots on the 2<sup>nd</sup> metasomal tergite* as a proxy for their dimension. We then weighted these measures by body size (head width). In this way, we weighted all the pigmentation values by the *apparent* size of the wasp. Overall, we used 261 records from iNaturalist.

## 2.4. Scanning electron microscopy

As we were analysing the intensity of yellow on the tergite, we also checked whether *P. dominula* had xanthopterin in its cuticle, similarly to other social vespids (Badejo et al., 2020a; Plotkin et al., 2009). Therefore, we visually inspected transverse sections of the yellow cuticle of *P. dominula* by scanning electron microscopy (SEM) to search for a thin layer of pigment granules in the hypocuticle. As a first control, we investigated by SEM the transverse sections of the black cuticle of *P. dominula*, where no granules are expected (Plotkin et al., 2009). As a second control, we observed with SEM yellow and black cuticle sections of a species of *Vespa*, which is known to possess xanthopterin granules (Badejo et al., 2020a; Plotkin et al., 2009) and compared their morphologies with those found in *P. dominula*.

For both wasp species, we manually detached the second tergite and broke it into tiny pieces, either yellow or black, using a pair of tweezers and a pair of scissors. This produced flake of cuticle which we placed on the mounting stubs for SEM analysis. The cuticle parts were then mounted perpendicular (i.e. with a cross section pointing upwards) on adhesive carbon pads attached to aluminium stubs and introduced in the SEM (Zeiss LEO 1430, Zeiss, Oberkochen, Germany), without previous gold coating. High vacuum conditions (resolution: 3.0 nm at 30 kV (Secondary electrons-SE), 10 nm at 3 kV (SE), and 4.0 nm at 30 kV (Backscattered electrons-BSEs) were used. The accelerating voltage was 26 kV, the high vacuum was 53.3–66.6 Pa, and the working distance was 10 mm. One individual *per species per colour* was analysed.

## 2.5. Statistical analysis

All the statistical analyses were carried out in R 4.2.2 (R Core Team, 2020). Firstly, we checked through Pearson correlation test that the hour in which each picture was taken did not significantly affected brightness or RGB values (all *P*-values >0.05). We analysed the variation in pigmentation along the urbanisation gradient with Linear Mixed Models using *lme4* (Bates, 2010) and *sjPlot* (Lüdtke, 2015). We selected temperature as the sole predictor and the sampling site as random effect. Only for the models regarding areas and lengths, we added the intertegular distance as covariate since we expect surfaces and distances to increase with body size. With the package “*performance*” (Lüdtke et al., 2021) no deviances from residual and random effects normality were detected (Cheng et al., 2010; Schielzeth et al., 2020).

To analyse the variation in pigmentation in the iNaturalist records, we build a full ordinary linear model using as predictors altitude, latitude, temperature, and interaction between month and year of the observation. Then, we used the package *MuMIn* (Barton, 2015) to generate all the possible combinations of predictors included in the full model and to rank the models based on Akaike’s information criterion (AIC). We selected the best model based on this ranking; we reported all the models differing by no more than two AIC units ( $\Delta AIC \leq 2$ ) from the

model ranked first in Table S2(e.g., Mayr et al., 2021; Polidori et al., 2020). For the number of yellow spots on the thorax, we used an ordinary generalised linear model with a binomial distribution, after re-coding the number of spots as 1 (in case of 4 spots) and 0 (in case of 2 spots). 0 spots was represented by only one observation and thus excluded. All the plots were made with *ggplot2* (Wickham et al., 2016). All the data are available in Dataset.xlsx

### 3. Results

SEM images showed that a similar structure of packed granules occurs in the hypocuticle of the yellow cuticle of both *Vespula* sp. and *P. dominula*. A cross section of a yellow portion of the 2<sup>nd</sup> metasomal tergite of *P. dominula* shows parallel layers forming the endocuticle and below a spongy layer of granules (Fig. 3A–C). This structure closely resembles the one we found in *Vespula* (Fig. 3B–D). Given the resemblance of this structure with that observed in the yellow cuticle of *Vespula* by Badejo et al. (2020a) and Plotkin et al. (2009), we can reasonably affirm that also *P. dominula* possess a form of pterin, likely xanthopterin. Conversely, on the black portion of the cuticle, only parallel layers of the endocuticle are visible, both in *Polistes* (Fig. 3E) and *Vespula* (Fig. 3D).

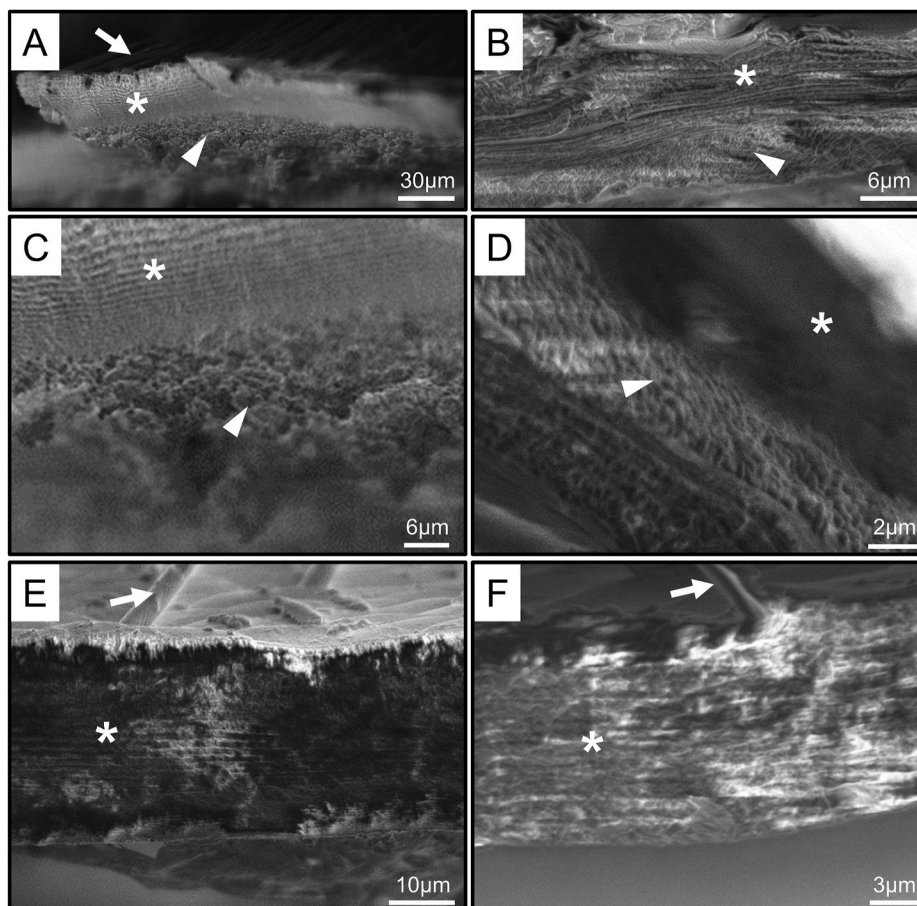
We have found variations in pigmentation both along the small (urban) gradient and the wider (Italian) gradient, partially in agreement with the thermal melanism hypothesis or with the heat storage need (xanthopterin) hypothesis. However, not all body parts were equally variable or responded in similar way to our predictors, and not all variations affected the same body parts at both spatial scales.

At the urban scale, we found that in warmer urban sites, the mean RGB of the yellow spots on the tergite was significantly lower than in cooler sites (Table 1, Fig. 4A). This is because the red component (Table 1, Fig. S2C) and the green component (Table 1, Fig. S2D) decreased in hotter sites. Marginally significantly, we also found that the area of the yellow spots on tergite decreased (Table 1, Fig. S2A) and that the length of the black area on the 2<sup>nd</sup> metasomal tergite was shorter (Table 1, Fig. S2B), both in warmer sites. However, we argue that the magnitude of these changes would not likely affect thermoregulation properties in wasps.

At the wider country-level scale, we found that wasps had significantly smaller yellow spots on the thorax – and therefore larger black surface for the same body size – at higher latitudes (i.e., in the north of Italy) (Table 2, Fig. 4B) and marginally significantly smaller yellow spots on the thorax – and thus larger black area – at higher altitudes (Table 2, Fig. S2E). Finally, we also found that wasps with only 2 yellow spots on the thorax were recorded at statistically significantly higher altitudes than those presenting 4 spots (Table 2, Fig. 4C).

### 4. Discussion

Here we presented a detailed study on the responses of pigmentation patterns in *Polistes dominula* to a local thermal gradient in a metropolitan city of northern Italy, likely associated with the UHI effect. We also investigated whether such responses are comparable to variations in melanism along geographical gradients retrieving iNaturalist observation of this species for Italy. For this part, we think that weighting the measurements by a good proxy of body size of the wasp (i.e., head

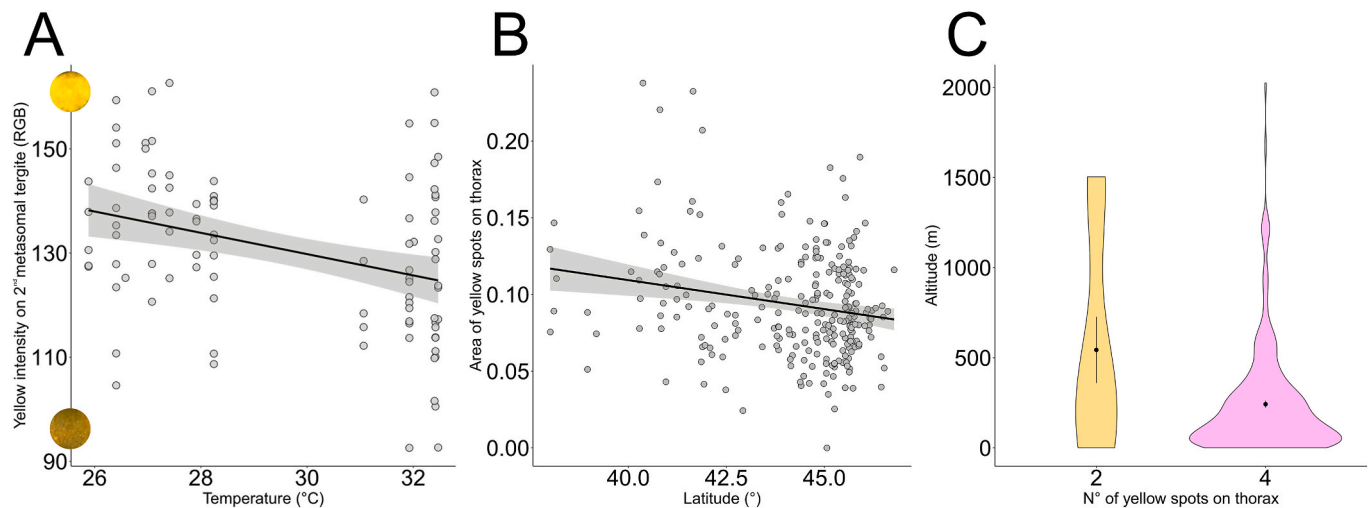


**Fig. 3.** SEM images showing an overview of a yellow portion of cuticle in *P. dominula* (A) and *Vespula* sp. (B). Close-up of the xanthopterin granules in *P. dominula* (C) and *Vespula* sp. (D). Finally, a black proportion of cuticle, used as control, in *P. dominula* (E) and *Vespula* sp. (F). Arrows: hairs extruding from the cuticle, asterisks: endocuticle, arrowhead: granules of xanthopterin.

**Table 1**

Summary statistics of the linear mixed models used to analyse the variations in melanisation across the urban thermal gradient Br: brightness, d.f.: degrees of freedom, N: sampling size,  $R_M^2$ : amount of variation explained by the fixed effects (i.e., temperature), P: p-value. Significant results are in bold, marginally significant results are noted with \*.

Trait	d.f.	N	$R_M^2$	Predictor	Estimate	Statistic	P
Proportion of black area on clypeus	93	97	0.004	Temperature (C°)	-0.002	-0.599	0.550
Area yellow spots on thorax (mm <sup>2</sup> )	92	97	0.215	Temperature (C°)	0.006	0.781	0.437
				<b>ITD</b>	<b>0.387</b>	<b>4.769</b>	<b>&lt;0.001</b>
Area yellow spots on 2 <sup>nd</sup> metasomal tergite (mm <sup>2</sup> )	92	97	0.129	Temperature (C°)	-0.034	-1.981	0.051*
				<b>ITD</b>	<b>0.807</b>	<b>3.441</b>	<b>0.001</b>
Length of the black area on 2 <sup>nd</sup> metasomal tergite (mm)	93	97	0.358	Temperature (C°)	-0.019	-1.753	0.083*
				<b>ITD</b>	<b>0.893</b>	<b>7.078</b>	<b>&lt;0.001</b>
Brightness black area on clypeus	75	79	0.035	Temperature (C°)	0.204	1.652	0.103
Brightness black area on thorax	93	97	0.001	Temperature (C°)	-0.008	-0.189	0.851
Brightness black area on 2 <sup>nd</sup> metasomal tergite	93	97	0.005	Temperature (C°)	-0.074	-0.590	0.556
Yellow intensity on clypeus (RGB)	93	97	0.001	Temperature (C°)	0.093	0.069	0.945
Red intensity on clypeus (R)	93	97	0.001	Temperature (C°)	-0.016	-0.009	0.993
Green intensity on clypeus (G)	93	97	0.001	Temperature (C°)	-0.486	-0.277	0.782
Blue intensity on clypeus (B)	93	97	0.042	Temperature (C°)	0.983	1.601	0.113
<b>Yellow intensity on 2<sup>nd</sup> metasomal tergite (RGB)</b>	<b>93</b>	<b>97</b>	<b>0.118</b>	<b>Temperature (C°)</b>	<b>-2.045</b>	<b>-3.581</b>	<b>0.001</b>
<b>Red intensity on 2<sup>nd</sup> metasomal tergite (R)</b>	<b>93</b>	<b>97</b>	<b>0.117</b>	<b>Temperature (C°)</b>	<b>-3.109</b>	<b>-3.558</b>	<b>0.001</b>
<b>Green intensity on 2<sup>nd</sup> metasomal tergite (G)</b>	<b>93</b>	<b>97</b>	<b>0.151</b>	<b>Temperature (C°)</b>	<b>-2.982</b>	<b>-3.596</b>	<b>0.001</b>
Blue intensity on 2 <sup>nd</sup> metasomal tergite (B)	93	97	0.017	Temperature (C°)	0.420	1.032	0.305



**Fig. 4.** Representations of the statistically significant models. A) Linear regression of the mean RGB on the second metasomal tergite along the urban temperature gradient. B) Linear regression of the area of the yellow spots on the second metasomal tergite along the Italian latitudinal gradient. Points are actual values; the line is a fitted linear model and in grey is the 95% confidence interval. C) Violin plots showing the mean altitude at which individuals with 2 or 4 spots were recorded. Points show the mean and bars standard error.

**Table 2**

Summary statistics of the ordinary linear models used to analyse the variations in melanisation across the geographical gradient in Italy. All traits are weighted by head size. d.f.: degrees of freedom, N: sampling size,  $R^2$ : amount of variation explained by the model, P: p-value. Significant results are in bold, marginally significant results are noted with \*.

Trait	d.f.	N	$R^2$	Predictor	Estimate	Statistic	P
<b>Area of yellow spots on thorax</b>	258	261	0.055	<b>Latitude</b>	<b>-0.004</b>	<b>-3.203</b>	<b>0.002</b>
				Altitude	-0.000	-1.896	0.059*
Distance between yellow spots on 2 <sup>nd</sup> metasomal tergite	259	261	0.004	Altitude	-0.001	-1.051	0.294
<b>N° of yellow spots on thorax</b>	259	261	0.066	<b>Altitude</b>	<b>-0.001</b>	<b>-2.730</b>	<b>0.006</b>

width) would make the comparison between different observations and scales reasonably robust. Additionally, our study proposed a new integrative method to take a broader view of pigmentation patterns in terms of both areas and hues of yellow and black portions of the cuticle. Overall, we found that the responses of the wasps were different at the two spatial scales we worked with. At the local scale, the yellow intensity on the tergite seems to be the most affected variable, while at the large scale it is the size and the number of the yellow spots on the thorax. However, at both scales, the responses indicate a possible adaptation to

better thermoregulation.

#### 4.1. Urban thermal gradient

At this scale, we did not find any statistically significant variations in the pigmentation on the clypeus, the only scale at which we studied this body part. This is somehow expected since in *P. dominula* and other social wasps the pattern on the clypeus is involved in intraspecific communication and dominance establishment within the colony (Cervo

et al., 2015; Tibbetts and Dale, 2004). Thus, it may be hypothesised that the colour pattern on the clypeus does not have pronounced phenotypic plasticity, as this could impair intraspecific communication (Tibbetts, 2010). This interpretation is supported by other studies on *Polistes* which found that the luminance in the clypeus did not track the elevation (and thus temperature) of wasp collection (De Souza et al., 2020). We found no response in the thorax, but we did find responses to the local urban thermal gradient – probably related to the UHI effect – in terms of the degree of yellowing on the 2<sup>nd</sup> metasomal tergite. It can be argued that the urban temperature gradient in which we sampled *P. dominula* (around 6 °C) was not sufficient to induce darkening of the thoracic cuticle. In fact, the thorax houses the flight muscles, which require a minimum temperature to contract and allow the insect to fly. We hypothesise that along the thermal urban gradient, the range of temperatures experienced by wasps is not large enough to induce darkening of the thorax, as the minimum temperature in more seminatural areas is still high enough to fly. Conversely, the abdomen has thermoregulatory functions and we hypothesise that some variation that makes thermoregulation more effective may also be seen along a local thermal gradient (De Souza et al., 2020).

Specifically, colder sites hosted wasps with more intense yellow given by a higher component of red and green. Based on our SEM analysis, this colour is probably due to an increased accumulation of xanthopterin. In animals, there are two types of pterin that give yellow colouration: sepiapterin and xanthopterin. However, the former has been only found in crustaceans and fishes, while the latter seems to be unique to insects (Andrade and Carneiro, 2021). Thus, if the yellow portion of the tergite serves as a biological heat sink for storing excess heat, reduced yellow pigmentation will dominate urban habitat where there is no need for heat storage due to the UHI effect (Badejo et al., 2020a). This is indeed what we found, wasps in warmer urban areas have a less intense yellow on the 2<sup>nd</sup> metasomal tergite. Interestingly, colder environments filtered for a yellow phenotype also in a *Colias* (Lepidoptera) species (Woronik et al., 2018). Moreover, in another *Colias* species, low temperature caused an increase in the concentrations of the coloured pteridines in the white females (Hoffmann, 1974). These results support our findings of a reduction in the yellow intensity in hotter sites. Joining this colourimetry result with the SEM evidence of the presence of xanthopterin also in *Polistes*, we may conclude that in warmer urban areas wasps likely possess less or thinner layers of xanthopterins, since they do not need effective heat storage abilities. In addition, similarly to Badejo et al. (2018), we found little-to-no variation in the length of the melanised area on the tergite along the urban thermal gradient. Nonetheless, our results are in contrast with what was found in different species of insects that present more melanised forms in more urbanised areas (reviewed by Leveau, 2021). However, the same author suggests that an increased melanisation in urban populations of these insects may be a response to a need of increasing camouflage in the urban matrix, rather than a response to the urban climate.

#### 4.2. Country-level gradient

On a large geographical cline, we find the pigmentation of only the thorax to be affected by the environmental conditions. We expected smaller yellow spots – and thus larger black areas, for the same body size – on the thorax in colder environments, as this body region houses the flight muscles. We found that latitude and altitude did affect thorax melanisation patterns, after having corrected for year and sampling month. These results are similar to what found in other studies on paper wasps (De Souza et al., 2017, 2020). In fact, we found that wasps further north (and less so at higher altitudes) had smaller yellow spots on their thorax when their body size was factored in. As a result, they have a larger black area on the thorax. This is to be expected since, at comparable altitudes, northern areas are colder than southern ones. Indeed, in colder environments, darker thoraxes are thought to improve the heating of the muscles associated with flight. This interpretation and our

findings are supported by other latitudinal gradient studies. For example, by a study on a *Drosophila* species in which higher latitudes (colder climates) hosted darker individuals (Parkash et al., 2008). In support of the temperature-related responses of the thorax, we also found that wasps with only 2, rather than 4, yellow spots on the thorax were recorded at significantly higher altitudes. However, it should be noted that the number of wasps with only 2 spots was smaller than that with 4 spots.

We may hypothesise that the two different patterns that we found at the urban- and country-level are due to different ecological needs that the wasps have. On the latitudinal gradient, there is an annual change in temperature, so the reduction in the size of the yellow spots on the thorax is probably due to a higher thermoregulatory efficiency in colder habitats (i.e., northern localities). Furthermore, there is also an increase in UV radiation along the altitudinal gradient, which may put additional pressure on melanisation patterns, and the wasps respond to this additional demand by reducing the number of spots. These hypotheses are supported by other studies, but we also argue that we may have overlooked other possible environmental factors and that ours may be a simplified overview. Nonetheless, in one species of butterfly, colder seasons and higher altitudes were found to produce more melanic individuals (Gautam and Kunte, 2020). Also, an *Agelaia* social wasp species presented progressively reduced yellow patterns on the thorax with increasing elevation (De Souza et al., 2020). So, mountain and northern wasps cope with lower temperatures and thus require darker thoraxes to properly warm up the flight muscles (De Souza et al., 2020). However, since it is known that UV radiation increases with altitudes, wasps at higher altitudes may have fewer yellow spots – and therefore larger black areas – to effectively protect themselves from intense solar radiation (Bastide et al., 2014; Bishop et al., 2016).

Conversely to what we found along the urbanisation gradient, there were no significant changes in the pigmentation of the abdomen along the geographical gradient (with a temperature range of around 20 °C). As explained earlier, large geographical gradients (and hence wider temperature ranges) are likely to exert greater pressure on thermoregulatory needs, resulting in changes in thoracic melanisation patterns.

## 5. Conclusions

Altogether, our results highlight how the pigmentation pattern in *P. dominula* – a common wasp species in anthropised environments – experiences a selective pressure from temperature both at local and large scales. We have shown how the local urban thermal gradient – due to the UHI effect – affects the degree of yellowing on the 2<sup>nd</sup> metasomal tergite, likely decreasing xanthopterin abundance. This would be one of the first pieces of evidence for a variation in the pigmentation in response to the local thermal gradient associated with the UHI effect. In addition, we confirmed previously known patterns of melanisation in response to geographical gradients, demonstrating that citizen science platforms such as iNaturalist can be effectively used for such studies. Interestingly, we showed that the thermal melanism hypothesis can be applied to geographical thermal gradients, while on the local gradient the heat storage need hypothesis seems more likely. Future studies should focus on more chemical analyses, for example to characterise and quantify in detail the pigments involved in the changes of melanisation patterns, as well as the physiological consequences of such variation for the wasps.

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## CRediT authorship contribution statement

**Andrea Ferrari:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Carlo Polidori:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2024.103840>.

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