Effects of land-use intensification and environmental stressors on intraspecific functional traits variability in bumblebees

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AUTHOR CONTRIBUTION

NT, EP and AG conceived the ideas and designed methodology; NT, EP and PB collected the data; NT and EP analysed the data, PB contributed to analyses checking/development and checked the final model outcomes; EP, NT and PB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ABSTRACT

Landscape anthropization replaces natural areas with agricultural or urban covers, leading to land-use intensification. This worldwide phenomenon affects biodiversity, but little is known about the effects on the intraspecific variation of functional traits related to ecosystem services. By combining field data collection, remote sensing and land cover analysis we investigated how land use intensification affects functional traits in two bumblebee species. Namely, the impact of different land use cover (i.e., semi-natural, urban, and agricultural) and of multiple biotic and abiotic stressors (i.e., temperature, resource availability, and air pollutants) was investigated. Along a gradient of landscape anthropization, we sampled populations of two European bumblebee species (*Bombus terrestris* and *B. pascuorum*) at 37 sites in Northern Italy. Through geometric morphometrics we investigated the variation of morphological traits related to flight performance (i.e., wing centroid size and shape and size fluctuating asymmetry FA), previously used as indicators of stress during insect development. Our results point out an idiosyncratic response of the two species to landscape anthropization. Smaller individuals of *B. pascuorum* were observed in response to increased impervious cover and temperature. No similar patterns were noticed in *B. terrestris*, which was characterized by larger individuals in response to floral resource availability. Wing size FA was positively associated with warmer temperatures and increased levels of NO_2 only in *B. terrestris*. Overall, this study found taxon-specific functional trait variation at the intraspecific level in syntopic organisms, expanding our understanding about the effects of land-use intensification on the ecological activity of pollinator model species.

Keywords: Functional diversity, heat islands, pollination, urban ecosystems, wing asymmetry

INTRODUCTION

Widespread phenomena, such as the progressive expansion of agricultural and urban lands are driving deep changes on ecosystems, among which the replacement of natural areas and the creation of novel ecosystem conditions Plants and animals respond to these marked environmental variations by shifting their distribution (Colla et al., 2012) and phenology (Huchler et al., 2020), and/or shaping some morphological traits considered "functional", i.e., relevant for their ecology, fitness and behaviour (Alberti et al., 2017; Eggenberger et al., 2019; Nooten & Rehan, 2020). Trait variation due to environmental alteration is particularly relevant in those organisms responsible for ecosystem services, such as pollinators, since changes in their traits could jeopardize the service provided (Buchholz & Egerer, 2020). For instance, land-use intensification and degradation (i.e., the growing proportion of anthropized or productive surfaces across landscapes) could lead to limited floral resource availability to pollinators (Steffan-Dewenter et al., 2001). This scenario, in turn, could impose changes in pollinator functional traits, such as body size decline due to less food supplied to larvae. Furthermore, anthropization of landscapes changes the local microclimate, thus determining an alteration of pollinator insects ecology, development and activities (Radmacher & Strohm, 2010). Specifically, higher degrees

of impervious land cover are often associated with increasing temperatures (Chun & Guldmann, 2018), a phenomenon known as “heat island effect”, that characterizes both urban and suburban areas. Observations from previous studies have strengthened the hypothesis that pollinator insects could face a shift towards smaller body size as an adaptation to reduce risk of overheating in presence of warmer conditions (Peters et al., 2016; Gérard et al., 2018a). Considering that the steady growth of the human population is driving a dramatic increase in the area allocated to intensive agriculture and urban expansion worldwide, new insights in the study of pollinator response are necessary to predict which scenarios would likely occur for urban biodiversity and its mediated services.

Previous studies investigated the morphological responses of pollinators to anthropogenic pressures, mainly focusing on key functional traits, such as body size (e.g., Chown & Gaston, 2010; Eggenberger et al., 2019; Theodorou et al., 2020). In bees, this character responds rapidly to environmental changes (Chown & Gaston, 2010), it shows little heritability and its variation mainly depends on the amount of food received during the larval development (Couvillon et al., 2010). Bee size directly influences their mobility and so their foraging range (Greenleaf et al., 2007), but also determines the metabolism rate and resource needs of adult imagos, with larger bees having higher metabolism rate (Kelemen et al., 2019) and thus potentially being more susceptible to variation in floral resource availability leading to starvation (Couvillon & Dornhaus, 2010). However, to date, the investigation of pollinator body size variation in anthropogenic habitats yielded heterogeneous results. A recent study on bumblebees found bigger workers in cities (Theodorou et al., 2020). This pattern has

been interpreted as an adaptation to longer flights for collecting resources, due to severe habitat fragmentation and dispersion caused by land use intensification (Greenleaf et al., 2007). Conversely, a study by Eggenberger et al. (2019) found smaller bumblebee foragers in cities, interpreted as an effect of both limited local resource availability and warmer temperature in urban areas.

A promising research field in the study of morphological traits is to identify possible stress biomarkers of habitat quality and population health status (Adams et al., 2001). The advantage of using trait variation to measure stress resides in the fact that changes of phenotypes are detectable before a decrease in population viability occurs (Hoffmann et al., 2005). Therefore, quantifying traits variation could become an essential practice when evaluating local- and landscape-level stressors. A metric that has grown in popularity is the fluctuating asymmetry (FA) (Klingenberg, 2001; Beasley et al., 2013; Alves-Silva et al., 2018), defined as the presence of small, randomly placed deviations from perfect bilateral symmetry due to the occurrence of developmental instability, driven by exogenous environmental conditions (Klingenberg, 2015). FA differs from another type of bilateral asymmetry, the directional asymmetry (DA), that occurs when one specific side tends to be steadily larger than the other. While DA have a genetic basis and therefore could be less impacted by the environment (Palmer & Strobeck, 2003), the FA is considered a valid proxy of stress exposure to conditions that typically occur in anthropized environments (e.g., temperature, air pollutants, and pesticides) (Beasley et al., 2013). For instance, laboratory-based studies have demonstrated that CO_2 or low temperature leads to wing FA, supporting the possible role of traffic pollutant in determining

developmental instability (Klingenberg et al., 2001; Hoffmann et al., 2002). Interesting, however, these studies also indicated how wing shape and wing size asymmetries had different responses to the same stressor type. This aspect is ecologically very relevant. Both wing size and shape are important functional traits in pollinators. This is because wing size is believed to be related to flight length and it influences metabolic costs (Fernandez et al., 2017; Soule et al., 2020), while shape is considered as important for flight maneuverability (Kolliker et al., 2003, Grilli et al., 2017). Thus, their asymmetry could reduce flight performance ability and thus could reduce fitness and even impact on species interactions and pollination efficiency.

In order to characterize the effects of landscape anthropization and related pressures on pollinator insects, we quantified the morphological variation in two species of bumblebee (i.e., *Bombus pascuorum* and *B. terrestris*), by investigating different populations across a gradient of growing land-use alteration, from semi-natural areas to rural sites and urban places. Specifically, we chose the region surrounding Milan (Northern Italy). This is one of the major European cities and lies on a territory that experienced a strong anthropogenic footprint and ongoing efforts are trying to mitigate it. We expected to find quantitative variation in bumblebee functional traits (i.e.body size and wing FA) in response to increased land-use intensification. Our hypothesis was that in anthropized areas, pressures eliciting a decrease in body size (e.g., high temperature and low floral resource availability) would be prevailing and would lead to smaller foragers in urbanized and agricultural habitats. We also expected to find increased wing size and shape FA in response to higher levels of biotic and abiotic stressors (e.g., floral resources abundance,

temperature, and air pollutants). Hence, our ultimate goal was to determine which type of response prevails and the putative pressures that are determining it to better understand the impact of landscape anthropization on the ecology of pollinator insects.

MATERIALS AND METHODS

Study species

This study was focused on two co-occurring species of bumblebee: *Bombus terrestris* (Linnaeus 1758) and *B. pascuorum* (Scopoli 1763). Both species are efficient pollinators, common in Europe, and can be found in different habitats from the natural to the agricultural and urban ones (Polce et al., 2018). Given these characteristics, these species are reliable models to investigate responses by pollinating insects to landscape anthropization (Eggenberger et al., 2019; Theodorou et al., 2020). The two selected species have slightly different foraging ranges, with estimated maximum dispersal range of 449 and 758 m for *B. pascuorum* and *B. terrestris*, respectively (Knight et al., 2005). Nesting habits are also dissimilar as *B. terrestris* builds its nest in subterranean holes, while *B. pascuorum* on top of or slightly beneath the soil surface (Goulson, 2010). Another important difference is represented by their dietary regimes with the *B. pascuorum* usually having a narrower trophic niche and a preference for deep-corolla flowers (Harder, 1985).

Study design and sampling

Samplings were conducted at 37 sites (Fig. 1), mainly lying within the metropolitan area of Milan in July 2019, only on days with sunny and not windy weather conditions.

The study sites were distributed in an area of about 1800 km^2 covering four administrative provinces, Milano, Monza e della Brianza, Lecco and Como. A minimum distance between sites of 1 km was imposed to avoid the non-independence of sites (Phillips et al., 2019) since it is above the mean foraging range observed for the two species (Knight et al., 2005). A Moran test was also applied to confirm the absence of spatial auto-correlation within sites ($P>0.05$).

Study sites spread along a gradient of landscape anthropization, ranging from areas dominated by semi-natural hay meadows close to forest, to agriculture dominated landscapes (i.e., sites at the edges of crop fields of mainly large monoculture) and sites characterized by a high degree of impervious surface (i.e., concrete, building, asphalt). For each species, five to six specimens, excluding queens and males, were captured in a plot of about 50 m x 50 m at each site using an entomological net. After collection, the insects were stored at -80 °C until further analysis.

Landscape and environmental variables

In order to assess landscape anthropization, land-use data were obtained from the latest version of land cover database provided by Lombardy region (2018-DUSAF 6.0; https://www.dati.lombardia.it/Territorio/DUSAF-6-0-Uso-del-suolo-

2018/7rae198 fng6). Using QGIS 3.10.11, a 1 km radius buffer area was created around each site where landscape composition was evaluated arranging DUSAF original level and sub-level of land-use classification into three macro categories: impervious (i.e., buildings, infrastructures, roads, and cemented surface), agricultural (i.e., arable land devoted to crop production) and semi-natural land (i.e., meadows, forest and urban green spaces) (Online resource 1 for a list of DUSAF codes assigned to each grouping). For each site, the ratio between impervious and semi-natural land (Impervious / Natural) as well as the ratio between agricultural and semi-natural land (Agricultural / Natural) was computed to describe the urbanization and agricultural intensification processes, respectively. Habitat fragmentation was quantified by computing the edge density (ED), as the ratio of edge length of green and semi-natural patches over their total area (Wang et al., 2014).

Other environmental biotic and abiotic features were considered to test for their potential effects on altering body size and wing size/shape FA.Specifically, land surface temperature was calculated as the mean value in the period June-July using data retrieved through remote sensing imaging spectroradiometer (MODIS) MOD11A2 from the NASA database (https://modis.gsfc.nasa.gov/data/dataprod/mod11.php) with a resolution of 1 km. The choice of considering temperature for these two months was dictated from the supposition that our sampled specimens developed during that period, according to the biology of two selected species (Goulson, 2010). Air pollution was estimated as the mean of daily concentrations of NO_2 over two months (June and July) registered by Regional agency for environmental protection (ARPA) through monitoring stations

located nearby our sampling sites (https://www.arpalombardia.it/Pages/Aria/qualita-aria.aspx).

Floral resources at each site (i.e., the total number of flowers) was estimated in sampling plots by using six randomly placed quadrats 1 m x 1 m (covering a proportion of sampling area similar to that reported in Fisher et al., 2017) and summing the number of flowers found within each quadrat.

Specimens imaging and wings measurement

The forewings of all individuals were detached at the base and scanned at high resolution. The obtained images were converted into TPS files using tps-UTIL 1.74 and digitised using the tps-Dig 2.31 software (Rohlf, 2015), with two-dimensional cartesian coordinates of 15 landmarks positioned at wing vein junction (Fig. 2) (as in Aytekin et al., 2007; Klingenberg et al., 2001). Bumblebees with damaged or badly worn wings were excluded from further analyses.

The analysis of landmark configuration was conducted in MorphoJ 1.07 software (Klingenberg, 2011). To remove all differences unrelated to shape (i.e., rotation, translation, and scale) a generalized least square Procrustes superimposition was applied (Klingenberg, 2011). The output of this procedure is a new set of superimposed landmark coordinates, called ‘Procrustes shape coordinates’, that contains all the shape information. Wing size was estimated as the centroid size (i.e., the square root of the sum of squared distances from the centroid of each landmark configuration) and used as a proxy of body size (hereafter “body size”, as in

Outomuro & Johansson, 2011 and Dellicour et al., 2017). Wing size asymmetry was computed by dividing the absolute difference between left and right centroid sizes by the mean centroid size and multiplying by 100 (Leonard et al., 2018). To estimate wing shape variation, Procrustes distances were computed for each individual (Klingenberg, 2015). These represent the measure of an individual's overall asymmetry (i.e., sum of DA and FA components), obtained by taking the square root of the sum of squared distances between corresponding right and left Procrustes' coordinates.

Statistical analysis

Since the levels of asymmetry in bilateral traits are subtle, we estimated the measurement error, which could possibly cause considerable variation in the assessment of asymmetry levels, by double-scanning wings and digitizing their landmarks for a subset of 50 specimens, in MorphoJ with the Procrustes ANOVA (Klingenberg, 2001), in order to evaluate if measurement error was negligible. The presence of directional asymmetry (DA) was also tested with the same approach, and if DA occurred, the total asymmetry measures were corrected subtracting the mean DA from the overall individual asymmetry, thus isolating the FA component as in Costa et al. (2015).

To investigate the relationship between morphological traits and covariates (i.e., ratio between impervious and natural cover, ratio between agricultural and natural cover, edge density, temperature, NO_2, and floral resources) a series of linear mixed models

were used. In order to improve the fit between the predictors and the response variable, mathematical transformations were applied on the covariates as reported in Table 1. The goodness of these transformations was assessed through the AIC criterion. (Δ AIC > 2).

The response of the two species was assessed separately, furthermore different models were used to evaluate the effects of highly correlated variables (r>0.7). Specifically, the response of centroid size was tested in two different models to account for covariates collinearity. The first one (Table 1, model id A) tested the effect of i) edge density of green areas, ii) ratio between impervious and semi-natural land cover, iii) ratio between agricultural and semi-natural land-cover, and iv) floral resources. Conversely, the second model (Table 1, model id B) evaluated separately the impact of temperature because of its correlation with the ratio between impervious and semi-natural land cover. Regarding FA, the effects of covariates known to influence the developmental stability as temperature (Table 1, model id C), and NO_2 (Table 1, model id D) were tested individually both for wing size FA and wing shape FA because of their correlation. In all models, the sampling site was included as the random effect. For all models we applied a stepwise model selection based on AIC values removing variables that did not improve the model fit (Zuur et al., 2009). Statistical analyses were conducted using MorphoJ 1.07 and JMP 14.2.0 (© SAS Institute Inc. Cary, North Carolina, US).

RESULTS

Overall, the sampling dataset was similar in the two bumblebee species, encompassing a total of 179 *B. pascuorum* (mean per site = 4.8±0.4) and 169 *B. terrestris* (mean per site = 4.5±0.3) that were considered for morphometric analyses. The measurement error was negligible because not significant for wing size (df = 79, F = 2.67 p = 0.4578) and shape (df = 2054, F = 0.51, p = 0.9976). Different patterns of size variation were found in the two bumblebee species. *B. pascuorum* decreased its body size in response to both the increasing temperature (Fig. 3a; Table 1: model id B) and the higher ratio of impervious on semi-natural land cover (Online resource 2 - Fig. S1 ; Table 1: model id A). Conversely, no significant effect of these two predictor variables was found on *B. terrestris*, that increased body size in response to the availability of floral resources (Fig. 3b; Table 1: model id A).

Concerning wing asymmetry, both species showed a significant level of shape DA (*B. pascuorum* df = 26, F = 4.66; p < 0.0001; *B. terrestris* df = 26, F = 5.60; p < 0.0001), while size DA was statistically significant only in *B. pascuorum* (df = 1, F = 29.77; p < 0.0001; in *B. terrestris* df = 1, F = 0.51; p = 0.4779). Measures of FA were then obtained correcting for directional components.

B. terrestris size FA was positively correlated to temperature (Fig. 4; Table 1: model id C) and concentration of NO_2 (Online resource2 - Fig. S2; Table 1: model id D), but no similar response was observed in *B. pascuorum.* None of the predictor variables (i.e., temperature, NO_2, resource abundance) showed any significant effect on wing shape asymmetry in both bumblebee species.

Table 1**:** Results of the regression analysis of centroid size and fluctuating size asymmetry as a function of landscape and biotic and abiotic covariates. Only final models selected through AIC criterion with significant covariates are reported. β_i: regression coefficient; F: F-value; P: p-values. Models and results of size FA for *B. pascuorum* and of shape FA for both species are not reported in the table as they were inconsistent.

Response variable	**Model ID**	**Covariates**	**Bi**	**Df; F**	**P > F**
B. pascuorum Centroid size	A	Impervious/Natural (Log)	-0.00869	1; 4.2661	0.0462*
	B	Temperature	-0.00341	1; 7.7271	0.0088*
B. terrestris Centroid size	A	Floral resources (Log)	0.0725	1; 11.0222	0.0026*
B. terrestris Fluctuating size asymmetry	C	Temperature	0.05259	1; 11.5565	0.0019*
	D	NO_2	0.01433	1; 7.2304	0.0118*

DISCUSSION

In this study, we quantified the spatial intraspecific variation of functional traits that can shape the ecology of two common bumblebee species, possibly altering their dispersion ability, and pollination efficiency. We focused on their variation along the main trajectories of land use alteration, namely urbanization and agriculture intensification of landscapes, by relating variation of body size and wing asymmetry with landscape features and environmental stressors.

Landscape anthropization was found to be associated with intraspecific variation of body size (i.e., estimated as wing centroid size) across bumblebees populations. Specifically, foragers of one of the two investigated species, *B. pascuorum*, showed a shift toward smaller body size in response to increasing proportion of impervious surfaces and temperature, two conditions that characterize urban landscapes and emerged as highly correlated from our analysis. A similar pattern of body size reduction in urban bumblebees was reported by Eggenberger et al. (2019) and it agrees with observations showing a general decrease of body size in urban insect communities (Merckx et al., 2018). These works suggest that a potential driver of size reduction in bumblebees is represented by the diminished, and spatially dispersed, availability of floral resources that is often associated with higher

impervious cover (Eggenberger et al., 2019). However, in our study, neither the estimation of floral resources, nor the fragmentation of green areas (i.e., the edge density), correlated with size variation in *B. pascuorum*, the species that responded to urbanization. Hence, a more reliable driver of urban-induced size shrinkage could be warmer temperatures linked to the urban "heat island effect" (Merckx et al., 2018), and this is also supported by the results of our study. Indeed, temperature has a strong effect on insect body size, with multiple historical series collections-based and experimental studies that revealed how higher environmental temperatures represent a driver of body size reduction in different species of bees (e.g., Nooten & Rehan, 2020; Theodorou et al., 2020). Higher temperature accelerates larval development, which likely result in smaller adults (Sibly & Atkinson, 1994). Furthermore, smaller sizes in warmer areas could also be a strategy for reducing overheating risks while foraging, due to an increased convective heat loss in smaller bees (de Farias-Silva & Freitas, 2020). Functionally, smaller foragers could travel shorter foraging distances (Greenleaf et al., 2007) and could also load less pollen and nectar (Goulson et al., 2002). As a consequence, the shift towards smaller body size in *B. pascuorum* in more urbanized environments could imply that it will pollinate less plants or handle flowers less efficiently (Földesi et al., 2020), a concerning aspect in view of colony provision and pollination.

In our study *B. terrestris* body size was found to respond only to the abundance of flower resources, with larger individuals observed where potentially more food is available. This is in accordance with the observation that adult size is strictly correlated with the amount of food received during larval development (Couvillon &

Dornhaus 2009). However, this trend seems to be not clearly confirmed by *B. pascuorum* probably due to the higher specialization of this species (Harder, 1985) that may prevent it from benefiting from higher availability of flowers, since this not necessarily results in higher possibility to exploit adequate resources for their diet.

Despite *B. pascuorum* and *B. terrestris* belong to the same genus, the responses of these two species were not equal. Similarly, idiosyncratic responses were also observed in other bumblebee species, where if the body size of some decreased over warming decades, others responded in the opposite way (Gérard et al., 2020). In our study the invariant size of *B. terrestris* in warmer conditions could be explained by the high heat tolerance of this species (Martinet et al., 2020). In addition, *B. terrestris* nests further underground compared to the other bumblebee, and might be less exposed to warm, urban air temperatures during larval stages. These aspects strengthen the hypothesis that temperature is a major determinant of pollinator size reduction in cities because they affected the body size of the more temperature-sensitive species, but not the heat-tolerant one. These species-level, idiosyncratic responses are very relevant for our understanding of the potential mechanism of intraspecific trait variation associated with landscape anthropization. This supports the need to consider a wider panel of species when investigating the impact of landscape anthropization on functional biodiversity.

Flight performance does not only depend on body size, but it is also affected by asymmetries in shape and size between wings (Grilli et al., 2017; Soule et al., 2020). Here, we found that wing size FA was positively correlated with environmental stressors such as increased temperatures and NO_2 concentration in *B. terrestris*. The

absence of variations in shape asymmetry registered for both the bumblebee species could confirm the results from other studies that have indicated shape variation as less susceptible to stressors than size asymmetry (e.g., Gérard et al., 2018b). Variation in wing asymmetry was observed in other insect taxa; the effect of temperature was previously investigated under controlled laboratory conditions (Mpho et al., 2002) while the role of pollutants was discussed interpreting the results of a field experiment evaluating the impact of road traffic exposure (Leonard et al., 2018). Studies associated the increased wing FA to environmental stressors, indicating that impairment of developmental processes might take place (e.g., Klingenberg et al., 2001; Kerr et al., 2013). Concerning pollutants, Klingenberg et al. (2001) hypothesized that they could alter development through mechanisms related to gas exchange. Trait variation does not only show developmental instability, but it also has ecological implications. Wing size FA impacts the management of lengthy flights (Fernandez et al., 2017, Soule et al., 2020), compared to wing shape which is often associated with flight maneuverability. Thus, size asymmetry could specifically impact flight performance, flight length in time and space, and even affect bumblebee interactions with plants, the provisioning of larvae with food and their pollination rate.

CONCLUSIONS

Overall, the experimental results obtained in this study suggest that landscape anthropization could affect different functional traits, and it occurs heterogeneously in different pollinator species. Eventually, these responses could bring to the alarming

outcome of decreased pollination efficiency, because the studied traits are often involved in flying abilities. Temperature emerged as one of the main drivers of these phenotypic variations, affecting the two species in a different manner. The multiple responses to the same stressor, observed for the two species, underline the necessity for future studies to consider a wider panel of taxa instead of single model species.

From a conservation perspective, the comprehension of how pollinators cope with the challenging conditions occurring in novel anthropogenic habitats, plays a key role in informing suitable policy efforts to conserve their biodiversity and the ecosystem service they provide. In the future, cities are predicted to expand constantly and thus designing of urban landscapes will become a fundamental step for achieving sustainability outcomes. The creation and the wise management of urban green spaces will allow not only to create conditions that could support pollinators species abundance and diversity but also sustain the biological interactions that are the basis of a correct ecosystem functioning. At the same time, urban forestry and greenery practices (e.g., plantation of street and residential trees and the creation of urban greenbelts or greenways) could represent a valid solution to mitigate stressful conditions related to the urban environment, such as the “heat island effect” (Chun & Guldmann, 2018) and the high level of air pollution (Ottosen & Kumar, 2020) that here were found to influence functional diversity issues.

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Authors' contributions: NT, EP and AG conceived the ideas and designed methodology; NT, EP and PB collected the data; NT and EP analysed the data, PB contributed to analyses checking/development and checked the final model outcomes; EP, NT and PB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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FIGURE LEGENDS

Fig.1: Map showing the distribution of the sampling sites and the border of the different provinces included in the study area.

Fig.2: Right forewing of *B. terrestris* with landmark locations

Fig. 3: Regression analysis of centroid size as a function of (a) temperature, and (b) floral resources . *B. pascuorum* is represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines. Continuous lines indicate the significant relationships while dashed lines represent the non-significant ones. Dots and triangles represent observed values. The two species were tested in separate models but represented together to facilitate the comparison.

Fig. 4: Regression analysis of fluctuating size asymmetry as a function temperature . *B. pascuorum* is represented by black dots and lines, *B. terrestris* is represented by grey triangles and lines. Continuous lines indicate the significant relationships, while dashed lines represent the non-significant ones.

Fig.1

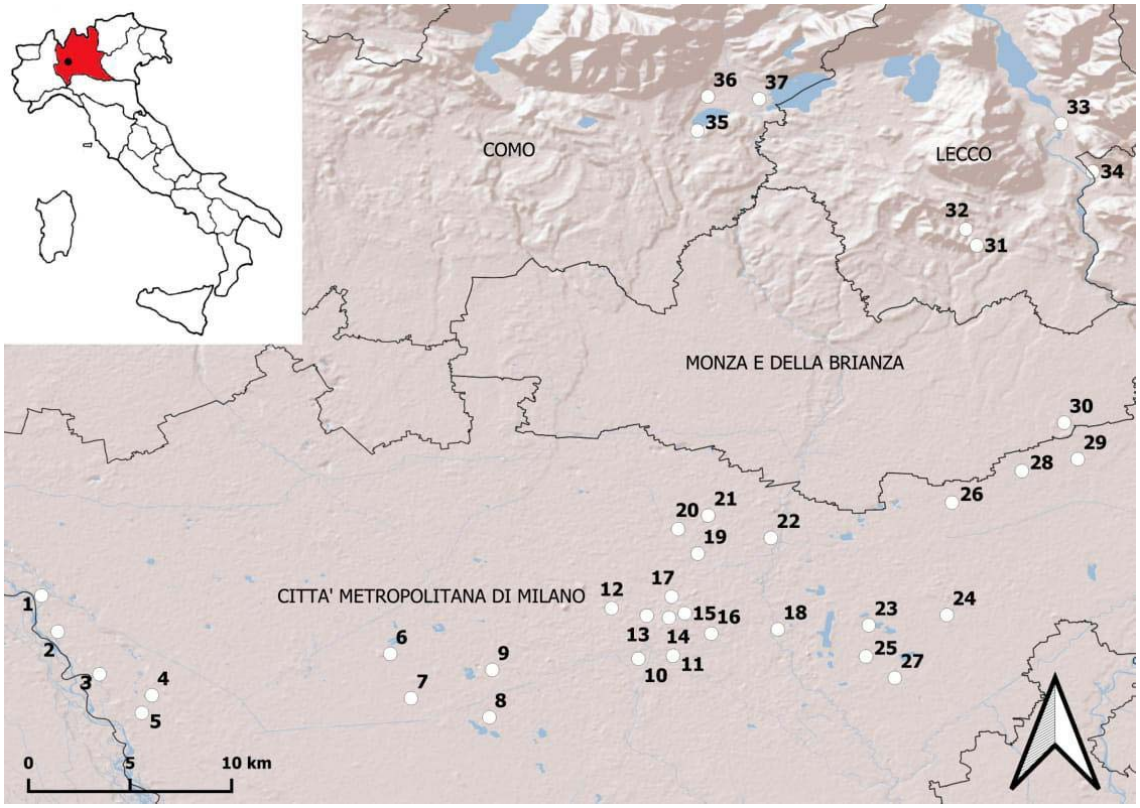
COMO
LECCO
MONZA E DELLA BRIANZA
CITTA' METROPOLITANA DI MILANO
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Fig.2

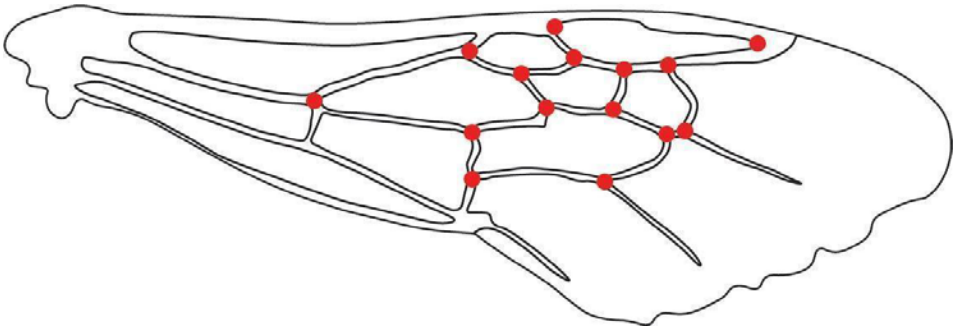


Fig.3

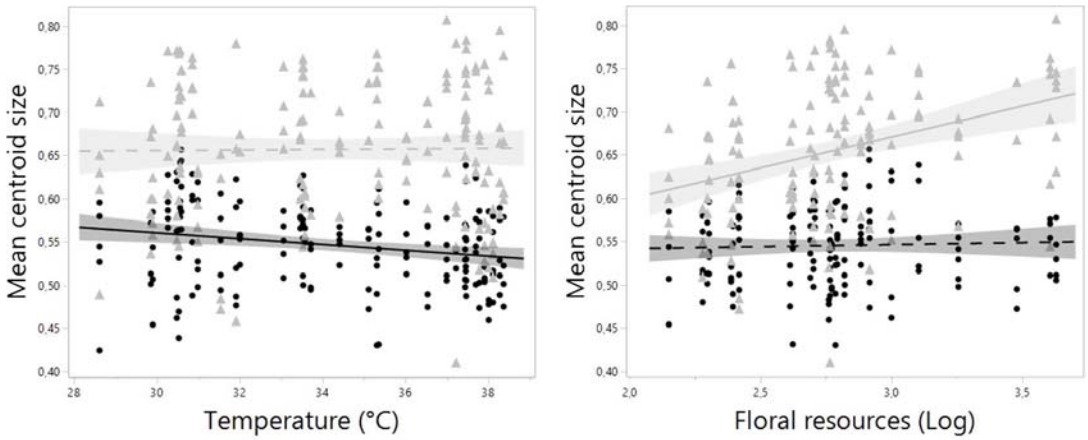
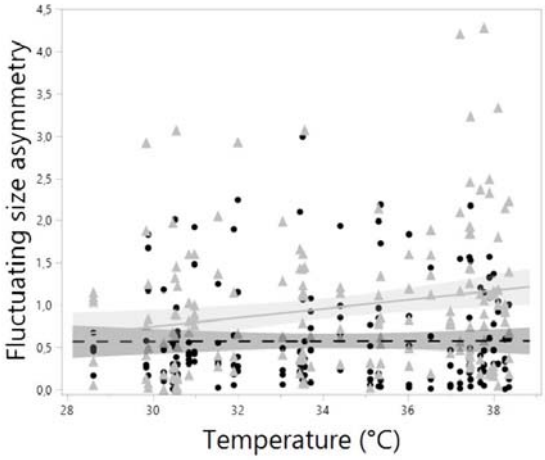

Mean centroid size
0,80
0,75
0,70
0,65
0,60
0,55
0,50
0,45
0,40
28
30
32
34
36
38
Temperature (°C)
Mean centroid size
0,80
0,75
0,70
0,65
0,60
0,55
0,50
0,45
0,40
2,0
2,5
3,0
3,5
Floral resources (Log)

Fig.4


Fluctuating size asymmetry
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
28
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32
34
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Temperature (°C)