No increase in fluctuating asymmetry in ground beetles (Carabidae) as urbanisation progresses

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Abstract

Environmental stress can lead to a reduction in developmental homeostasis, which could be reflected in increased variability of morphological traits. Fluctuating asymmetry (FA) is one possible manifestation of such a stress, and is often taken as a proxy for individual fitness. To test the usefulness of FA in morphological traits as an indicator of environmental quality, we studied the effect of urbanisation on FA in ground beetles (Carabidae) near a Danish city. First, we performed a critical examination whether morphological character traits suggested in the literature displayed true fluctuating asymmetry in three common predatory ground beetles, *Carabus nemoralis, Nebria brevicollis* and *Pterostichus melanarius*. Eight metrical (length of the second and third antennal segments, elytral length, length of the first tarsus segment, length of the first and second tibiae, length of the proximal and distal spines on the first femurs) and one meristic (the number of spines on the second tibiae) traits were examined. Most of them showed FA but not consistently. Females generally displayed a higher level of FA than males. Finally, we examined the changes in the level of FA in bilateral morphological traits along an urbanisation gradient (forest - suburban forest - forest fragments in urban park) to test whether environmental stress created by urbanisation is reflected in FA. Ground beetles common along a Danish urbanisation gradient did not seem to indicate differences in habitat quality by their level of FA.

Abbreviation: FA- fluctuating asymmetry; ANOVA- analysis of variance; R- right; L- left; ME- measurement error

Nomenclature: Hurka, 1996

Introduction

Urban ecosystems are formed after the “colonising” human populations profoundly transform the original habitat; they remain under consistent and pervasive anthropogenic influence (McIntyre et al. 2001). During the process of urbanisation, many of the original plant and animal species disappear or become rare (Marzluff et al. 2001), and are often replaced by non-native species (Kowarik 1995, Blair 1996, 2004). The filters and drivers of this profound rearrangement are only partially known; and we do not yet have a detailed understanding of the effects of urbanisation on biodiversity nor its functioning.

At the assemblage level, the effects of human activities on the abundance, species richness and diversity are not always negative (Magura et al. 2010a,b). Modified urban landscapes might have increased habitat diversity, resulting in higher species richness than in the less disturbed and more rural sites (Eversham et al. 1996, Elek and Lövei 2007). Habitat specialist species (i.e. forest specialists) are more affected by the urbanisation than generalists, and decline in urban habitats (Magura et al. 2010a).

One of the most sophisticated body of evidence concerning the effects of urbanisation on invertebrate diversity arises from the Globenet Project (Niemelä et al. 2000) that uses a uniform methodology in terms of study group, experimental design and sampling regime. However, the focus of these studies is at supra-individual level with their inevitable time constraints:effects on populations and assemblages need one or more generations to unfold (Magura et al. 2008). However, organisms can react to conditions in their habitats at lower organisational levels, including behavioural or physiological characteristics, which are more immediate. One such potentially useful measure reflects particular changes experienced during the individual developmental process (Parsons 1992). Several morphological traits are symmetrical, and theoretically should display perfectly identical halves. Deviations from this ideal leads to different types of asymmetry (Palmer and Strobeck 1986, 1992). One of these, the fluctuating asymmetry (FA) is a measure of developmental stability of an organism, and refers to random and subtle deviations from perfect bilateral symmetry (Palmer and Strobeck 1986, 1992). FA can increase when environmental stress disturbs developmental processes that normally promote symmetrical growth (Floate and Fox 2000), and as it is easy to measure, FA was suggested as a cost-effective indicator of environmental quality or stress (Valentine et al. 1973, Clarke and Ridsdill-Smith 1990, Clarke 1993, 1994). The applicability of FA to assess habitat quality has been widely discussed (Clarke 1995, Møller 1995, Møller and Thornhill 1997, Leung and Forbes 1996, Leung et al. 2000, Van Dongen and Lens 2000), but remains controversial. While a wide range of organisms and characters show increased FA due to exposure to a variety of environmental stressors (Hendrickx et al. 2003, Labrie et al. 2003, Weller and Ganzhorn 2004, Vilisics et al. 2005, Garnier et al. 2006, Henriquez et al. 2009), in some cases, the approach seems unsatisfactory (Floate and Coghlin 2010).

To extend the methodological toolkit examining the effects of urbanisation, we aimed to test the usefulness of FA in bilateral traits to signal habitat quality in ground beetles living in forested habitats in different urbanisation stages. First, we examined which of the commonly suggested morphological characters showed fluctuating (or true) asymmetry in ground beetles. Second, we examined the behaviour of these characters, whether these showed consistency (*sensu* Soulé 1967) in their reaction to the different levels of disturbance. Furthermore, we tested whether females were more sensitive than males to changes in habitat quality (Bots et al. 2009). Females need more resources than males for reproduction, because they have to successfully form and lay fertile eggs. Fitness in females is often size-related, and is profoundly influenced by conditions during larval development (McCabe and Partridge 1997). We hypothesised that females will be more sensitive than males to changes in habitat quality, stress or disturbance during their larval development, resulting in higher levels of FA as adults. This hypothesis would predict a higher level of FA in females than males. Finally, we tested if common ground beetle species showed a higher level of FA in more urbanised habitats than in the original, rural one. This is a version the *Habitat Disturbance Hypothesis*

(Gray 1989), but our response parameter is different from Gray's. We assumed that habitat quality was highest in the forested area. With progressing urbanisation, general conditions for forest-associated species deteriorate (Magura et al. 2010b). However, for some species, conditions may remain favourable, and they can have a lower level of FA in urbanised habitats as hypothesised by Weller & Ganzhorn (2004).

We found that several (but not all) of the suggested morphological characters showed FA, but not all of these showed consistency. Females had a higher level of FA than males, but in general, the ground beetles studied did not show a consistent increase in FA (at least under Danish conditions) as urbanisation intensified.

Material and methods

Study area and sampling design

Our study area was in and around the town of Sorø a regional centre about 80 km west of Copenhagen, on the island of Zealand. The city arose from a medieval monastery established in 1140 in this extensively forested area, and was gradually carved out of the original forest. The chosen study habitat was dominated by beech (*Fagus sylvatica*) , of which there are still extensive stands, as well as parcels of different size, and isolation in and around the town. The *rural stage* of the urbanisation gradient was represented by this forest, in parts now under non-intensive forestry management. The forested area extended to the shores of Lake Sorø, and continued for several kms in all directions. An area of 6000ha is under the ownership of the Sorø Akademi, the foundation currently managing the area. Our selected sites started ca. 3 km from the current town centre. There were only a few houses, with dirt access roads in the forest; covering < 3% of the total area. The *suburban stage* was represented by suburbia positioned northeast of the town centre, with an old cemetery, a weekend garden allotment zone, an old ditch, a dirt road and a marsh with trees. The edge of this area was ca. 1 km from the edge of the Sorø Akademi park complex. The built-up area and covered road surface in this urbanisation stage was approximately 20%. The understory indicated nitrogen-rich soil (plenty of nettle, *Urtica dioica*). The *urban stage* was represented by forest patches in the park complex of the Sorø Akademi. The park is "softly" managed: there are only gravel paths, rotting logs are usually left in place, and the mown grass, litter and cut branches are returned to the understory of the forested patches; there is no fertilisation, and no use of herbicides (which is forbidden in public areas in Denmark). The built-up area was about 40%; the forest patches occupied about 15% of the park. The park complex was isolated: on one side, by Sorø Lake, on the other, by the city centre. At each urbanisation stage, four sites were selected, with 10 pitfall traps per site, according to the Globenet protocol (see Niemelä et al. 2000). In the urban (park) stage, traps were set only in the forest patches, and at least 5 m from an edge (as well as min. 10 m from each other). Due to these size and inter-trap distance constraints, no more than 5 traps were set in one forest patch. Here, we selected four pairs of forest patches; which were always closer to each other than the distance to the next pair of patches, at least by a factor of two.

The traps were operated every second fortnight, starting in April and ending in mid-October, 2005 (Sapia et al. 2006). Further details about the sampling, handling of material and identification are in Elek and Lövei (2005).

From the overall catch (details in Elek and Lövei 2005, 2007), we selected species that a) were common in the forested area, and b) min. 10 individuals were trapped in at least two of the three urbanisation stages during the first half of the season. Three predatory carabids fulfilled these criteria: *Carabus nemoralis* Müller 1764, *Nebria brevicollis* (F. 1792), and *Pterostichus melanarius* (Illiger, 1783). We selected individual beetles randomly from the catch in April - June 2005: 137 individuals of *C. nemoralis* (56 males, 81 females), 152 individuals of *N. brevicollis* (21 males, 131 females), and 176 individuals of *P. melanarius* (100 males, 76 females). This sample size was adequate to test FA differences (Smith et al.

1982, Palmer 1994). The selected beetles were kept at -18ºC in individual containers until measurements were taken.

The measurement of all morphological traits involved two independent mounting and photographing of the specimens (Palmer 1994). Before taking measurements, beetles were thawed, and individually put into a plastic Petri dish filled with small (diameter 0.08 mm) glass beads which allowed to arrange them into a standard, horizontal position using a plastic gauge. Beetles were placed under a stereomicroscope (Nikon SMZ 800, 10-63x magnification) mounted with a digital camera (Nikon Coolpix 4500); two photographs were taken using identical aperture and exposure, at maximum resolution (2272×1704 pixels, in JPEG format). These digital photos were used to measure (precision of 0.0001 mm) the studied morphological traits, with the assistance of the software ImageJ (Rasband 2003). We measured morphological characters used in earlier studies. These included eight metrical and one meristic characters (Fig.1):

- the length of the second (*antsegment2*) and third (*antsegment3*) segments of the left and right antennae (Labrie et al. 2003)
- the lengths of the left and right elytrae (*elytra*, Weller and Ganzhorn 2004)
- lengths of the first tarsi (*tarsus1*), the first (*tibia1*) and second tibiae (*tibia2*) on both sides (modified after Garnier et al. 2006)
- the distance between the end of the femur and the proximal (*proxi*) and distal (*distal*) spines on the two first femurs (adapted from Hendrickx et al. 2003) the number of spines on the second tibia (*spine*, Labrie et al. 2003)

Data analysis

Data analysis followed the procedure recommended by Palmer (1994). The measured specimens were grouped according to urbanisation stage (rural, suburban, urban) and sex (female, male).

The detection of outliers in the dataset is an important issue in the beginning of the analyses to control biases such as measurement error, directional- or antisymmetry. We estimated outliers by the Grubb and Dixon's test (corrected for small sample sizes) with the use of scatterplot for the average trait size. This protocol was applied for the raw data as well as for the estimated FA data. When consistency occurred in the two approaches, the data of that individual (a total of eight *C. nemoralis*, five *N. brevicollis* and four *P. melanarius*) were removed from the dataset. In order to exclude the developmental noise based on genomic stress, we tested if any of the traits showed directional asymmetry or antisymmetry. Directional asymmetry, (when bilateral variance is statistically significant among the sides, but the direction of the difference is consistent, i.e. the left side is consistently larger than the right one), was tested by factorial ANOVA for the overall samples and nested ANOVA (sides within individuals) for individuals as samples and for the estimation of skewness. Antisymmetry (when bilateral variance is statistically significant among the sides, but this variation is larger between the sides than between individuals, i.e. platykurtosis), was tested by calculating kurtosis. The true asymmetry (free from any biases) of a morphological trait was confirmed by the Shapiro test for normality. An estimate of measurement error (ME) is essential for FA analyses (Palmer 1994). Measurements of each trait were compared using a two-way analysis of variance (ANOVA) with sides (right versus left) and individuals as factors. These tests measured whether non-directional asymmetry (i.e., FA, antisymmetry) could be distinguished from ME (Palmer 1994). If the heterogeneity occur in ME (as in our case, see Table S4 in the Appendix), it should be partitioned out in order to properly estimate the degree of FA (Palmer 1994).

We tested the size dependence of FA within samples by a linear regression of the absolute difference of Right vs. Left (|R – L|) values against the elytral length as an independent measure of body size.

We calculated a size-corrected and unsigned FA index (FA = |R – L|/mean [(R + L)/2], Vilisics et al. 2005 after Palmer 1994), where the unsigned difference is divided by the sample mean of the average trait size, where the sample referred to the combination of urbanisation stages and sexes . General linear mixed-effect models were used to study the relationship between the response variables (FA), and the supposed explanatory variables (the urbanisation stages and sex, as well as their interaction) (GLMM, Bolker et al. 2009). We added a nested random effect to account for the variance structure among the replicates (measurements 1 and 2) and individuals, to control the heteroscedasticity (Table S4 in the Appendix) by measurement error: the individuals were nested in the replications. The differences among the levels of the tested factors (urbanisation stages, sexes) were evaluated by multiple comparisons (with Tukey computed contrast matrices for several multiple comparisons procedures) after a single argument ANOVA for the tested model. For the meristic trait, we used the G-test (log-likelihood ratio test) with Williams' correction to calculate the differences in the number of spines (i.e. frequencies) between the right and left side of the second tibia, using the R-scripts by Hurd (2010). When more than two morphological traits showed real asymmetry, a test of consistency in FA values was performed using the Kendall concordance analysis with a Bonferroni correction (Légendre 2005, Palmer, 1994). We estimated the model parameters by using *nlme* (Pinheiro et al. 2012). Graphs were created using the *gplots* package (Warnes 2012) in R 2.15.2 (R Development Core Team 2012).

Results

Usefulness of morphological traits for asymmetry studies

Seven of the examined characters showed real FA in at least one of the selected carabid species (Table 1), but none of them was universally suitable. *antsegment2* and *antsegment3* showed directional asymmetry in one and three species respectively; *elytra*, *proxi, distal*, *tarsus1* showed antisymmetry in two species, while *tibia1* for one species (Table 1). The *tibia2* showed antisymmetry and direction asymmetry for one species each. The character *spine* did not show any variation, and was unsuitable as a potential FA character.

Consistency of the asymmetry profiles within species

The analysis of concordance for *C. nemoralis* showed that there was no relationship among the traits showing real asymmetry (W=0.15, p=0.63). The test of the contribution of the traits to the overall concordance (Table 2) revealed that the asymmetries of the studied morphological traits were independent of each other.

Assessment of fluctuating asymmetry among different species, urbanisation stages and sexes

Asymmetry for proximal distance of spines on the femur in *C. nemoralis* were significantly influenced by the interaction of the urbanisation stage and sex (Tables 3 and S1). The degree of the asymmetry for forest females was lower than for females in the urban forest fragment (T_{169}=2.269, p=0.024, Fig. 2). The asymmetry of the second tibia length was different by urbanisation stage only (Table 3), and was higher in the forest than in the urban forest fragments (T_{169}=-1.67, p=0.096, Fig. 2).

In the case of *N. brevicollis*, the asymmetry of the first tibia was higher (T_{277}=8.70, p=0.000) in females than males. Females in suburban and urban sites had higher asymmetry (T_{277}=-2.30, p=0.02; T_{277}=-2.56, p=0.01 respectively) than males, while the forest males were unaffected (i.e. confidence interval included zero, Fig.3). The interaction of sex and urbanisation stage was also significant (Tables 3 and S2).

For *P. melanarius*, the asymmetry for first tarsus length was significantly influenced by the urbanisation stage; the interaction of urbanisation stage and sex was marginally significant (Tables 3 and S3). The FA value for males was higher in the suburban stage (T_{245}=2.081, p=0.038) than either in the forest or urban forest fragment (Fig. 4).

Discussion

Usefulness of morphological traits for FA studies

Most of the published FA studies (Hendrickx et al. 2003, Labrie et al. 2003, Garnier et al. 2006, Henriquez et al. 2009) were performed on a single species, using one or several closely related traits. The relationship among the selected traits was not always analysed. We found that seven out of nine morphological traits showed true asymmetry in one or more of the common carabid species at our study site. The character *tibia2* showed directional and antisymmetry in one species each, similar to another European ground beetle, *Carabus soleri* (Garnier et al. 2006). This suggests that this type of asymmetry may have a genetic background. Two morphological traits (*antsegment2, tibia1*) that showed real asymmetry in two studied species could be useful in further FA studies.

Sex-specific differences in fluctuating asymmetry

Fitness in females is often size-related, and is profoundly influenced by conditions during larval development (McCabe and Partridge 1997). Most of the published studies on FA (Hendrickx et al. 2003, Labrie et al. 2003, Weller and Ganzhorn 2004, Garnier et al. 2006) did not consider sex as a factor in their analysis. Vilisics et al. (2005) and Henríquez et al. (2009) included this factor, but found no effects in isopods and carabids, respectively. In our study, *C. nemoralis* and in *N. brevicollis* females were more asymmetric than males, indicating that females could indeed be more sensitive than males to disturbance.

Fluctuating asymmetry and urbanisation

Higher developmental instability in more urbanised habitats was reported from Northern Germany (Weller and Ganzhorn 2004) and this can be considered support for the *Habitat Disturbance Hypothesis* (Gray 1989). Although FA in the length of the *tibia2* in *C. nemoralis* showed differences among the studied urbanisation stages, this asymmetry was higher in the forest than in the other urbanisation stages , while *tarsus1* showed lower FA values in the urban stage than elsewhere for *P. melanarius*. Finding similar inconsistencies, Weller and Ganzhorn (2004) speculated that habitat size and the degree of isolation could play a role in this phenomenon. Weller and Ganzhorn (2004) also suggest that the species that occur at all studied sites are more "robust" and are less affected by urbanisation-related stress than species which do not occur at all studied sites. If this suggestion is correct, the less common species, in general, should show a higher degree of FA than the common ones, which remains to be tested. We did not find unequivocal support for the hypothesis that as urbanization progresses, the conditions create higher levels of FA in ground beetles present in more than one urbanization stage. This makes it difficult to suggest that measuring FA is useful to indicate environmental disturbance in ground beetles. Females showed a higher level of FA than males, which warrants the separation of sexes in further studies of this type. The theoretical advantages using within-individual characters as markers of habitat quality are still upheld, but the question whether measuring FA in carabids is useful in this context remains open.

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Table 1. Performance of nine morphological characters as potential measures of fluctuating asymmetry in the carabids *Carabus nemoralis, Nebria brevicollis and Pterostichus melanarius* collected in pitfall traps at Sorø, Denmark, during spring 2005.

Morphological trait	Type of asymmetry		
	Carabus nemoralis	*Nebria brevicollis*	*Pterostichus melanarius*
antsegment2	fluctuating	directional (R<L)	fluctuating
antsegment3	directional (R<L)	directional (R<L)	directional (R<L)
elytra	antisymmetry	fluctuating	antisymmetry
proxi	fluctuating	antisymmetry	antisymmetry
distal	fluctuating	antisymmetry	antisymmetry
tarsus1	antisymmetry	antisymmetry	fluctuating
tibia1	fluctuating	fluctuating	antisymmetry
tibia2	fluctuating	antisymmetry	directional (R<L)
spine	no variation	no variation	no variation

Table 2. Summary table of concordance analysis in *Carabus nemoralis,* collected in pitfall traps at Sorø, Denmark, during spring 2005. For the species, the result of global analysis is given, for traits, the posterior test of the contribution of individual judges (morphological traits) to the overall concordance. Legend: W - Kendall's coefficient of concordance; p- permutational probabilities, uncorrected; cor.-p.= permutational probabilities corrected, calculated only if there are more than one group.

Morphological trait	Concordance/congruity (W=0.15, p=0.63)
antsegment2	W=0.16/ p=0.54/cor.-p. =1
antsegment3	no true asymmetry detected
elytra	no true asymmetry detected
proxi	0.12/0.85/1
distal	0.17/0.44/1
tarsus1	no true asymmetry detected
tibia1	0.13/0.86/1
tibia2	0.16/0.53/1

Table 3. Results of linear mixed-effect models relating urbanisation stages and sexes to fluctuating asymmetry in ground beetles collected in pitfall traps at Sorø, Denmark, spring 2005. Significant values are in bold.

Morphological trait/species	*d.f.*	*F*	*p*
Carabus nemoralis			
antsegment2			
intercept	**1, 169**	**402.372**	**<0.0001**
Site	2, 169	0.285	0.751
Sex	1, 169	0.121	0.727
Site:Sex	2, 169	2.261	0.106
sd (random effect residuals)			0.048
proxi			
intercept	**1, 169**	**369.468**	**<0.0001**
Site	2, 169	1.133	0.324
Sex	1, 169	0.454	0.501
Site:Sex	**2, 169**	**3.449**	**0.034**
sd (random effecs residuals)			0.08
distal			
intercept	**1, 169**	**317.067**	**<0.0001**
Site	2, 169	0.245	0.782
Sex	1, 169	1.169	0.281
Site:Sex	2, 169	0.784	0.458
sd (random effect residuals)			0.049
tibia1			
intercept	**1, 169**	**408.3**	**<0.0001**
Site	2, 169	0.369	0.691
Sex	1, 169	0.007	0.931
Site:Sex	2, 169	0.937	0.393
sd (random effect residuals)			0.017
tibia2			

intercept	**1, 169**	**352.753**	**<0.0001**
Site	**2, 169**	**4.011**	**0.019**
Sex	1, 169	2.673	0.103
Site:Sex	2, 169	1.396	0.25
sd (random effect residuals)			0.013
Nebria brevicollis			
elytra			
intercept	**1, 187**	**453.599**	**<0.0001**
Site	2, 187	1.235	0.292
Sex	1, 187	1.712	0.191
Site:Sex	2, 187	0.671	0.512
sd (random effect residuals)			0.006
tibia1			
Intercept	**1, 187**	**285.639**	**<0.0001**
Site	2, 187	2.292	0.102
Sex	**1, 187**	**4.296**	**0.039**
Site:Sex	**2, 187**	**3.334**	**0.037**
sd (random effect residuals)			0.027
Pterostichus melanarius			
antsegment2			
Intercept	**1, 245**	**460.24**	**<0.0001**
Site	2, 245	1.629	0.198
Sex	1, 245	0.059	0.808
Site:Sex	2, 245	2.194	0.113
sd (random effect residuals)			0.088
tarsus1			
Intercept	**1, 245**	**489.739**	**<0.0001**
Site	**2, 245**	**4.578**	**0.011**
Sex	1, 245	1.887	0.17
Site:Sex	2, 245	2.419	0.091
sd (random effect residuals)			0.09

Figure legends

Fig. 1. The method of measurement of the studied morphological traits. Abbreviations: *antsegment2, antsegment3*- the length of the second, and third segments of the antennae respectively; *elytra* - the length of the elytrae; *tarsus1*- length of the first tarsi; *tibia1, tibia2* – the length the first and second tibiae respectively; *proxi, distal*- the distance between the end of the femur and the proximal and distal spines respectively on the first femur; *spine* - the number of spines on the second tibia. Drawings modified from Aukema (1998) and Csiki (1908).

Fig. 2. Distribution of asymmetry index for *proxi* and the *tibia2* in male and female *Carabus nemoralis* in the studied urbanisation stages in Sorø, Denmark. Data are means; vertical lines indicate ± 95% confidence intervals.

Fig. 3. Distribution of asymmetry index for the *tibia1* in male and female *Nebria brevicollis* in the studied urbanisation stages in Sorø, Denmark. Data are means; vertical lines indicate ± 95% confidence intervals.

Fig. 4. Distribution of asymmetry index for *antsegment2* and *tarsus1* in male and female *Pterostichus melanarius* in the studied urbanisation stages in Sorø, Denmark. Data are means; vertical lines indicate ± 95% confidence intervals.

Fig. 1. (top)

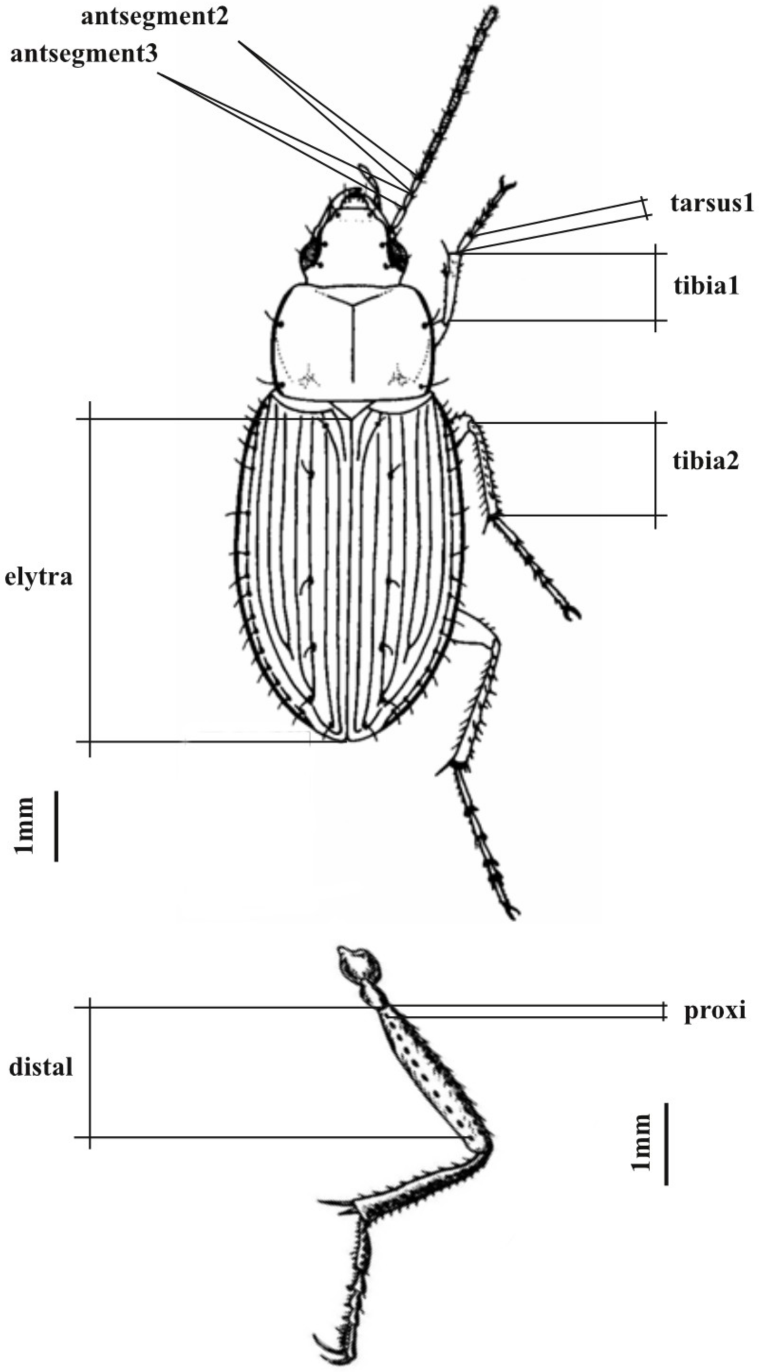

antsegment2
antsegment3
tarsus1
tibia1
tibia2
elytra
1mm
proxi
distal
1mm

Fig. 2. (top)

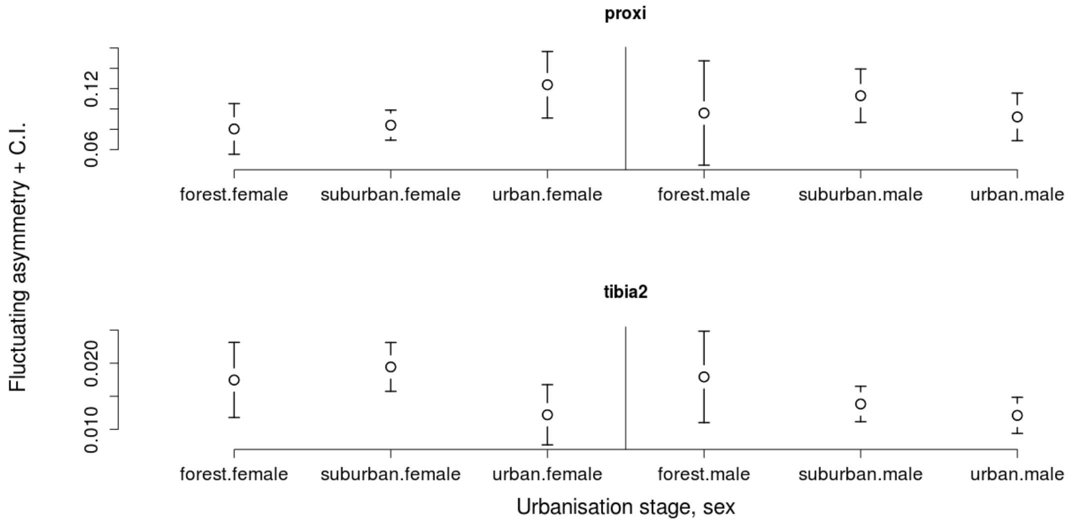

proxi
0.06
0.12
forest.female
suburban.female
urban.female
forest.male
suburban.male
urban.male
tibia2
0.010
0.020
forest.female
suburban.female
urban.female
forest.male
suburban.male
urban.male
Fluctuating asymmetry + C.I.
Urbanisation stage, sex

Fig. 3. (top)

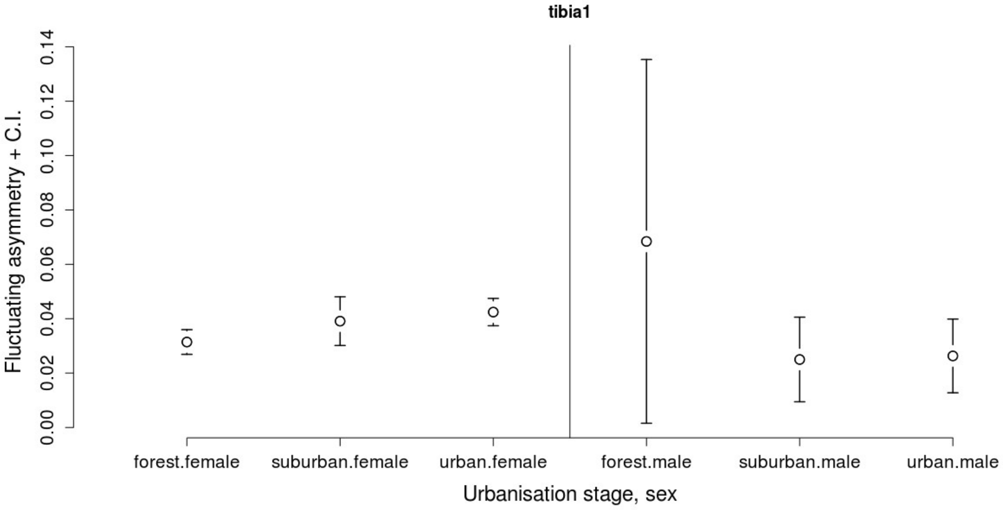
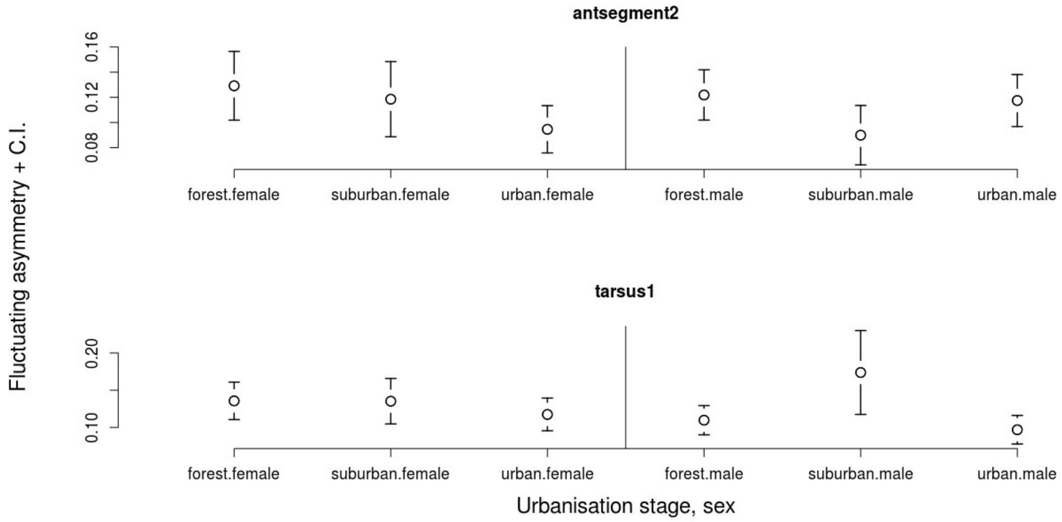

tibia1
Fluctuating asymmetry + C.I.
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
forest.female
suburban.female
urban.female
forest.male
suburban.male
urban.male
Urbanisation stage, sex

Fig. 4. (top)


antsegment2
0.08
0.12
0.16
forest.female
suburban.female
urban.female
forest.male
suburban.male
urban.male
tarsus1
0.10
0.20
forest.female
suburban.female
urban.female
forest.male
suburban.male
urban.male
Fluctuating asymmetry + C.I.
Urbanisation stage, sex