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Urban environment and climate condition-related phenotypic plasticity of the common wasp *Vespula vulgaris*

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Abstract

Environmental alteration for urban development prompts ecological changes across urban centres, ranging out towards the surrounding undisturbed areas. These impact on organisms living across the urban-rural gradient and insects, being widely distributed in nature, are confronted with adaptive choices in such situations. The temperature in urban cores is generally higher due to the urban heat island effect. Thus, insects might modify their behaviour or morphology to cope with the urban environment. The common wasp *Vespula vulgaris* (L.) has shown adaptive capability as its colour pattern varies between years and geographic distribution. Our study assessed the impact of the built urban environment and summers of differing weather conditions on the common wasp by studying the melanic pigmentation on the abdomen. Samples were collected from urban zones in three cities and their adjacent rural zones in Finland. In one location samples were also collected from two different summers to see if climate condition difference between years can drive morph variation in the 1st and 2nd tergite. We also studied the structure of the cuticle and discovered a structure similar to xanthopterin granules embedded in the yellow stripes of the cuticle, this pigment acts as solar cells and is useful in thermoregulation. Our results showed that the common wasps had differences in the frequency of colour morphs of 2nd tergite between years with different weather and between urban and rural zones in the largest city, Helsinki. Wasps from urban and rural zones showed similar trend for the proportion of black and yellow pigmentation. Common wasps seem to have capability to adapt their pigmentation to correspond with prevailing summer and urban climate conditions, thus reaching better balance between heating and energy storing components in their cuticle.

Key words: climate effects, melanisation, xanthopterin, urban-rural gradient, yellowjacket wasp.

Introduction

One of the important phenotypic traits associated with temperature and thermoregulation in insects is colouration. Melanisation has been linked with thermoregulation in some insects (Pereboom and Biesmeijer, 2003; Abe *et al.*, 2013; Bishop *et al.*, 2016), as darker individuals have the potential to heat up faster and forage better in a colder environment.

Wasps of the genera *Vespula*, *Dolichovespula*, *Vespa* and *Polistes* have conspicuous colour patterns on different parts of their body; the clypeus, thorax and abdomen that are mostly black and yellow. Climate-induced intraspecific variation in colouration has been reported in *Polistes* wasps (Tibbetts *et al.*, 2011; de Souza *et al.*, 2017) and Badejo *et al.* (2018) recently found that melanisation and body size also varied geographically in the common wasp, *Vespula vulgaris* (L.). The common wasp is an important social insect species with various ecological roles, such as its role as a predator and its role in seed dispersal (Jules, 1996; Richter, 2000; Brodmann *et al.*, 2008). They have great ability to adapt to local conditions in the environment through modification of traits. These characteristics have enabled the wide dispersal of the species outside its native range; the species has successfully colonised new environments in New Zealand, Australia, Hawaii, North and South America (Donovan, 1984; Beggs *et al.*, 1998; Mathews *et al.*, 2000; Lester and Beggs, 2019).

The abdominal colour patterns of Vespine wasps can be classed into colour morphs. Colour morph categorisations have been described earlier by Clapperton *et al.* (1989) for *Vespula* spp. in an invaded ecosystem. These authors used the colour markings on the clypeus and tergites (the first and second) to differentiate between the German wasp -*Vespula germanica* (F.)- and the common wasp and grouped the identified species into different morphs. In addition, Pawlikowski (1986) showed that in the German wasp, variation in the colouration of the clypeal and first abdominal tergite in new emerging wasps was linked to changes in weather from late summer to autumn. This implies that the development of different colour morphs in *Vespula* wasps might not be solely genetic; thus, temperature and other environment factors may contribute to these phenotypic and morphological variations. Apart from the trend of colour variation reported by Pawlikowski (1986), there is the possibility of similar variation between years with different summer weather conditions as well as locations with varying levels of urbanisation.

The development of urban environments involves an alteration of the natural ecosystem; this creates an entirely different microclimate due to drastic changes in landscape structure and ground surface materials. This change influences the weather of an urbanised location by reducing humidity and increasing the temperature relative to the surrounding, less disturbed ecosystems, an effect termed the *urban heat island effect* (UHI) (Kourtidis *et al.*, 2015; Du *et al.*, 2016; Gunawardena *et al.*, 2017).

The increased temperature that is expected in an urbanised ecosystem may alter the ecology and morphology of organisms living in the environment. The adaptation of organisms to the UHI effect might take different forms, ranging from phenotypic modification to changes in behaviour (Hasegawa *et al*., 2014). One of the common groups of organisms in urban environments are insects, which are ectotherms. In the case of *Vespula* wasps, the temperature-related phenotypic plasticity might be visible as changes in the black-yellow colour patterns. Urban environment presents a conducive ecosystem for most insect species (Shochat *et al*., 2004; 2006) due to climatic characters, human activities and waste generated from urban consumption.

The quality of food in an urban environment varies in nutritional content compared to that in the rural surroundings. Studies have shown that plumage colouration in different species of birds in urban centres was paler than in the rural species because micronutrients, such as carotenoids, are reduced in the diet of urban nestlings (Biard *et al*., 2017; Sumasgutner *et al*., 2018). Insects in urban areas may also be faced with a similar challenge of nutrient deficiency regarding the nutrients required for the synthesis of important colour traits, combined with the UHI effect. The urban food for insects is higher in energy content than the food in rural areas (Penick *et al*., 2015) but may be poorer in the essential nutrients needed by insects.

The effect of the UHI and human originated food stuff waste reduces with increasing distance from the urban centres, thus forming urban-rural gradient. In insects this urban-rural gradient may prompt different responses, which can be expressed with morphological or phenotypic modification in order to cope with the local environmental conditions. Such modifications were shown in our previous study (Badejo *et al*., 2018) linking the degree of black pigmentation to thermoregulatory advantage in *V. vulgaris*. Similar adaptive ability was discovered in the oriental hornet *Vespa orientalis* L., yellow pigments showed ability to absorb UV radiation (Plotkin *et al*., 2010) and serve as solar cells (Ishay and Pertsis, 2002) to store excess heat. The yellow stripes contain xanthopterin pigments (Plotkin *et al*., 2009) which has embedded photoelectric receptors (Ishay, 2004) that enhances its function as cuticular solar cells.

The common wasp is native to Finland; while the species forages freely in both urban and rural areas across the country, it mostly prefers rural areas (Sorvari, 2018). Thus, the urban environment might be slightly challenging for the common wasp, which might be visible in different colour patterns. The variation in temperature between urban and rural areas provides an opportunity to detect if phenotypic variation expressed as colour morphs are selected for thermoregulation in different ecosystems. Our previous study has shown that highly melanic individuals of *V. vulgaris* exist in colder environment in Finland; exploiting the possibility of melanic variation and morph differentiation across the urban-rural gradient and between years with different temperatures will provide new information on the possible impact of the UHI effect and climate change on *V. vulgaris* within their native range. Also, this study will provide information on the thermoregulatory significance of the relative proportion of black and yellow colour patterns on abdominal tergites.

We predict that if the yellow portion of abdominal tergite serves as a biological heat sink (xanthopterin) for storing excess heat, the morphs with reduced proportion of yellow pigmentation will dominate urban habitat where there is no need for heat storage due to UHI and morphs with greater proportion of yellow pigmentation relative to melanic pigmentation will dominate the rural habitat to utilize the heat storing potential of the morphs. Also, UHI is expected to present an unfavourable growth condition in urban location; therefore, we expect an opposite trend in adult worker size in urban and rural sampling zones. The aim of this study is to determine: i) whether the cuticle of *V. vulgaris* contain xanthopterin, ii) whether melanisation pattern is influenced by body size, iii) whether the colour morphs of the common wasp are different between years with different temperatures, and iv) whether the colour morphs of the common wasp are different between urban and rural zones using landscape to classify the zones.

Materials and methods

Wasp trapping and trapping sites

For traps we used modified transparent plastic beer pint glasses (0.5 L) covered with a transparent plastic petri-dish top and with a circular 2.5 cm hole on the upper part of the plastic glass to allow wasps to enter. The trap type and beer-based bait were identical to those in an earlier study of Sorvari (2013). The beer bait was enriched with brown sugar and fresh yeast. The traps were hung on trees and bushes (at a height of 1-4 m) with a steel wire.

In the study between years, the samples were collected from Turku in 2011 and 2012, being the same traps and wasps used in the work of Badejo *et al*. (2018). The traps were located along the river Aurajoki, 1-3 km from the city centre where the habitat was a semi-open riverside park. The average distance between nearest neighbour traps was approximately 200 m and the same locations for traps were used in both sampling years. Nine traps were on the northern side of the river and 11 on the southern side of the river.

The trapping period was 04-11th August in 2011 and 07-14th August in 2012. The traps remained in their positions for seven days in both years. In the study 262 workers were collected: 73 worker specimens from 2011 and 189 from 2012. The weather information of Turku on average daily temperature, average daily precipitation and monthly number of rainy days (rainfall of a day exceeds 1 mm) was collected from the Finnish Meteorological Institute. The months were chosen based on the phenology of the common wasp: colonies are mainly established in May and early June, and the colony grows in June and July (Pawlikowski and Pawlikowski, 2009). The number of days with average precipitation exceeding 1 mm and average quantity of daily rainfall were used in the assessment of weather conditions in both years (table 1) because quantity of rain in the early season can affect establishment and success of colonies (Dejean *et al*., 2010). The weather information for August was excluded because the samples were collected early in the month.

In the urban versus rural study, the traps and *V. vulgaris* wasps sampled were the same as in Sorvari (2018). We used four sets of five traps in southern Finland cities of

Helsinki, Tampere and Turku (figure 1A) totalling 20 traps in each town. For the urban-rural study, we collected 361 workers of *V. vulgaris*; Helsinki traps provided 148, Tampere 108 and Turku 105 specimens.

The urban and rural zones were visually determined from a map and in site by the density of urban-cover and natural environment in the locations. In each city two sets of traps were placed in managed parks or on street-side trees within the grid plan of city centres (classified as 'urban') and two sets of traps were placed in natural forest margins, well outside the city centres (classified as 'rural'). The distance between the nearest neighbour traps was approximately 250 metres. The trapping periods were 07-14th August 2012 in Turku, 09-16th August 2014 in Tampere and 10-17th August 2014 in Helsinki.

Table 1. Average daily temperature (°C), the number of rainy days (days with rainfall of over 1 mm) and average daily precipitation (mm) in May, June and July in Turku (2011 and 2012).

	Temp. (°C)		Rainy days (N)		Rainfall (mm)	
	2011	2012	2011	2012	2011	2012
May	10.2	11.0	12	5	1.8	0.9
June	17.5	13.3	9	11	2.5	2.3
July	20.5	17.6	10	12	3.4	2.4

Helsinki is a city of circa 620 000 inhabitants, being clearly bigger than Tampere with 233 000 inhabitants and Turku with 190 000 inhabitants. In Helsinki, the urban trap sets were set on the western side of the city

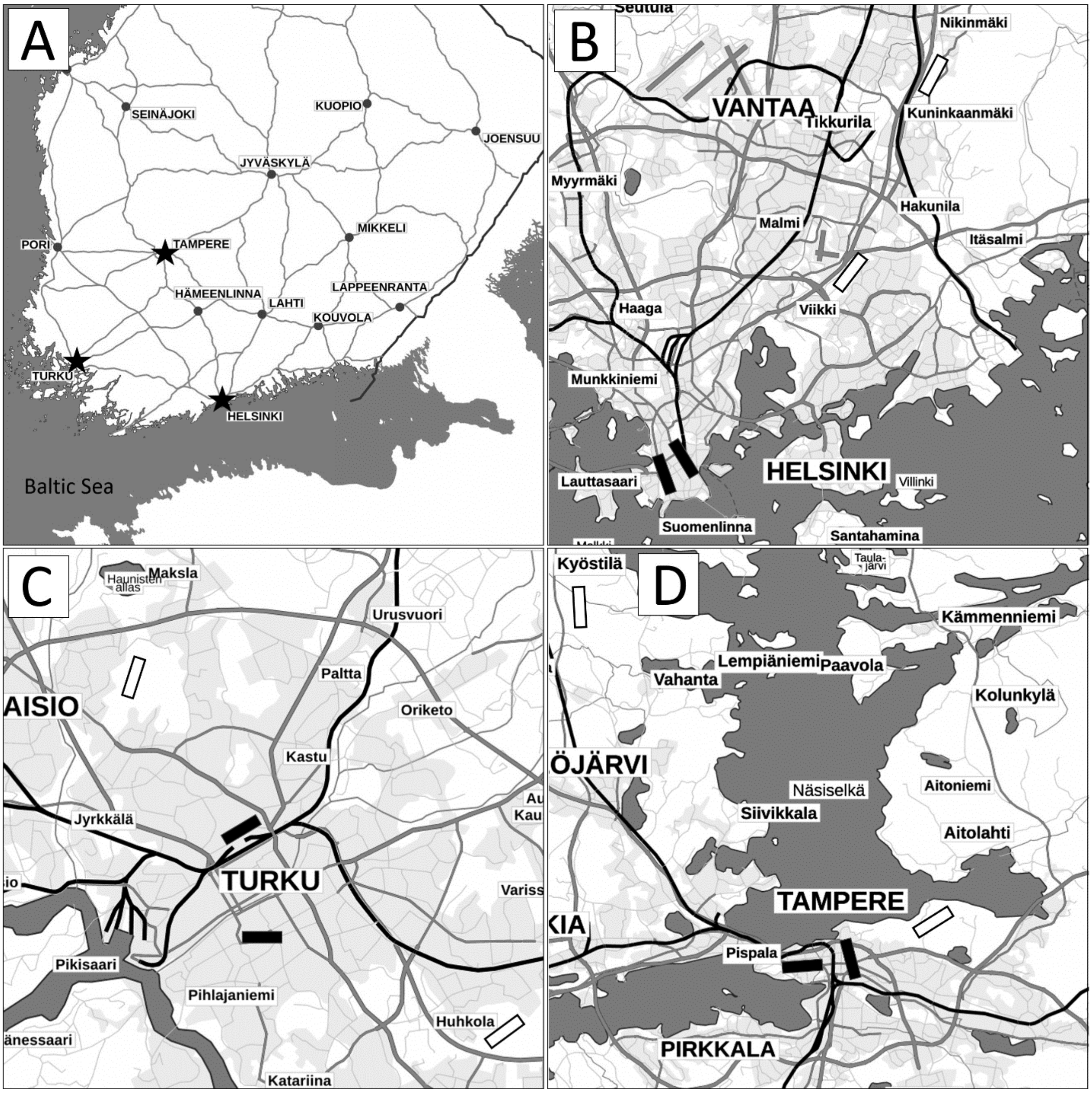

Figure 1. Locations of (**A**) study cities in southern Finland (marked with stars), and urban and rural trap sets (filled and open squares, respectively) in (**B**) Helsinki, (**C**) Turku and (**D**) Tampere.

centre and the northern side of the city centre, and the rural trap sets were located about 12 km north-east from the city centre and 19 km north-east from the city centre in the Vantaa district (figure 1B).

In Turku, the urban trap sets were set on the southern side of the city centre and on the northern side of the city centre, and the rural transects were set about 4.5 km north from the city centre and about 5 km south-east from the city centre (figure 1C). In Tampere, the urban trap sets were set in the western city area and in the eastern city area. The rural trap sets were set 7 km east from the city centre and 19 km north-west from the city centre in the Ylöjärvi district (figure 1D).

Digital photography and SEM imaging

Digital photographs of the thorax and abdomen of individual specimens were obtained under constant lighting in a room without natural light penetration, using a binocular microscope camera setting with a Nikon DS-Fi1 microscope camera attached to an Olympus SZX9 microscope and using Nikon NIS-Elements BR version 3.2 software. The photographs obtained were used for the analysis of individual body size, melanisation and categorisation into different morphs. The camera was set at 80X magnification and 125 ms exposure, and the photos were saved as 8-bit RGB colour JPG images.

A scanning electron microscope (SEM) Zeiss Sigma HD|VP (Carl Zeiss NTS, Cambridge, UK) was used for structural investigation of the wasp chitin specimen. The cross section of the black and yellow chitin coat was obtained by fracturing the wasp cuticle in two pieces with a help of liquid nitrogen (LN_2). Thereafter, the specimen was mounted onto a custom-made cryoholder with 10 µl silicon vacuum grease (Wacker Hochvakuumfett Schwer, Wacker Chemie AG, Burghausen, Nünchritz, Germany). The cryoholder consisted of a 0.5 kg LN_2 cooled cool reserve made of copper and a heat insulator made of PTFE to insulate the heat conduction from the SEM framework. The electron acceleration voltage of 0.5 kV was applied for surface-sensitive structural imaging. The specimens were kept stable in high vacuum ($P < 0.01$ Pa) condition using cryogenic fixation and SEM micrographs were recorded using Everhart-Thornley type II secondary electron detector with +300 V grid bias and working distance of 3.5 mm.

Image analysis

We used ImageJ software (Schneider *et al*., 2012) to process the photographs obtained for each wasp. The intertegular distance (ITD) was measured in mm and used to estimate the wasp's body size; ITD is the distance between the bases of the wings on the thorax (Cane, 1987; Badejo *et al*., 2018). The photos of the abdomen were pre-processed with Adobe Photoshop CS5 software before cropping out the first and second tergites. The cropped images of the second tergites was used in the analyses of the morph pattern of wasp samples of different years and the first and second tergites was used in the analyses of the morph pattern of wasp samples for urban-rural study. In addition, in the urban-rural study, the proportion of the second tergite with black (melanin) and yellow (xanthopterin) pigmentation was measured in relation to the total area of the tergite. Our analysis focused more on the second tergite because it is the most conspicuous segment of the abdomen and it has continuous interaction with the environment throughout the lifespan of the wasp.

Colour pattern morph categorisation

We used the type of morph categorisation used by Clapperton *et al*. (1989) with some modifications. Visual observation of the shape of the melanised part on the first and second abdominal segments was used for morph categorisation. Clapperton *et al*. (1989) classified the fusion (the side peaks) on the second tergite separately, while we classified the morphs using the pattern of the entire melanised part of the tergite.

Statistical methods

The association of ITD with colour pattern morph, region and zone was analysed using residual maximum likelihood-based linear mixed models (LMMs) and using the trap as a random factor with Kenward and Roger calculation for the degrees of freedom. Non-significant interactions were removed from the models before the final results. Differences in morph frequencies between urban and rural zones were analysed using Pearson's chi-square tests (χ^2) and, in cases where assumptions for cell frequencies were not fulfilled, we used likelihood ratio chi-square tests (G^2). Urban-rural zone and regional differences in the proportion of yellow area on the dorsal surface of second tergite was analysed with linear mixed models using the proportion of yellow area as a response variable and zone and city region in separate models as explanatory variables. Trap identity and in one case trap identity nested within city was used as a random factor and Kenward-Roger approximation was used for the degrees of freedom. Normality of the residuals and the homogeneity of variances were assessed visually from residual plots in linear mixed models and not any significant deviations were found. All statistical tests were performed using SAS 9.4 statistical software (SAS Inc., Cary, IL, USA).

Results

Xanthopterin layer and colour morphs

We found a layer of xanthopterin in the SEM image of the yellow strip on the cuticle of *V. vulgaris*. The pigments were arranged below the layers of chitin lamellae (figure 2), similar in size and shape, like the barrel-shaped cylindrical yellow granules identified in the oriental hornet by Ishay *et al*. (2004). Although, the magnification used in this study was lower and preparation method cruder, it was sufficient to show the presence and features of xanthopterin pigment granules. Features were not visualized below the black strips.

By assessing visually from photos, we found three different and easy to separate colour pattern morphs in the first gaster tergite (figure 3) and six in the second gaster tergite (figure 4) and these categories are used in the further analyses. Our categorisation resembles greatly that of Clapperton *et al*. (1989) but with some minor modifications.

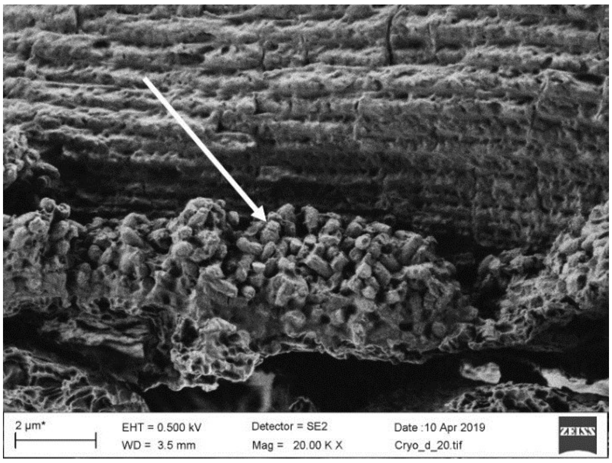

Figure 2. SEM image showing xanthopterin pigment granules in the yellow stripe of the common wasp *V. vulgaris* cuticle. The pigment granules pointed by an arrow are located below the cuticle layer.

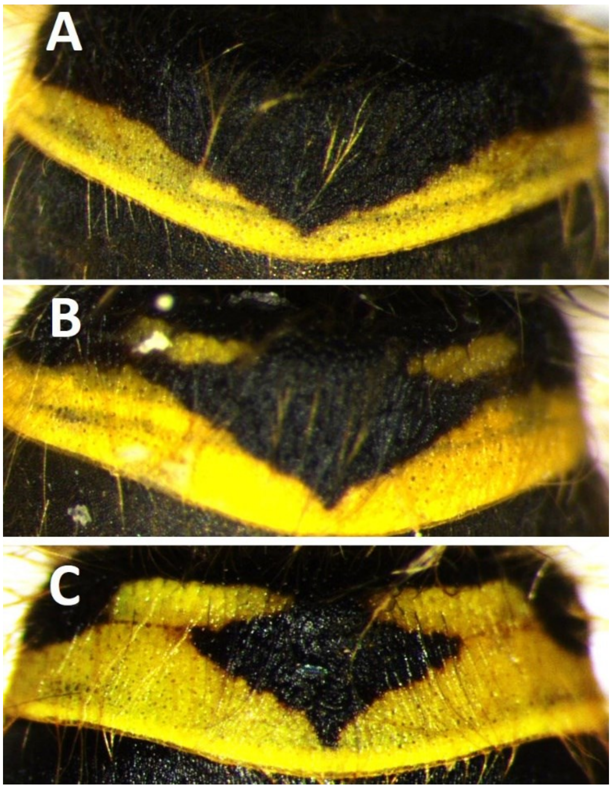

Figure 3. The colour morph categories in the first gaster tergite of the studied Finnish population of the common wasp, *V. vulgaris*. Morph A shows a triangle-shaped pattern on the centre of the dorsal side of the tergite, morph C resembles an arrowhead and morph B is intermediate of morphs A and C, showing reduced yellow spots in the base of triangle but not forming an arrowhead.

Comparison between the two years

The frequencies of the colour pattern morphs of workers of the common wasp collected in 2011 and 2012 along the Aurajoki river in the semi-rural area in Turku differed between the years ($df = 5$, likelihood ratio $\chi^2 = 15.402$, exact $P = 0.013$; figure 5). In both years, colour pattern morphs B and C were the most common. In the warmer and low wasp abundance year of 2011, morph A was relatively more common compared to 2012 and morph E was present in eight percent of the samples in 2012, whereas it was totally absent in 2011 (figure 5).

Comparison between regions and urbanisation zones

ITD was used as a measurement of morphological size and it varied between 1.68 mm and 2.86 mm. Mean ITD was 2.42 ($SD = 0.18$). ITD was similar between workers with different patterns in the first tergites ($F_{2,354} = 0.006$, $P = 0.94$). Due to the rarity of A, E and F patterns in the second tergite, the ITD was compared among B-, C- and D-pattern workers and there was no significant difference between those morphs ($F_{2,347} = 0.006$, $P = 0.76$).

The association between ITD and zone (urban, rural) was similar between regions (interaction term zone × region: $F_{2,61.1} = 2.02$, $P = 0.14$). ITD did not differ between urban and rural zones but there was a marginally non-significant difference among the three studied regions (zone: $F_{2,55.4} = 0.87$, $P = 0.36$; region: $F_{2,46.6} = 3.11$, $P = 0.054$). On average the ITD was biggest in Tampere and smallest in Turku, but in the pairwise comparisons only Tampere and Turku differed significantly (Tukey's test $P = 0.044$; figure 6).

In the second gaster tergite, the colour patterns A, E and F were rare (table 2). This might be because the total area of the melanised part combined with the proportion of yellow pigmentation was important for thermoregulatory fitness. Thus, the other morphs were selected for combining a greater portion of yellow pigmentation and significant melanised area with conspicuous side peaks.

When city regions were pooled together, there was no difference between zones in morph frequencies in the first tergite nor in the second tergite (1^{st} tergite: $df = 2$, $\chi^2 = 3.63$, $P = 0.16$; 2^{nd} tergite: $df = 5$, $G^2 = 7.23$, $P = 0.20$). However, as the cities were of different size, especially Helsinki is larger than the two other cities, it was reasonable to analyse the three city regions separately.

In Helsinki, the morph frequencies of workers collected from urban zone and rural zone in the first tergite did not differ, but the frequencies in the second tergite did so (figure 7; table 3). The difference was mostly due to opposite trends between morphs B and C, as morph B was the most common in urban areas and C in rural areas. The morph frequencies of the first and second tergite between urban and rural samples did not differ in Tampere and Turku (table 3).

The proportion of yellow-pigmented (xanthopterin) area on the dorsal surface of second gastral tergite was on average smaller in urban areas compared to that of rural areas in Helsinki, but the comparison only showed a trend (proportion of yellow-pigmented area, estimated marginal mean ± 95% CI, urban: 0.243 ± 0.017, rural 0.262 ± 0.012; $F_{1,15.7} = 3.94$, $P = 0.065$). In Tampere and Turku there was no difference between urban and rural areas (Tampere: urban 0.274 ± 0.032, rural 0.269 ± 0.024, $F_{1,18.9} = 0.08$, $P = 0.78$; Turku: urban 0.265 ± 0.021, rural 0.268 ± 0.026, $F_{1,21.1} = 0.02$, $P = 0.88$). Overall, there was no difference between urban and rural area in the proportions of yellow (urban 0.261 ± 0.013, rural

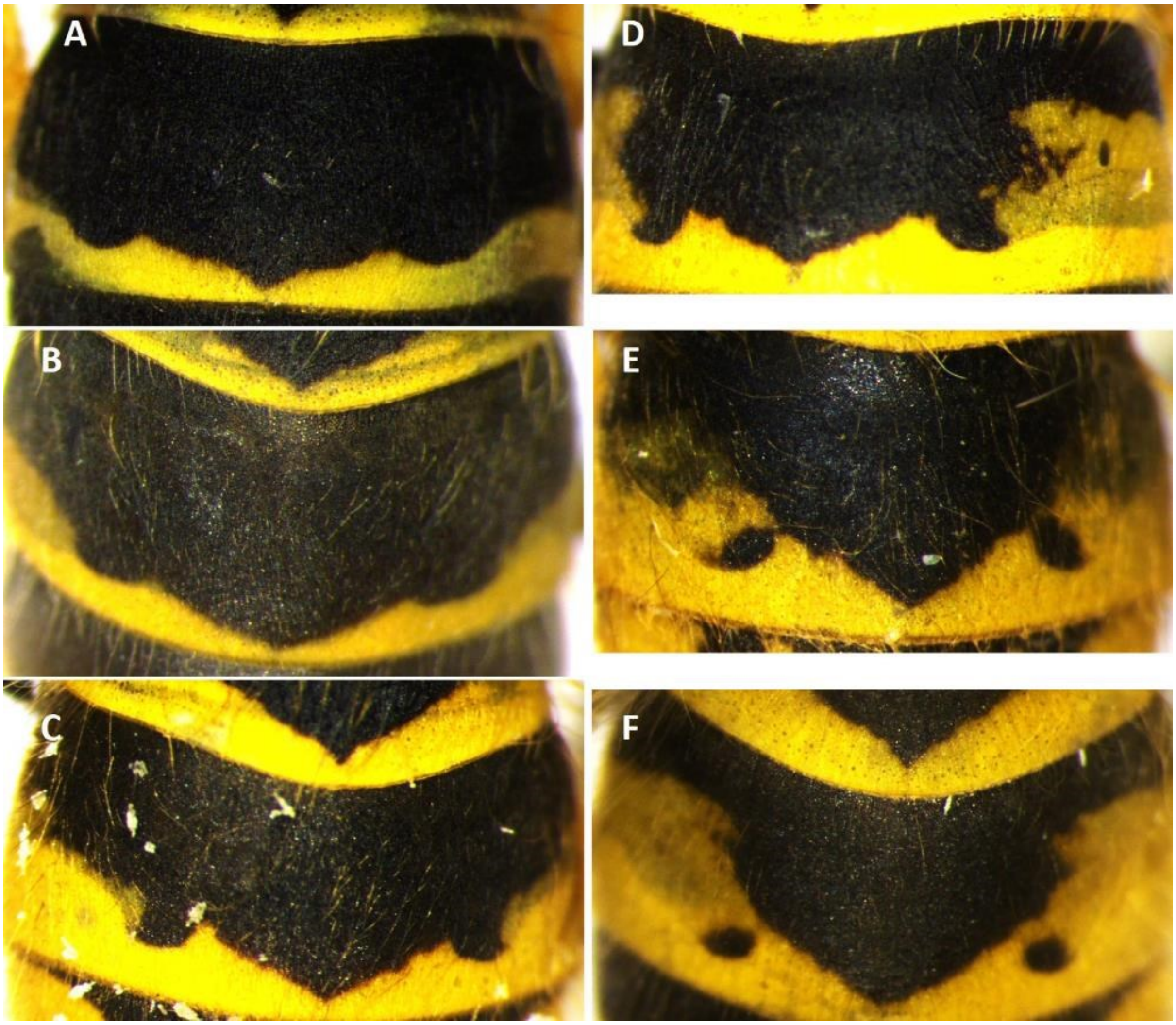

Figure 4. The colour morph categories in the second gaster tergite of the studied Finnish population of the common wasp, *V. vulgaris*. The narrowing central black area and finally separated spots on the sides separate the morphs.

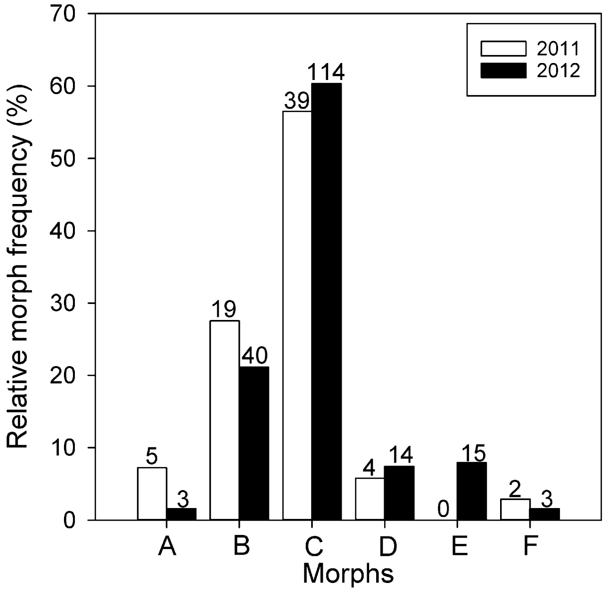

Figure 5. The relative colour pattern morph frequencies of the common wasp *V. vulgaris,* collected in 2011 and 2012 in the park-like Aurajoki river valley in Turku, southwest Finland.

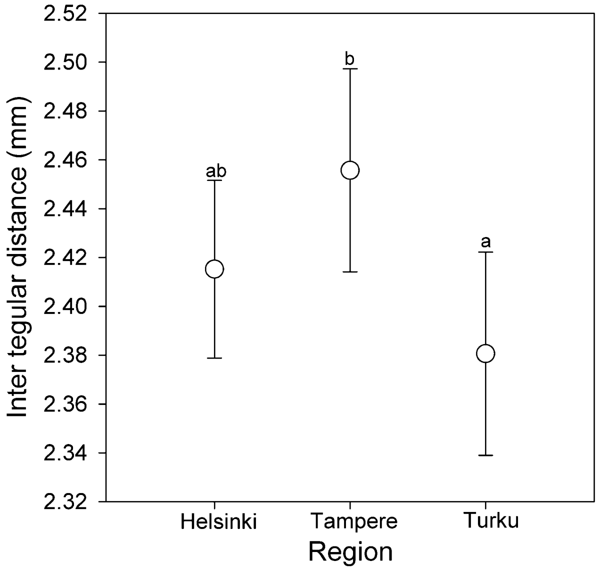

Figure 6. Estimated marginal means of common wasp (*V. vulgaris*) worker body sizes (ITD in mm) in Helsinki, Tampere and Turku. The same letter above the symbols indicates non-significant difference in the Tukey's test for pairwise comparisons ($P > 0.05$).

Table 2. The frequencies of different tergite colour patterns in Helsinki, Tampere and Turku in urban and rural areas. In second tergite morphs, morph A was absent in Tampere and morphs E and F were absent in Turku. The Helsinki and Tampere samples were collected in summer 2014 and the Turku samples were collected in 2012.

		First tergite			Second tergite					
		A	B	C	A	B	C	D	E	F
Helsinki	Urban	35	4	9	1	28	16	2	1	0
	Rural	68	16	16	1	32	55	10	1	1
Tampere	Urban	16	2	8	0	2	21	2	1	1
	Rural	57	6	18	0	20	51	4	5	1
Turku	Urban	52	5	22	3	25	42	9	0	0
	Rural	16	3	6	0	5	19	2	0	0
Total		244	36	79	5	112	204	29	8	3

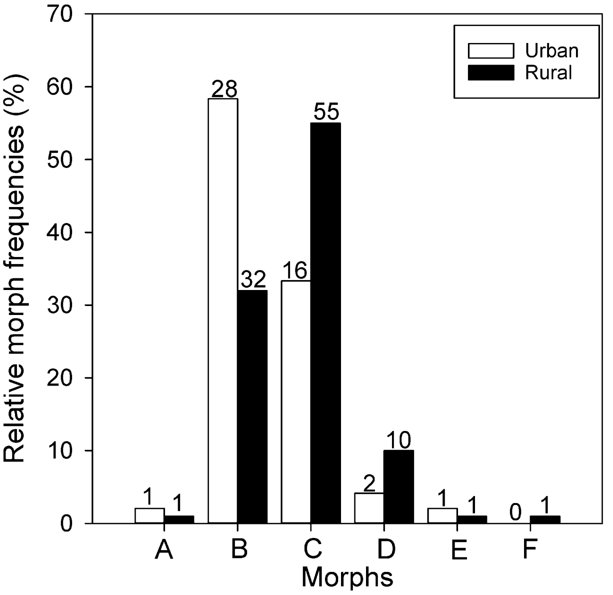

Figure 7. Relative colour pattern morph frequencies and the observed numbers appear on the top of the bars of urban and rural samples of the common wasp *V. vulgaris* in Helsinki 2014.

0.266 ± 0.011; LMM, trap nested within city in the mixed model, $F_{1,46.7} = 0.37$, $P = 0.55$). The proportion of yellow pigmented area on second tergite did not differ between the studied city regions (Helsinki: 0.255 ± 0.013, Tampere: 0.272 ± 0.015, Turku: 0.266 ± 0.015; $F_{2,41.6} = 1.47$, $P = 0.24$).

Discussion

We found that the yellow part of the wasp cuticle contained a pigment layer that was morphologically identical to xanthopterin that is important in heat storage process. In addition, we found that climatic and urban conditions affected phenotypic plasticity in the common wasp *V. vulgaris* in terms of shape and proportion of black pigment, but not in body size. However, there was difference in size between regions as observed in our previous study across geographical zones in Finland (Badejo *et al.*, 2018).

Table 3. Test for the frequencies of colour pattern morphs in different towns and their surroundings. The *P*-values are probabilities from exact tests.

	Tergite	*N* urban	*N* rural	*df*	χ^2	*P*
Helsinki	1st	48	100	2	1.669	0.50
	2nd	48	100	5	11.465†	**0.045**
Tampere	1st	26‡	81	2	0.819	0.70
	2nd	27	81	4	5.319†	0.36
Turku	1st	79	25‡	2	0.908	0.71
	2nd	79	26	3	4.400†	0.23

† The likelihood ratio chi-square test G^2 was applied due to low frequencies in some morphs.

‡ One sample was omitted due to poor quality of the first tergite.

Ishay and Pertsis (2002) described the xanthopterin granules as a battery which stores excess heat absorbed by the melanin pigments. The authors described the wasp cuticle as an electric capacitor where the melanin stripe acts as n-type semiconductor which donates extra electrons to the p-type semiconductor (yellow xanthopterin stripe) for energy storage. Our study presents information of structural similarity in xanthopterin pigments described in oriental hornet within the yellow stripe of the cuticle of *V. vulgaris*. Therefore, the xanthopterin pigmentation in *V. vulgaris* is expected to perform similar photoreceptive functions. Thus, we suggest that a balance between the proportion of black and yellow pigmentation might be advantageous for effective location-specific thermoregulation. This is the first report of xanthopterin in other social insects apart from the oriental hornet, more studies are needed to substantiate our findings.

Skaldina and Sorvari (2017) associated proportion of pigmented area with individual body size in ants but there was no sign of such in our result, while there was clear variation in ITD (1.68-2.86 mm). The ITD was similar between morphs and across rural-urban gradient, so there was no signs of trade-off between growth and melanisation.

The variation in the expression of pigmentation was studied by grouping samples into morphs using the first and second tergites. The identified morphs showed a difference in the proportion and shape of the melanised part on the assessed first and second tergites, the difference in the frequency of the different morphs across temperature and climate zones points at ecological and genetic drivers within the different environments. Studies have shown that lower temperature of the environment can affect the selection of more melanised individuals in insect species (Fedorka *et al*., 2013; Badejo *et al*., 2018; Sibilia *et al*., 2018), because highly melanised individuals heat up faster in cold environment than lighter ones; however, in the wasp case, the balance between heating speed and heat storage may affect the thermal melanism process. Other studies have stated that melanised individuals are more resistant to parasites and pathogens (Armitage and Siva-Jothy, 2005; Bailey, 2011); this might be the reason why the morphs with the highest frequency were those with a larger melanised portion.

There was no significant difference in the distribution and frequency of morph when classified based on the first tergite; this gives the second tergite more phenotypic significance in wasp morph classification. We also assessed the proportion of melanised and yellow pigmented area on the second tergite because the first tergite is very narrow and the second tergite does not go much inside the first tergite. Although, the small difference in contraction or angle of photographing could cause error for both directions, the proportion of pigmentation area is still useful in quantifying xanthopterin pigmentation present in the yellow stripes. Due to possible errors, it is easier and perhaps more reliable to use morph categories in studies like ours. The second tergite was also identified in our previous study as the most conspicuous in aposematic display (Badejo *et al*., 2018). The morphs with low frequency had a reduced melanised portion on the second tergite. The melanised portion on the second tergite might give thermoregulatory advantage to morphs with a higher melanised portion combined with conspicuous yellow portion, thus, increasing the survival rate of morphs B and C. This indicates that the total melanised portion of the insect tergite is important in thermoregulation, not just the degree of melanisation.

In the studies between the years 2011 and 2012 in Turku, morphs B and C were also the most dominant, but the frequency of the morphs differed between years; the frequency of morph B was lower, and C was higher in 2012 compared to 2011. In addition, the morph A was slightly higher in frequency in 2011 compared to 2012. These morphs were possibly selected to improve the thermoregulation of the common wasps in this geographic location. The two years differed in weather; rainfall in Turku was low early in the season and progressively increased through the season in 2012, while in 2011 the rainfall trend was opposite (table 1). In addition, summer 2012 was generally cooler than summer 2011 (Badejo *et al*., 2018). DeJean *et al*. (2010; 2011) identified rainfall as an important factor that affects the success of colonies; the quantity of rainfall had a correlation with temperature and relative humidity. The significance of the proportion of yellow pigmentation was obvious, morph C had higher abundance in 2012 due to the need to store heat to compensate for the cooler summer temperature while morphs A and B with reduced proportion of yellow pigmentation dominated in warmer 2011. There might be other factors in addition to temperature driving the expression of melanisation in the common wasp; therefore, we intend to study the heritability of melanisation in our future studies.

The temperature of urban areas is typically higher than that of the surrounding areas; studies have shown that some insect species establish their colonies first in urban areas in invaded environments because of this UHI effect (Mieneke *et al*., 2013; Branco *et al*., 2019). This scenario was suggested also for the German wasp *V. germanica* in Finland (Sorvari, 2018). The city centre of Helsinki can be about 8 °C warmer than nearby rural areas during the daytime in August (Savijärvi, 1985), whereas in Turku the UHI effect can be about 3-4 °C (Suomi and Käyhkö, 2002). There is no such data from Tampere, but as it is slightly larger and more continental than Turku, the UHI effect might be at least the same as in Turku.

Helsinki - being the most urbanised location in our study - showed some morph differences between urban and rural wasps. The second tergite morph B, which had reduced side peaks (fusion) and smaller proportion of yellow pigmentation, was more abundant in the urban areas, while morph C, with conspicuous fusions and larger proportion of yellow pigmentation, was predominant in the rural part of the city. The dominance of morph C in the rural area might link the occurrence of melanin fusion and larger yellow portion on the second tergite to thermoregulatory functions as the rural part of Helsinki is expected to be cooler than the city centre. Reduced fusion and yellow portion in morph B, might be due to the UHI effect and the need for the modification of morph C in order to reduce unnecessary heat storage and overheating in warmer environment. In other words, morph C may present a standard morph for *V. vulgaris* while morphs A and B are variants of it which could be induced by the temperature fluctuations of the location.

The proportion of the melanised area on the second tergite was slightly different in marginally non-significant level in Helsinki samples across the urban and rural zones. The yellow area tended to be smaller in the urban samples than in the rural ones. This difference in yellow area points to the advantage of the relative proportion of the yellow pigmentation on the abdominal segments in rural wasps for heat energy storage to improve thermoregulation. Although, we have previous data on temperature for Helsinki and Turku (Savijärvi, 1985; Suomi and Käyhkö, 2002), the association between colour morph and Helsinki urban area is indirect due to lack of simultaneous temperature data. Moreover, our results on urban vs. rural colour morphs are preliminary and this should be tested with larger data sets and additional city regions.

The intensity of the UHI effect has a significant positive relationship with surface area of urban cover (Du *et al*., 2016). Thus, the result from Turku and Tampere - with no significant difference in morph frequencies - was expected. These cities have smaller urban cover compared to Helsinki and the UHI effect is likely less conspicuous. This suggests that there is a level of influence of the UHI effect in morph selection and more pronounced morph variation is expected in larger cities. Access to human originated food and grid plan habitat was seemingly similar among the cities; thus, the observed difference between cities points more towards the UHI effect.

Adverse UHI effect in larger cities might reduce the yellow proportion on the tergite to a great extent because wasps population within a microclimate can select suitable phenotypic properties as shown in morphs A and B. Therefore, extremely melanic individuals of *V. vulgaris* can lose their aposematic characteristics and become exposed to predation. Thus, future planning of urban centres should incorporate green areas and minimize the alteration of ecology of the location to enable conservation of native species.

To conclude, our prediction of phenotypic plasticity in colouration in *V. vulgaris* across the climatic gradient due to UHI effect and between year variation was partly supported. This provides evidence of the thermoregulatory modification of melanic and xanthopterin pigment expression in order to suit the environmental conditions across the urban-rural gradient, and the impact of the UHI effect might provide more striking morph differentiation across the urban-rural gradient in larger European cities.

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