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Notes on biometric variability in invasive species: the case of *Psacothea hilaris hilaris*

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Abstract

Species morphometric variability is the result of the combined effect of genes and environment. This is emphasized in insects, especially in ones that rely on discrete food resources, such as xylophagous insects. *Psacothea hilaris hilaris* (Pascoe) (Coleoptera Cerambycidae Lamiinae), an exotic beetle already established in Italy, is used as a model species for the study. The findings presented in this research increase knowledge of morphological and colourimetric traits in *P. h. hilaris* and support the hypothesis that environmental cues can impact certain important morphometric features of exotic insects. Principal component analysis (PCA) and Bayesian's posterior probability applied to the dimensions of specimens collected over a four-year period showed that some morphological parameters changed significantly over the years. According to PCA the most meaningful morphometric variables were body length, elytral length, and antenna-to-body length ratio. One of the most significant results is the variability of the antenna-to-body length ratio over the period of the study. In cerambycids longer antennae allow for better detection of host tree, oviposition site, and favour mating strategies. Consequently variability in this physical trait can influence the ability of the species to adapt to a new habitat.

Key words: phenotype, environmental induction, morphometry, colourimetry, image processing, ecology.

Introduction

Phenotype is the combined result of genetic and environmental cues during the development of an organism (Willmore *et al*., 2007). The relationship between genetic and environmental variability is not defined and sometimes arbitrary. However, both contribute to alter the dynamic and timing of developmental processes, resulting in an outcome different from that which would develop if only genotype induction were present (Nijhout, 1999). Hence plasticity within populations and species is an important mechanism of adaptation to variable environments (Wund, 2012; Gomez-Mestre and Jovani, 2013). It can facilitate colonization and expansion into new areas (Fox and Savalli, 2000) and is a key issue in ecological and evolutionary studies (Agrawal 2001; Price *et al*., 2003; Olendorf *et al*., 2006). This concept is true for all animals, but is emphasized in insects, as they exhibit great variability within species and even within populations.

Insects use a variety of environmental factors as cues for their polyphenic development; among them temperature and photoperiod play major roles, but food quality, season, humidity and population density can also be implicated (Pener and Yerushalmi, 1998; Evans and Wheeler, 2001; Hochkirch *et al*., 2008). Variation in host availability could result in the evolution of phenotypic plasticity, in which the same genotype expresses different phenotypes on different plant hosts (Amarillo-Suárez and Fox, 2006). Species distributed over a broad geographic range often exhibit thermal clines in body size, with the majority of species showing larger adult size in colder environments (Azevedo *et al*., 2002; Angilletta *et al*., 2004). However, great variability in size is often found within the same population in one environment. A classic example is the honeybee *Apis mellifera* which in different environments has developed different ecotypes (Andere *et al*., 2008; Alattal *et al*., 2014). For insects that use discrete resources, including parasitoids, seed feeders, and xylophagous insects, host size and host quality are major sources of phenotypic variation among species and may constrain offspring growth, influencing the evolution of body size and life history traits (Hardy *et al*., 1992; Mackauer and Chau, 2001; Tsai *et al*., 2001). This fact is strictly influenced by cross-generational (or trans-generational) phenotypic plasticity (Mousseau and Dingle, 1991; Mousseau and Fox, 1998), a phenomenon in which parents modify the phenotype of their offspring in response to environmental conditions through the level of care they provide (Mondor *et al*., 2005; Hunt and Simmons, 2007).

A valuable tool for the study of phenotypic plasticity is provided by morphometry, the measure and analysis of the physical form (Howell, 1985): in insects the exoskeleton can be easily measured as it is not subjected to physical distortions undergone by the bodies of many other animals. Another tool for the study of insect population variability is colour, which may also depend from both genetic diversity and from external inputs such as temperature and photoperiod (Hazel, 2002). Colour polymorphism within a species is common in insects and may depend from the season and temperature in many Hemiptera (Stewart, 1986; Musolin and Numata, 2003; Wenninger and Hall, 2008) as well as in Coleoptera (Osawa and Nishida, 1992; Davis *et al*., 2008). Colour variability may be also an informative feature for insect taxonomy providing additional traits that aid subspecies identification. The association between behaviour and recognition of mates in a single population is important because it may enhance the probability of speciation and provides an example of the missing link between an interbreeding population and isolated spe-

cies (Chamberlain *et al*., 2009).

The effect of environmental variables on the phenotype of insects could be especially manifested in an exotic species subjected to new external inputs. The early stage of the invasion process is generally led by individuals deriving from few ancestors; thus the species shows reduced genetic variability in the new environment compared to that of the native countries. Distance from an insect's native range often results in low genetic variability of the exotic population, and in addition to the novel selection pressure in a new habitat, these two factors can cause the species to undergo changes in physical form (Vellend *et al*., 2007).

Among insects, Cerambycidae represent classical objects for the study of the effect of environmental variables on morphological polymorphism (Shoda *et al*., 2003). Their larvae complete development in the plant where the egg was oviposited, hence larval development is directly related to the plant nutritional values, which varies according to the species, the diameter of the plant and the season (Hanks *et al*., 2005; Reagel *et al*., 2012). Specimens developing inside vigorous, large hosts use more alimentary resources and generally become bigger than those in small hosts. This was demonstrated with *Cerambyx cerdo* L. and *Phoracantha semipunctata* (F.) adults (Starzyk and Strojny, 1985; Hanks *et al*., 2005; Michalchewicz and Ciach, 2012).

As a model species for the study, the polymorphic yellow-spotted longicorn beetle *Psacothea hilaris* (Pascoe) (Coleoptera Cerambycidae Lamiinae) was selected. *P. hilaris* is autochthonous in various countries from far East Asia (Kim *et al*., 2009) and was detected in Italy in two municipalities in 2005 (Jucker *et al*., 2006), where it has now established on a wider area (Lupi *et al*., 2013). This xylophagous insect is mostly associated with mulberry trees in its countries of origin, but with *Ficus carica* L. in Italy (Lupi *et al*., 2013).

P. hilaris adults are elongate and subcylindrical beetles characterized as many adult longicorn beetles by long antennae, longer in males than in females; black cuticle covered by a green-greyish pubescence with spots and stripes of yellow hair; pronotum with two yellow stripes, elytra truncated at the apex and with several small spots and a row of five larger spots on each outside edge; antenna light grey, with the two first segments completely black, the following eight black at the apex, and the last one black in the middle; and the body pubescent beneath with yellow spots (Pascoe, 1857; Shintani *et al*., 2003). The species is known to have remarkable intraspecific variability and the different subspecies are quite easily recognizable in relation to their colour variability. The subspecies detected in Italy is *Psacothea hilaris hilaris*, also present in Japan and China (Jucker *et al*., 2006). Extreme phenotypic variability is also present in *P. h. hilaris*; two different ecotypes have been detected in Japan that are characterized by differences in distribution, seasonal life cycle, photoperiodic and temperature responses to diapause and spot patterns on the pronotum of adults (Iba, 1976; Sakakibara, 1995; Shintani *et al*., 1996; Shintani and Ishikawa, 1997).

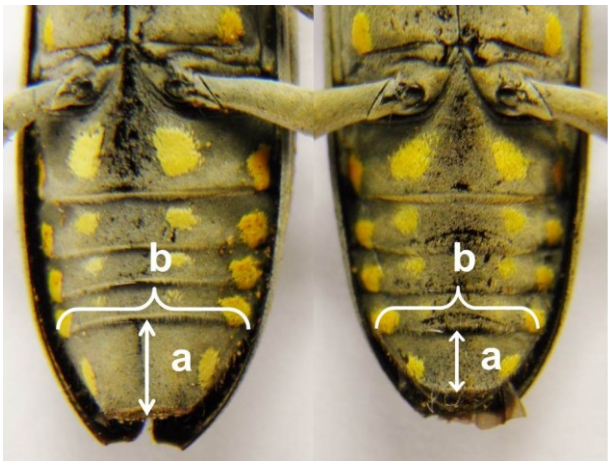

Figure 1. Morphometric detail of the last ventrites in female (left) and male (right).
(in colour at www.bulletinofinsectology.org)

Considering the potential variability of the species, the recent settlement in the new area and the preference for fig instead of mulberry trees as hosts in Italy, *P. h. hilaris* is a suitable candidate species to highlight the importance of phenotypic variability in invasion biological studies, to evaluate the influence of time with regards to this variability, and to improve knowledge of invasive species polymorphism.

Materials and methods

Insect collection

P. h. hilaris adults were collected from 2010 to 2013 in eight different sampling sites in a small area south of Como Lake, one of the largest hydrographic basins in Northern Italy, where the xylophagous insect has settled (Lupi *et al*., 2013). The zone is characterized by a subcontinental climate (Pinna, 1978), with milder winter due to its vicinity to the lake.

Some specimens were collected among vegetation on fig trees. Field-direct capture can be influenced by operators and insect characteristics (sex, mobility, period of survey and greater visibility of larger adults among vegetation). Thus, to obtain more detailed information about population variability, branches and trunks infested with larvae were cut in late spring and maintained at room temperature inside cages at the DeFENS laboratory (University of Milan). This operation allowed the collection of all specimens that would have emerged. The infested wood trunks was checked three times per week and the adults were collected, sexed and preserved in the freezer at −18 °C.

Insect measures

Data including the year of emergence, sex, and morphological measurements were registered for each sample. Adults were measured using an electronic digital caliper (Stainless Hardened®) to the nearest 0.01 mm.

The following biometric traits were measured: 1) the body length (from the head to the apex of the abdomen); 2) the elytral length (from pronotum insertion to apex of abdomen); 3) the length and 4) the width of the last ventrite (figure 1). The first two parameters were acquired

positioning specimens on their ventre, while the last two were measured positioning them on their back. Moreover, 5) the length of the first antennomere and 6) the total length of the antenna (fully extended from the scapus to the apex) were acquired; this last characteristic was measured only on specimens with at least one complete antenna. Data were then compared with the ones available in literature in native countries (Fukaya, 2004).

Colourimetric variability

As colourimetric variability in *P. h. hilaris* is found in the pubescence covering the cuticle, damaged individuals were discarded. 100 specimens (50 males and 50 females) were then randomly chosen in the pool of the undamaged insects and were individually photographed to acquire standardized data on the colourimetric pattern variability of the elytra. Photographs were taken with a Canon EOS 50D camera fitted with a Canon 60 mm EF-S macro lens and Canon MT-24EX flash. All images were taken positioning the camera perpendicular to the mounted specimen retaining the same physical distance and focal length for every picture. The resultant images were then re-coloured in a black and white scale to clarify elytral spots, and resized to allow image superimposition, with Adobe Photoshop® (9.0.2 ver.). Analysis of superimposed specimens allows a better description of spots' presence/absence and their position and to remove differences due to adult size.

All specimens were visually inspected to evaluate if the stripes on the pronotum were continuous or broken. The image processing program SigmaScan Pro® (SPSS) was used to estimate the following parameters on each of the larger spots in the row on the outer side of each elytra that in literature is described as representative of *P. hilaris* specimens (von Breuning, 1943) (figure 2, left):

1- Presence/absence of a well-defined spot. Calculated as percentage on the entire range of males/females.
2- Spot location. Calculated for the spots in the same position on both elytra as distance from the elytral suture to the center of each spot (figure 2, right).
3- Spot area. Calculated as mean value ± standard error for the spots in the same position.

To better standardize measurements, the length of the elytra, calculated from the pronotum insertion to the apex, was considered as reference unit.

In order to acquire new information about the colourimetric variability of the subspecies, the pattern of all the spots was analyzed in detail evidencing characteristics and recurrent spots in different specimens in the two sexes.

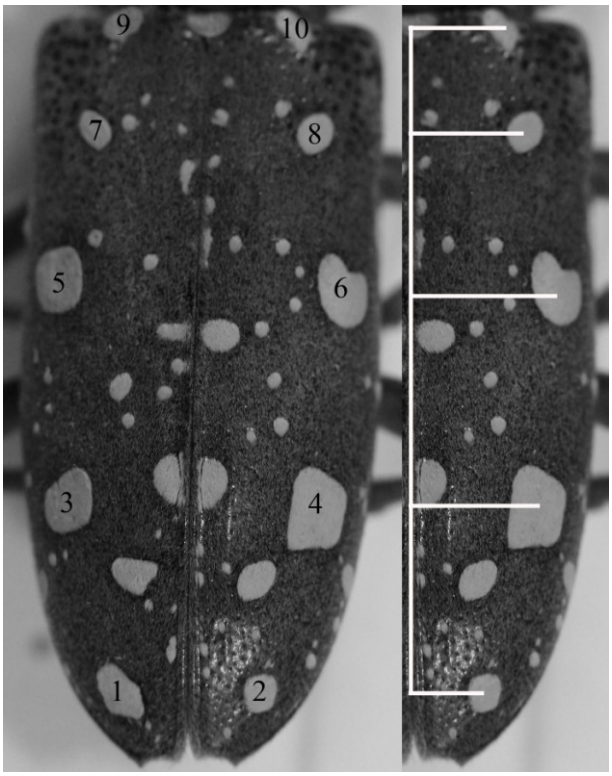

Figure 2. Distribution of the ten larger spots on the elytra (left) and distance from the elytral suture (right).

Statistical analysis

Sexes variability

Data from all four-years' specimen collection were pooled together to evaluate population variability. Analyses were performed with IBM SPSS® Statistics 21 and the following statistical parameters were analyzed concerning morphometry and colourimetry: distribution frequency, mean, and standard error. The relationship between body length and antennal length and the relationship between last ventrite length and body length were evaluated as possible characteristics allowing for discrimination of sexes. *F*-tests were used to investigate differences in variance between phenotypic traits and sexes (Sokal and Rohlf, 1995).

Four-year effect on the phenotype

To evaluate if the environment can affect the phenotype, the evolution of different parameters over the years was studied separatedly in the two sexes. The specimens collected were first pooled by sex and year. Both univariate and multivariate procedures were used for analyzing the data: (i) one-way ANOVA with replications and means separated by Tukey's Test ($P < 0.05$); (ii) principal component analysis (PCA); (iii) Bayesian's posterior probability was then applied to verify the probability of each specimen to be assigned to one of the four-year classes.

The data of the linear measurements were log transformed before multivariate analysis. PCA was first performed to examine the strength of the associations between morphological characters, and to select a minimum set of meaningful variables capturing most of the variation in the complete data set (Ripley, 1996; Venables and Ripley, 2002). Analyses were performed with R package for multivariate analyses ade4 (Dray and Dufour, 2007).

Results

A total of 400 adults of *P. h. hilaris* (200 males and 200 females) were collected in the period of the study; specifically 43 (30 males and 13 females) specimens in 2010, 156 (85 males and 71 females) in 2011, 146 (72 males and 74 females) in 2012 and 55 (13 males and 42 females) in 2013.

Table 1. Morphometric measurements of males and females of *P. h. hilaris* and ANOVA results.

Biometric parameter	Sex	Specimens	ANOVA	Mean ± SE	Max- value	Min value
Body length (mm)	M	200	F = 0.737; P = 0.391	22.914 ± 0.229	29.595	13.030
	F	200		23.161 ± 0.175	28.520	14.020
Elytral length (mm)	M	200	F = 27.614; P < 0.005	14.791 ± 0.167	19.000	8.390
	F	200		15.870 ± 0.178	20.230	9.800
Antennal length (mm)	M	147	F = 341.164; P < 0.005	58.338 ± 1.915	77.780	27.63
	F	150		42.196 ± 1.336	51.950	26.66
First antennomere length (mm)	M	197	F = 199.913; P < 0.005	8.876 ± 0.133	13.000	4.300
	F	196		7.093 ± 0.098	9.000	4.450
Last ventrite length (mm)	M	200	F = 700.628; P < 0.005	1.694 ± 0.028	2.860	0.500
	F	200		2.694 ± 0.031	3.750	1.740
Last ventrite width (mm)	M	200	F = 340.623; P < 0.005	3.692 ± 0.054	5.260	1.500
	F	200		4.954 ± 0.059	6.630	2.550
Antennae/body	M	147	F = 1573.426; P < 0.005	2.551 ± 0.080	2.88	2.10
	F	150		1.840 ± 0.057	2.12	1.43
First antennomere/body	M	197	F = 668.172; P < 0.005	0.390 ± 0.004	0.48	0.27
	F	196		0.300 ± 0.004	0.41	0.26
Last ventrite/body	M	200	F = 922.815; P < 0.005	0.073 ± 0.001	0.12	0.04
	F	200		0.116 ± 0.001	0.17	0.08

Sexes variability

P. h. hilaris exhibited great variability in body dimension. The spectrum of dimensional variability was broad in both sexes with a range of variability of 16.565 mm in males (mean 22.914 ± 0.229) and of 14.500 mm in females (mean 23.161 ± 0.175). Even so, this did not result in significant differences between the two sexes (F = 0.737; P = 0.391) (table 1). This result is different from the one obtained in Japan (Fukaya, 2004) where the observation of a set of 174 males and 154 females resulted in major variation in male dimensions. However, all the other biometric parameters analyzed resulted in significant differences among sexes. As in many Lamiinae, *P. h. hilaris* males have significantly longer antennae than females, both considering the entire length (mean 58.338 ± 1.915 mm in males and 42.196 ± 1.336 mm in females) and the length of the first antennomere (mean 8.876 ± 0.133 mm in males and 7.093 ± 0.098 mm in females). Males displayed an overall higher ratio of antenna length to body length (mean 2.551 ± 0.080 mm in males and 1.840 ± 0.057 mm in females). Males also were found to have a higher ratio of first antennomere length to body length (mean 0.390 ± 0.004 mm in males and 0.300 ± 0.004 mm in females). Another morphologic characteristic considered was the last abdominal ventrite: the mean value of its length (mean 1.694 ± 0.028 mm in males and 2.694 ± 0.031 mm in females), width (mean 3.692 ± 0.054 mm in males and 4.954 ± 0.059 mm in females) and the ratio with the body length was always significantly higher in females than in males (mean 0.073 ± 0.001 mm in males and 0.116 ± 0.001 mm in females). More details can be observed in figure 3 that allows comparison of the frequency diagrams of the different biometric parameters and shows the ratios among different traits. Morphological separation of the sexes is more evident in the graphs showing the relationship between antenna and body length, first antennomere and body length, and terminal ventrite and body length. It is possible to note that in the first the curves are completely separated (F = 1573.426; P < 0.05). The curves representing the ratios of first antennomere/body and ventrite/body are only slightly superimposed (F = 668.172; P < 0.05; F = 922.815; P < 0.05): values common to both sexes are between 0.31 and 0.37 in first antennomere/body and between 0.08 and 0.11 in ventrite/body. Notwithstanding that, the histogram representing the body length frequencies appears to be nearly symmetrical.

Colourimetric pattern variability

Visual inspection indicated that all specimens showed the broken stripe pattern on the *pronotum.* The elytra appeared more or less pointed in relation to each specimen. The spots that in literature are considered as representative of *P. h. hilaris* were present in nearly all the specimens examined. Individual observation indicated some asymmetry in shape and dimension that did not result in significant differences among the areas. Spots 7 and 8, positioned in the third superior area, sometimes were absent. In detail, spot number 7 was lacking in 6% of males and in 4% of females while spot number 8 was lacking in 6% of both sexes. Moreover spots 1 and 2 were absent only in 2% of males. As spots 9 and 10 proved to be always elliptical and slanted up toward the elytral suture, the software Measure Inc® was then used to define their angle of slant. No differences were detected between males and females (F = 3.346; P = 0.07); the mean slope of these spots towards the elytral suture was 45.51 ± 3.20° (maximum 51.0°, minimum 37.2°) in males and 44.45 ± 2.46° (maximum 50.7°, minimum 40.0°) in females.

Numerous lesser spots could be found on the elytra, variable and unique to each specimen examined. However, two particular spots per elytra were detected in the majority of the specimens observed: the first one is present on both elytra in 98% of females and in 100% of males, located between the spots 1-3 and 2-4; the second, having a semilunar shape that creates a circular

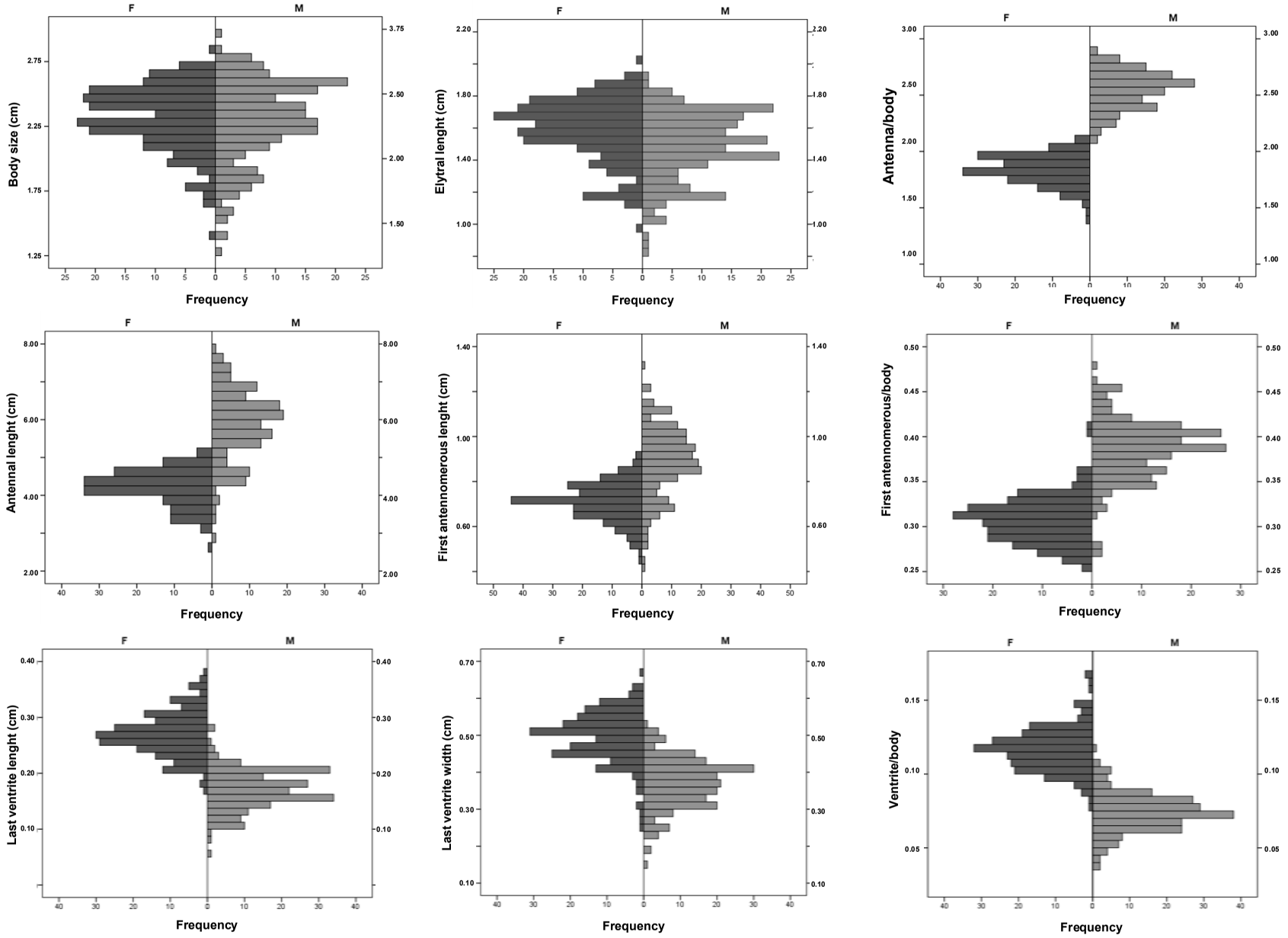

Figure 3. Frequency histograms of the biometric traits measured in *P. h. hilaris* and of the ratio among different traits (dark grey = female; light grey = male).

Figure 4. Spots detected as characteristic of *P. h. hilaris* in the present research (indicated by the arrows). (in colour at www.bulletinofinsectology.org)

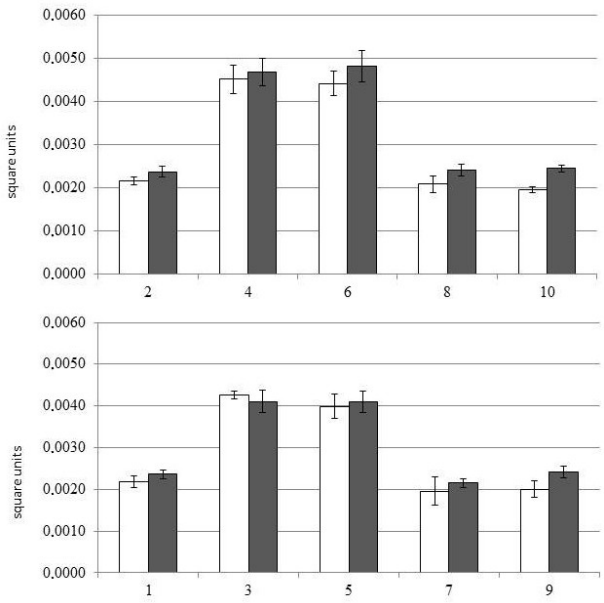

Figure 5. Mean area of the spots on the right elytra (top) and on the left (bottom) in females (white) and in males (dark grey).

spot across the two elytra when they are closed, is positioned between spots 3 and 4 (figure 4). This characteristic spot was observed in 68% of males and in 80% of the females examined. Sometimes it is present only on one elytra (in 32% of males and in 20% of females) while on the other elytra the semilunar spot is replaced by two small separate spots.

The spots numbered 3-4-5-6 were consistently larger than the others ($F = 59.305$; $P < 0.05$) with an area which is nearly twice that of the spots 1-2-7-8-9-10 in both males and females (0.004 ± 0.0002 *vs* 0.0022 ± 0.0007 square units) (figure 5). No differences were observed among the sexes related to spot dimensions ($F = 3.235$; $P = 0.72$).

The analyses of the distance from spot center to elytral suture revealed significant differences in spot localization. Two different groups can be found: spots 1-2-7-8-9-10 belong to the same row parallel to the suture (whose distances correspond to 1/9 of the length of the elytra) while the larger ones (3-4-5-6) are slightly shifted from this axis to an outer line (whose distances correspond to 1/6 of the length of the elytra).

Four-year effect on the phenotype

The study of the phenotype variability over a four-year period showed that some parameters changed significantly over time. In table 2 the results of Tukey's post hoc test are reported. For males, significant differences were observed in elytral length ($F = 6.61$; $P < 0.05$), in first antennomere length ($F = 5.39$; $P < 0.05$), and especially in the ratios of antenna/body length ($F = 14.95$; $P < 0.05$) and first antennomere/body length ($F = 22.31$; $P < 0.05$). For females, significant differences were observed in the same biometric characters: elytral length ($F = 5.36$; $P < 0.05$), first antennomere length ($F = 3.60$; $P < 0.05$), and again especially in the ratios of antenna/body length ($F = 21.40$; $P < 0.05$) and first antennomere/body length ($F = 20.82$; $P < 0.05$). Multivariate analysis allowed evaluation of the importance of different variables in the phenotype expression. In the PCA, in the eigenvalues barplot, more than 80.82% of the variance in males and 78.89% in females was accounted for in the first two axes and thus presented a good initial summary of the structure of the datasets. According to the PCA, the most meaningful variables were body, elytral and first antennomere length in both sexes, and antenna length in females only. The length and the width of terminal ventrite seems to have less importance in variation among years (figure 6c and 6d). For both males and females, data from specimens of 2013 manifested in a different quadrant of the plot of analysis, hence are established as significantly different from data of the other years (figure 6a and 6b). 2010 and 2011 occurred in the same quadrant evidencing more similarities: longer antennae and longer first antennomere characterized the specimens in this quadrant (figure 6c and 6d). Bayesian's clustering posterior confirmed the PCA results. More than 91% of the population collected in 2013 manifested traits that allowed assignment to the cluster identified as characteristic of the year 2013. The affinity of the specimens to the year-classes decreased to 71% (2012-males), to 61% (2011 and 2012-females; 2010-males), and to 56% (2011-males and 2010-females). The remaining percentage showed characteristics that included them in two-year classes.

Daily temperature (mean data per month) and pluviometric data (mm per month) were used to compare different years (data provided by the Regional Agency For Environmental Protection of Lombardy region). Differences appeared in early 2012 as the temperature dropped to −11.5 °C in February but the following months were comparable to the corresponding ones of 2010 and 2013.

Table 2. Variability in males' and females' morphological parameters over the years of study and ANOVA results. (Means followed by different letters in the same column are significantly different at $P < 0.05$).

Parameter	Sex	Year	Specimens	ANOVA	Mean ± SE
Body length (mm)	M	2010	30	F = 2.70; P = 0.052	23.500 ± 0.447 a
		2011	85		22.190 ± 0.232 a
		2012	72		23.534 ± 0.240 a
		2013	13		22.861 ± 1.102 a
	F	2010	13	F = 2.46; P = 0.065	23.346 ± 0.601 a
		2011	71		22.614 ± 0.695 a
		2012	74		23.709 ± 0.700 a
		2013	42		23.053 ± 0.840 a
Elytral length (mm)	M	2010	30	F = 6.61; P < 0.05	15.417 ± 0.416a
		2011	85		14.528 ± 1.161 a
		2012	72		15.201 ± 0.401 a
		2013	13		12.779 ± 0.430 b
	F	2010	12	F = 5.36; P < 0.05	15.958 ± 0.601 ab
		2011	71		15.737 ± 0.594 ab
		2012	73		16.461 ± 0.564 b
		2013	42		15.001 ± 0.612 a
Antennal length (mm)	M	2010	15	F = 1.01; P = 0.393	62.400 ± 2.830 a
		2011	80		57.782 ± 2.010 a
		2012	40		58.042 ± 2.915 a
		2013	12		57.954 ± 1.521 a
	F	2010	5	F = 1.70; P = 0.170	43.800 ± 2.901 a
		2011	68		42.985 ± 2.222 a
		2012	43		41.148 ± 2.241 a
		2013	34		41.706 ± 2.201 a
First antennomere length (mm)	M	2010	30	F = 5.39; P < 0.05	9.883 ± 0.143 b
		2011	85		8.910 ± 0.156 a
		2012	69		8.660 ± 0.112 a
		2013	13		8.908 ± 0.450 ab
	F	2010	12	F = 3.60; P < 0.05	7.750 ± 0.276 b
		2011	71		7.118 ± 0.131 a
		2012	72		6.927 ± 0.132 a
		2013	40		7.154 ± 0.199 a
Last ventrite length (mm)	M	2010	30	F = 1.94; P = 0.124	1.750 ± 0.101a
		2011	85		1.629 ± 0.234 a
		2012	70		1.723 ± 0.091 a
		2013	13		1.828 ± 0.089 a
	F	2010	12	F = 0.70; P = 0.053	2.708 ± 0.097 a
		2011	71		2.718 ± 0.104 a
		2012	74		2.643 ± 0.111 a
		2013	42		2.740 ± 0.123 a
Last ventrite width (mm)	M	2010	30	F = 1.79; P = 0.150	3.567 ± 0.432 a
		2011	84		3.612 ± 0.071 a
		2012	71		3.010 ± 0.089 a
		2013	13		3.902 ± 0.194 a
	F	2010	12	F = 1.87; P = 0.136	4.542 ± 0.198 a
		2011	70		4.919 ± 0.245 ab
		2012	74		5.021 ± 0.198 b
		2013	42		5.017 ± 0.156 b
Antennae/body	M	2010	15	F=14.95; P<0.05	2.621 ± 0.039 b
		2011	80		2.610 ± 0.042 b
		2012	40		2.419 ± 0.050 a
		2013	12		2.512 ± 0.061 ab
	F	2010	5	F = 21.40; P < 0.05	1.821 ± 0.051 ab
		2011	68		1.909 ± 0.047 b
		2012	43		1.742 ± 0.054 a
		2013	34		1.819 ± 0.048 ab
First antennomere/body	M	2010	30	F = 22.31; P < 0.05	0.423 ± 0.014 c
		2011	85		0.389 ± 0.009 b
		2012	69		0.373 ± 0.011 a
		2013	13		0.394 ± 0.013 b
	F	2010	12	F = 20.82; P < 0.05	0.342 ± 0.011 c
		2011	71		0.319 ± 0.011 b
		2012	72		0.294 ± 0.014 a
		2013	40		0.313 ± 0.012 b
Last ventrite/body	M	2010	30	F = 1.10; P = 0.350	0.072 ± 0.031 a
		2011	85		0.071 ± 0.032 a
		2012	70		0.073 ± 0.031 a
		2013	13		0.078 ± 0.039 a
	F	2010	12	F = 4.493; P = 0.055	0.111 ± 0.011 a
		2011	71		0.121 ± 0.012 a
		2012	74		0.114 ± 0.013 a
		2013	42		0.123 ± 0.011 a

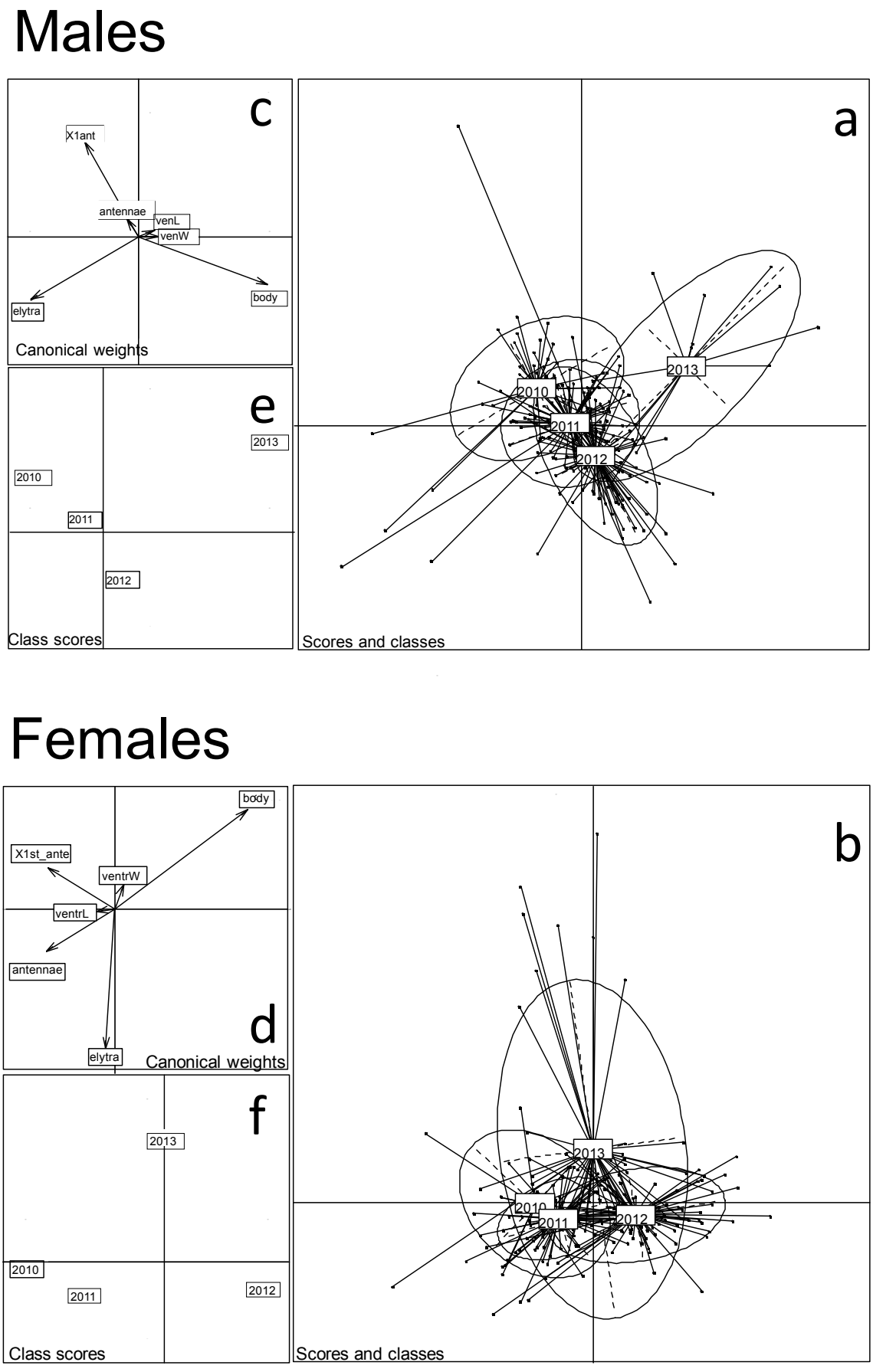

Figure 6. Principal component analysis carried out with the years as instrumental variables on males and females (a) and (b). Plots (c) and (d) indicate the most the most meaningful variables in males and females respectively; (e) and (f) show the position of the years using morphological variables (origin of the arrows).

In 2013 the temperatures from February to May were significantly lower according to Tukey's Test ($F_{February} = 7.4$; $F_{March} = 12.51$; $F_{April} = 9.83$; $F_{May} = 11.19$; $P < 0.05$). According to pluviometric data the periods of the heaviest rain were from midsummer to late autumn (July-November) 2010 and during spring (March-May) 2013.

Discussion

The results of the study provided detailed information on *P. h. hilaris* polymorphism, confirmed the extreme phenotypic variability associated with the subspecies, and supported the hypothesis that environmental cues can affect some morphometric traits of this exotic insect.

Even though sexual dimorphism is common in cerambycids with females larger than males (Linsley, 1961), and according to Fukaya (2004) also in *P. h. hilaris*, this was not the case of the population collected in Italy, where no significant differences have been found in body size comparison between sexes. This is a first evidence of the influence of a different environment and hosts (mulberry in Japan and fig tree in Italy) on adult population traits. However, the set of 400 specimens collected in Italy showed a dimensional variability with mean values in line with the ones obtained in Japan (Fukaya and Honda, 1996; Fukaya, 2004). This is very important as body size variability reflects the adaptability of a species to a certain environment. Great body size variation within populations is found in wood borers, as it is strongly dependent on the condition of the host tree in which they hatch and mature (e.g. Basset *et al*., 1994; Dmitriew and Rowe, 2011). For cerambycids it is difficult to distinguish the influences of diet and environment as the two are the same during this beetle's early life stages (Jermy, 1984; Trematerra *et al.*, 2013). Larger males are capable of searching a wider area for mates than smaller ones and are also more accepted by females (Yokoi, 1990; Fukaya, 2004). In addition, a strong positive correlation exists between adult female body mass and fecundity (Allison *et al.*, 2004). As for colour, even though Italian specimens possess the broken line on the pronotum found in the Japanese ecotype, there is an important difference in larval development between these two populations. In Japan, this ecotype undergoes diapause during the larval stage, while in Italy it does not. Instead, *P. h. hilaris* in Italy overwinters as eggs or larvae able to hatch and/or develop in the subsequent spring (Lupi *et al.*, 2013). This behaviour confirmed the great flexibility of the polymorphic *P. h. hilaris*, and its good adaptability to a changing environment.

The results show that the phenotype of *P. h. hilaris* can be influenced by the environment. PCA analysis separated specimens from different years in relation to different morphometric traits: major separation is evidenced for 2013 specimens in both sexes, while a little superimposition between 2010-2011 and 2011-2012 was observed. Among the variable characters in the years examined the most important appears to be the ratio of antennal/body length. It is noteworthy that this morphometric trait is related to location of hosts, oviposition sites and mating strategies; *P. h. hilaris* mating involves contact pheromones that allow males to locate a female when the male antennae and/or tarsi are in contact with the female. Consequently, longer antennae may facilitate female location (Fukaya and Honda, 1992). The causes of this variability are probably due to environmental conditions, and to the quality of the food the larvae consumed. Bayesian's Posterior analysis revealed the overall variation among specimens within a year decreases from 2010 to 2013. This could be due to progressive adaptation of the exotic species to its new environment. Another important consideration is weather; there was unusually high rainfall in 2013 and, as the radial growth of the sapwood is correlated with the spring rains from March to May (Coile, 1936; Lambers *et al*., 2008), *P. h. hilaris* larvae from that year probably had access to richer, more nutritious plant tissue during that critical period in their early development. The spring season in Italy simultaneously marks generation of new plant tissue and the start of larval feeding under the bark. As a result, pluviometric phenomena affecting tree growth can greatly influence insect development, hence the insect's phenotypic characteristics. Considering that capture of specimens over the years were made in the same localities where plant attack steadily augmented, the morphometric traits could have been differently influenced by the quality of decaying plant tissue where the larvae lived. Moreover, the increasing population density in these places can influence sexual selection: with a high population density females are less selective in the choice of the males (McLain, 1982; Jirotkul, 1999) and males can easily find the females independently from the length of their antennae and their size. Hence a population's phenotypic variability can reflect the variation in genotypic selection.

Time did not show any influence across the 4 years considered on elytra colourimetric pattern. The five spots per elytra described by von Breuning (1943) and found in the present research coexisted in the years and with the same percentage of presence. However the colour, according to Fukaya and Honda (1996), seems not to have influence on mating behaviour and so its variability is probably not involved in the mechanisms of specimen adaptability and selection in a new environment.

Some valuable biometric traits that allow distinction of the sexes were identified during the study. The first one is common in Cerambycidae and also in Lamiinae (Duffy, 1952; Lingafelter and Hoebeke, 2002): males of the yellow spotted longicorn beetle in fact exhibited antennae more than twice the length of the body, while females less than twice the length of the body. The second character is the last ventrite, which is longer in females than in males. The combination of the observation of both characters allows to segregate sexes, even in individuals which are borderline for one of the two parameters.

Adult variability in a recently settled population can be important to evaluate if the species is well adapted to the new environment and to establish how the environment can influence the phenotype significantly. This is emphasized in polymorphic species as they are able to

slightly change their phenotype demonstrating their plasticity to different habitats. Insect polyphenism provides the opportunities to study the evolution of developmental processes, showing how a single species can evolve in a new environment.

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