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

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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.

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

ME- measurement error

Nomenclature: Hurka, 1996

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49   Introduction

50   Urban ecosystems are formed after the "colonising" human populations profoundly

51   transform the original habitat; they remain under consistent and pervasive anthropogenic

52   influence (McIntyre et al. 2001). During the process of urbanisation, many of the original

53   plant and animal species disappear or become rare (Marzluff et al. 2001), and are often

54   replaced by non-native species (Kowarik 1995, Blair 1996, 2004). The filters and drivers of

55   this profound rearrangement are only partially known; and we do not yet have a detailed

56   understanding of the effects of urbanisation on biodiversity nor its functioning.

57   At the assemblage level, the effects of human activities on the abundance, species

58   richness and diversity are not always negative (Magura et al. 2010a,b). Modified urban

59   landscapes might have increased habitat diversity, resulting in higher species richness than in

60   the less disturbed and more rural sites (Eversham et al. 1996, Elek and Lövei 2007). Habitat

61   specialist species (i.e. forest specialists) are more affected by the urbanisation than generalists,

62   and decline in urban habitats (Magura et al. 2010a).

63   One of the most sophisticated body of evidence concerning the effects of urbanisation

64   on invertebrate diversity arises from the Globenet Project (Niemelä et al. 2000) that uses a

65   uniform methodology in terms of study group, experimental design and sampling regime.

66   However, the focus of these studies is at supra-individual level with their inevitable time

67   constraints:effects on populations and assemblages need one or more generations to unfold

68   (Magura et al. 2008). However, organisms can react to conditions in their habitats at lower

69   organisational levels, including behavioural or physiological characteristics, which are more

70   immediate. One such potentially useful measure reflects particular changes experienced

71   during the individual developmental process (Parsons 1992). Several morphological traits are

72   symmetrical, and theoretically should display perfectly identical halves. Deviations from this

73   ideal leads to different types of asymmetry (Palmer and Strobeck 1986, 1992). One of these,

74   the fluctuating asymmetry (FA) is a measure of developmental stability of an organism, and

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75   refers to random and subtle deviations from perfect bilateral symmetry (Palmer and Strobeck

76   1986, 1992). FA can increase when environmental stress disturbs developmental processes

77   that normally promote symmetrical growth (Floate and Fox 2000), and as it is easy to

78   measure, FA was suggested as a cost-effective indicator of environmental quality or stress

79   (Valentine et al. 1973, Clarke and Ridsdill-Smith 1990, Clarke 1993, 1994). The applicability

80   of FA to assess habitat quality has been widely discussed (Clarke 1995, Møller 1995, Møller

81   and Thornhill 1997, Leung and Forbes 1996, Leung et al. 2000, Van Dongen and Lens 2000),

82   but remains controversial. While a wide range of organisms and characters show increased FA

83   due to exposure to a variety of environmental stressors (Hendrickx et al. 2003, Labrie et al.

84   2003, Weller and Ganzhorn 2004, Vilisics et al. 2005, Garnier et al. 2006, Henriquez et al.

85   2009), in some cases, the approach seems unsatisfactory (Floate and Coghlin 2010).

86   To extend the methodological toolkit examining the effects of urbanisation, we aimed

87   to test the usefulness of FA in bilateral traits to signal habitat quality in ground beetles living

88   in forested habitats in different urbanisation stages. First, we examined which of the

89   commonly suggested morphological characters showed fluctuating (or true) asymmetry in

90   ground beetles. Second, we examined the behaviour of these characters, whether these

91   showed consistency (*sensu* Soulé 1967) in their reaction to the different levels of disturbance.

92   Furthermore, we tested whether females were more sensitive than males to changes in habitat

93   quality (Bots et al. 2009). Females need more resources than males for reproduction, because

94   they have to successfully form and lay fertile eggs. Fitness in females is often size-related,

95   and is profoundly influenced by conditions during larval development (McCabe and Partridge

96   1997). We hypothesised that females will be more sensitive than males to changes in habitat

97   quality, stress or disturbance during their larval development, resulting in higher levels of FA

98   as adults. This hypothesis would predict a higher level of FA in females than males. Finally,

99   we tested if common ground beetle species showed a higher level of FA in more urbanised

100 habitats than in the original, rural one. This is a version the *Habitat Disturbance Hypothesis*

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101 (Gray 1989), but our response parameter is different from Gray's. We assumed that habitat

102 quality was highest in the forested area. With progressing urbanisation, general conditions for

103 forest-associated species deteriorate (Magura et al. 2010b). However, for some species,

104 conditions may remain favourable, and they can have a lower level of FA in urbanised

105 habitats as hypothesised by Weller & Ganzhorn (2004).

106   We found that several (but not all) of the suggested morphological characters showed

107 FA, but not all of these showed consistency. Females had a higher level of FA than males, but

108 in general, the ground beetles studied did not show a consistent increase in FA (at least under

109 Danish conditions) as urbanisation intensified.

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111 Material and methods

112 *Study area and sampling design*

113   Our study area was in and around the town of Sorø a regional centre about 80 km west

114 of Copenhagen, on the island of Zealand. The city arose from a medieval monastery

115 established in 1140 in this extensively forested area, and was gradually carved out of the

116 original forest. The chosen study habitat was dominated by beech (*Fagus sylvatica*) , of which

117 there are still extensive stands, as well as parcels of different size, and isolation in and around

118 the town. The *rural stage* of the urbanisation gradient was represented by this forest, in parts

119 now under non-intensive forestry management. The forested area extended to the shores of

120 Lake Sorø, and continued for several kms in all directions. An area of 6000ha is under the

121 ownership of the Sorø Akademi, the foundation currently managing the area. Our selected

122 sites started ca. 3 km from the current town centre. There were only a few houses, with dirt

123 access roads in the forest; covering < 3% of the total area. The *suburban stage* was

124 represented by suburbia positioned northeast of the town centre, with an old cemetery, a

125 weekend garden allotment zone, an old ditch, a dirt road and a marsh with trees. The edge of

126 this area was ca. 1 km from the edge of the Sorø Akademi park complex. The built-up area

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127   and covered road surface in this urbanisation stage was approximately 20%. The understory

128   indicated nitrogen-rich soil (plenty of nettle, *Urtica dioica*). The *urban stage* was represented

129   by forest patches in the park complex of the Sorø Akademi. The park is "softly" managed:

130   there are only gravel paths, rotting logs are usually left in place, and the mown grass, litter

131   and cut branches are returned to the understory of the forested patches; there is no

132   fertilisation, and no use of herbicides (which is forbidden in public areas in Denmark). The

133   built-up area was about 40%; the forest patches occupied about 15% of the park. The park

134   complex was isolated: on one side, by Sorø Lake, on the other, by the city centre. At each

135   urbanisation stage, four sites were selected, with 10 pitfall traps per site, according to the

136   Globenet protocol (see Niemelä et al. 2000). In the urban (park) stage, traps were set only in

137   the forest patches, and at least 5 m from an edge (as well as min. 10 m from each other). Due

138   to these size and inter-trap distance constraints, no more than 5 traps were set in one forest

139   patch. Here, we selected four pairs of forest patches; which were always closer to each other

140   than the distance to the next pair of patches, at least by a factor of two.

141   The traps were operated every second fortnight, starting in April and ending in mid-

142   October, 2005 (Sapia et al. 2006). Further details about the sampling, handling of material and

143   identification are in Elek and Lövei (2005).

144   From the overall catch (details in Elek and Lövei 2005, 2007), we selected species that

145   a) were common in the forested area, and b) min. 10 individuals were trapped in at least two

146   of the three urbanisation stages during the first half of the season. Three predatory carabids

147   fulfilled these criteria: *Carabus nemoralis* Müller 1764, *Nebria brevicollis* (F. 1792), and

148   *Pterostichus melanarius* (Illiger, 1783). We selected individual beetles randomly from the

149   catch in April - June 2005: 137 individuals of *C. nemoralis* (56 males, 81 females), 152

150   individuals of *N. brevicollis* (21 males, 131 females), and 176 individuals of *P. melanarius*

151   (100 males, 76 females). This sample size was adequate to test FA differences (Smith et al.

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152   1982, Palmer 1994). The selected beetles were kept at -18°C in individual containers until

153   measurements were taken.

154   The measurement of all morphological traits involved two independent mounting and

155   photographing of the specimens (Palmer 1994). Before taking measurements, beetles were

156   thawed, and individually put into a plastic Petri dish filled with small (diameter 0.08 mm)

157   glass beads which allowed to arrange them into a standard, horizontal position using a plastic

158   gauge. Beetles were placed under a stereomicroscope (Nikon SMZ 800, 10-63x

159   magnification) mounted with a digital camera (Nikon Coolpix 4500); two photographs were

160   taken using identical aperture and exposure, at maximum resolution (2272×1704 pixels, in

161   JPEG format). These digital photos were used to measure (precision of 0.0001 mm) the

162   studied morphological traits, with the assistance of the software ImageJ (Rasband 2003). We

163   measured morphological characters used in earlier studies. These included eight metrical and

164   one meristic characters (Fig.1):

165   -   the length of the second (*antsegment2*) and third (*antsegment3*) segments of the

166   left and right antennae (Labrie et al. 2003)

167   -   the lengths of the left and right elytrae (*elytra*, Weller and Ganzhorn 2004)

168   -   lengths of the first tarsi (*tarsus1*), the first (*tibia1*) and second tibiae (*tibia2*) on

169   both sides (modified after Garnier et al. 2006)

170   -   the distance between the end of the femur and the proximal (*proxi*) and distal

171   (*distal*) spines on the two first femurs (adapted from Hendrickx et al. 2003) the

172   number of spines on the second tibia (*spine*, Labrie et al. 2003)

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175   *Data analysis*

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176   Data analysis followed the procedure recommended by Palmer (1994). The measured

177   specimens were grouped according to urbanisation stage (rural, suburban, urban) and sex

178   (female, male).

179   The detection of outliers in the dataset is an important issue in the beginning of the

180   analyses to control biases such as measurement error, directional- or antisymmetry. We

181   estimated outliers by the Grubb and Dixon's test (corrected for small sample sizes) with the

182   use of scatterplot for the average trait size. This protocol was applied for the raw data as well

183   as for the estimated FA data. When consistency occurred in the two approaches, the data of

184   that individual (a total of eight *C. nemoralis*, five *N. brevicollis* and four *P. melanarius*) were

185   removed from the dataset. In order to exclude the developmental noise based on genomic

186   stress, we tested if any of the traits showed directional asymmetry or antisymmetry.

187   Directional asymmetry, (when bilateral variance is statistically significant among the sides,

188   but the direction of the difference is consistent, i.e. the left side is consistently larger than the

189   right one), was tested by factorial ANOVA for the overall samples and nested ANOVA (sides

190   within individuals) for individuals as samples and for the estimation of skewness.

191   Antisymmetry (when bilateral variance is statistically significant among the sides, but this

192   variation is larger between the sides than between individuals, i.e. platykurtosis), was tested

193   by calculating kurtosis. The true asymmetry (free from any biases) of a morphological trait

194   was confirmed by the Shapiro test for normality. An estimate of measurement error (ME) is

195   essential for FA analyses (Palmer 1994). Measurements of each trait were compared using a

196   two-way analysis of variance (ANOVA) with sides (right versus left) and individuals as

197   factors. These tests measured whether non-directional asymmetry (i.e., FA, antisymmetry)

198   could be distinguished from ME (Palmer 1994). If the heterogeneity occur in ME (as in our

199   case, see Table S4 in the Appendix), it should be partitioned out in order to properly estimate

200   the degree of FA (Palmer 1994).

16

201   We tested the size dependence of FA within samples by a linear regression of the

202   absolute difference of Right vs. Left ($|R - L|$) values against the elytral length as an

203   independent measure of body size.

204   We calculated a size-corrected and unsigned FA index (FA = $|R - L|$/mean [(R + L)/2],

205   Vilisics et al. 2005 after Palmer 1994), where the unsigned difference is divided by the sample

206   mean of the average trait size, where the sample referred to the combination of urbanisation

207   stages and sexes . General linear mixed-effect models were used to study the relationship

208   between the response variables (FA), and the supposed explanatory variables (the urbanisation

209   stages and sex, as well as their interaction) (GLMM, Bolker et al. 2009). We added a nested

210   random effect to account for the variance structure among the replicates (measurements 1 and

211   2) and individuals, to control the heteroscedasticity (Table S4 in the Appendix) by

212   measurement error: the individuals were nested in the replications. The differences among the

213   levels of the tested factors (urbanisation stages, sexes) were evaluated by multiple

214   comparisons (with Tukey computed contrast matrices for several multiple comparisons

215   procedures) after a single argument ANOVA for the tested model. For the meristic trait, we

216   used the G-test (log-likelihood ratio test) with Williams' correction to calculate the differences

217   in the number of spines (i.e. frequencies) between the right and left side of the second tibia,

218   using the R-scripts by Hurd (2010). When more than two morphological traits showed real

219   asymmetry, a test of consistency in FA values was performed using the Kendall concordance

220   analysis with a Bonferroni correction (Légendre 2005, Palmer, 1994). We estimated the model

221   parameters by using *nlme* (Pinheiro et al. 2012). Graphs were created using the *gplots*

222   package (Warnes 2012) in R 2.15.2 (R Development Core Team 2012).

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224

225   Results

226   *Usefulness of morphological traits for asymmetry studies*

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227   Seven of the examined characters showed real FA in at least one of the selected

228   carabid species (Table 1), but none of them was universally suitable. *antsegment2* and

229   *antsegment3* showed directional asymmetry in one and three species respectively; *elytra,*

230   *proxi, distal, tarsus1* showed antisymmetry in two species, while *tibia1* for one species (Table

231   1). The *tibia2* showed antisymmetry and direction asymmetry for one species each. The

232   character *spine* did not show any variation, and was unsuitable as a potential FA character.

233

234   *Consistency of the asymmetry profiles within species*

235   The analysis of concordance for *C. nemoralis* showed that there was no relationship among

236   the traits showing real asymmetry (W=0.15, p=0.63). The test of the contribution of the traits

237   to the overall concordance (Table 2) revealed that the asymmetries of the studied

238   morphological traits were independent of each other.

239

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

241   Asymmetry for proximal distance of spines on the femur in *C. nemoralis* were

242   significantly influenced by the interaction of the urbanisation stage and sex (Tables 3 and S1).

243   The degree of the asymmetry for forest females was lower than for females in the urban forest

244   fragment (T_{169}=2.269, p=0.024, Fig. 2). The asymmetry of the second tibia length was

245   different by urbanisation stage only (Table 3), and was higher in the forest than in the urban

246   forest fragments (T_{169}=-1.67, p=0.096, Fig. 2).

247   In the case of *N. brevicollis*, the asymmetry of the first tibia was higher (T_{277}=8.70,

248   p=0.000) in females than males. Females in suburban and urban sites had higher asymmetry

249   (T_{277}=-2.30, p=0.02; T_{277}=-2.56, p=0.01 respectively) than males, while the forest males were

250   unaffected (i.e. confidence interval included zero, Fig.3). The interaction of sex and

251   urbanisation stage was also significant (Tables 3 and S2).

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252   For *P. melanarius*, the asymmetry for first tarsus length was significantly influenced

253   by the urbanisation stage; the interaction of urbanisation stage and sex was marginally

254   significant (Tables 3 and S3). The FA value for males was higher in the suburban stage

255   (T_{245}=2.081, p=0.038) than either in the forest or urban forest fragment (Fig. 4).

256

257   Discussion

258   *Usefulness of morphological traits for FA studies*

259   Most of the published FA studies (Hendrickx et al. 2003, Labrie et al. 2003, Garnier et al.

260   2006, Henriquez et al. 2009) were performed on a single species, using one or several closely

261   related traits. The relationship among the selected traits was not always analysed. We found

262   that seven out of nine morphological traits showed true asymmetry in one or more of the

263   common carabid species at our study site. The character *tibia2* showed directional and

264   antisymmetry in one species each, similar to another European ground beetle, *Carabus soleri*

265   (Garnier et al. 2006). This suggests that this type of asymmetry may have a genetic

266   background. Two morphological traits (*antsegment2, tibia1*) that showed real asymmetry in

267   two studied species could be useful in further FA studies.

268

269   *Sex-specific differences in fluctuating asymmetry*

270   Fitness in females is often size-related, and is profoundly influenced by conditions

271   during larval development (McCabe and Partridge 1997). Most of the published studies on FA

272   (Hendrickx et al. 2003, Labrie et al. 2003, Weller and Ganzhorn 2004, Garnier et al. 2006) did

273   not consider sex as a factor in their analysis. Vilisics et al. (2005) and Henríquez et al. (2009)

274   included this factor, but found no effects in isopods and carabids, respectively. In our study,

275   *C. nemoralis* and in *N. brevicollis* females were more asymmetric than males, indicating that

276   females could indeed be more sensitive than males to disturbance.

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278 *Fluctuating asymmetry and urbanisation*

279   Higher developmental instability in more urbanised habitats was reported from

280 Northern Germany (Weller and Ganzhorn 2004) and this can be considered support for the

281 *Habitat Disturbance Hypothesis* (Gray 1989). Although FA in the length of the *tibia2* in *C.*

282 *nemoralis* showed differences among the studied urbanisation stages, this asymmetry was

283 higher in the forest than in the other urbanisation stages , while *tarsus1* showed lower FA

284 values in the urban stage than elsewhere for *P. melanarius*. Finding similar inconsistencies,

285 Weller and Ganzhorn (2004) speculated that habitat size and the degree of isolation could play

286 a role in this phenomenon. Weller and Ganzhorn (2004) also suggest that the species that

287 occur at all studied sites are more "robust" and are less affected by urbanisation-related stress

288 than species which do not occur at all studied sites. If this suggestion is correct, the less

289 common species, in general, should show a higher degree of FA than the common ones, which

290 remains to be tested. We did not find unequivocal support for the hypothesis that as

291 urbanization progresses, the conditions create higher levels of FA in ground beetles present in

292 more than one urbanization stage. This makes it difficult to suggest that measuring FA is

293 useful to indicate environmental disturbance in ground beetles. Females showed a higher level

294 of FA than males, which warrants the separation of sexes in further studies of this type. The

295 theoretical advantages using within-individual characters as markers of habitat quality are still

296 upheld, but the question whether measuring FA in carabids is useful in this context remains

297 open.

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423

424   Table 1. Performance of nine morphological characters as potential measures of fluctuating asymmetry in the carabids *Carabus nemoralis,*

425   *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

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429   Table 2. Summary table of concordance analysis in *Carabus nemoralis,* collected in pitfall

430   traps at Sorø, Denmark, during spring 2005. For the species, the result of global analysis is

431   given, for traits, the posterior test of the contribution of individual judges (morphological

432   traits) to the overall concordance. Legend: W - Kendall's coefficient of concordance; p-

433   permutational probabilities, uncorrected; cor.-p.= permutational probabilities corrected,

434   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

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437   Table 3. Results of linear mixed-effect models relating urbanisation stages and sexes to

438   fluctuating asymmetry in ground beetles collected in pitfall traps at Sorø, Denmark, spring

439   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			

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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

440   Figure legends

441

442   **Fig. 1.** The method of measurement of the studied morphological traits. Abbreviations:
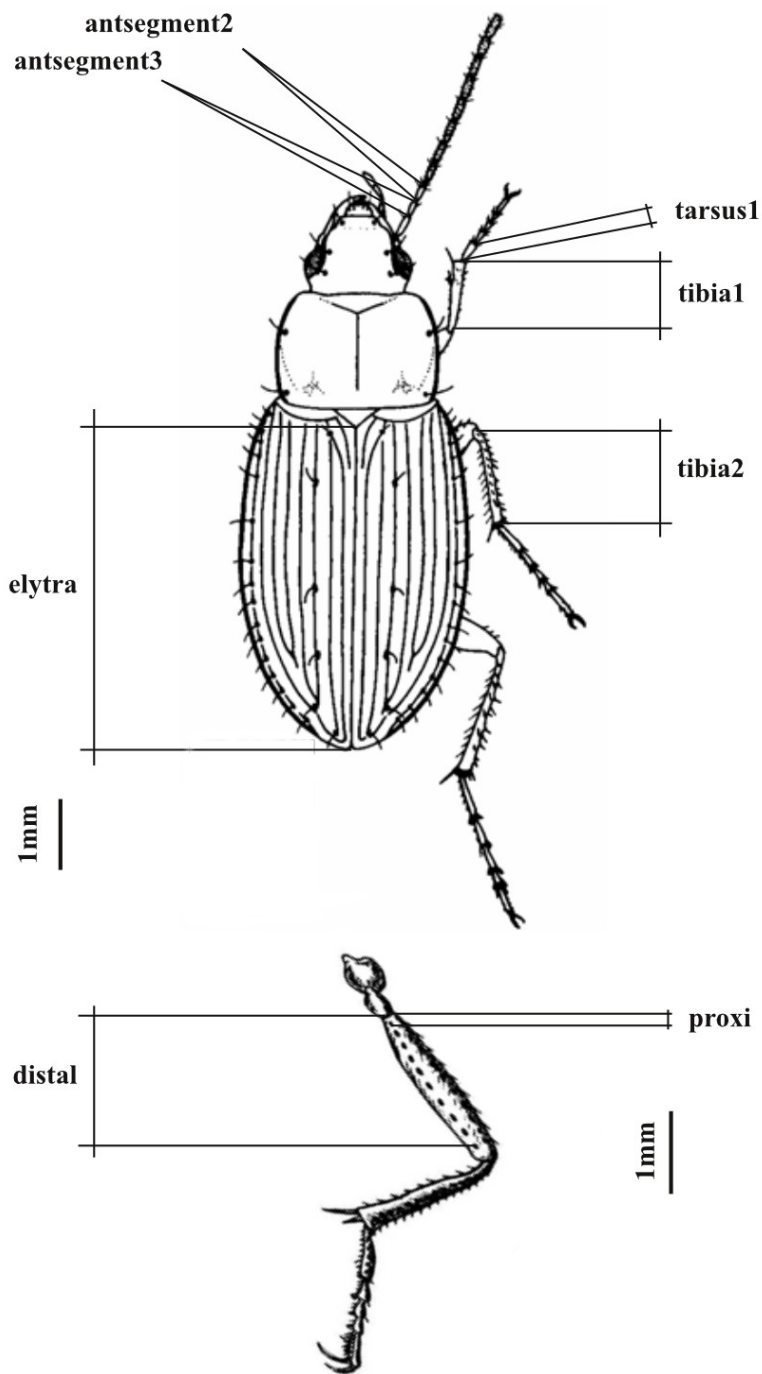
443   *antsegment2, antsegment3*- the length of the second, and third segments of the antennae

444   respectively; *elytra* - the length of the elytrae; *tarsus1*- length of the first tarsi; *tibia1,*

445   *tibia2* – the length the first and second tibiae respectively; *proxi, distal*- the distance

446   between the end of the femur and the proximal and distal spines respectively on the first

447   femur; *spine* - the number of spines on the second tibia. Drawings modified from

448   Aukema (1998) and Csiki (1908).

449   **Fig. 2.** Distribution of asymmetry index for *proxi* and the *tibia2* in male and female *Carabus*

450   *nemoralis* in the studied urbanisation stages in Sorø, Denmark. Data are means; vertical

451   lines indicate ± 95% confidence intervals.

452   **Fig. 3.** Distribution of asymmetry index for the *tibia1* in male and female *Nebria brevicollis*

453   in the studied urbanisation stages in Sorø, Denmark. Data are means; vertical lines

454   indicate ± 95% confidence intervals.

455   **Fig. 4.** Distribution of asymmetry index for *antsegment2* and *tarsus1* in male and female

456   *Pterostichus melanarius* in the studied urbanisation stages in Sorø, Denmark. Data are

457   means; vertical lines indicate ± 95% confidence intervals.

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458
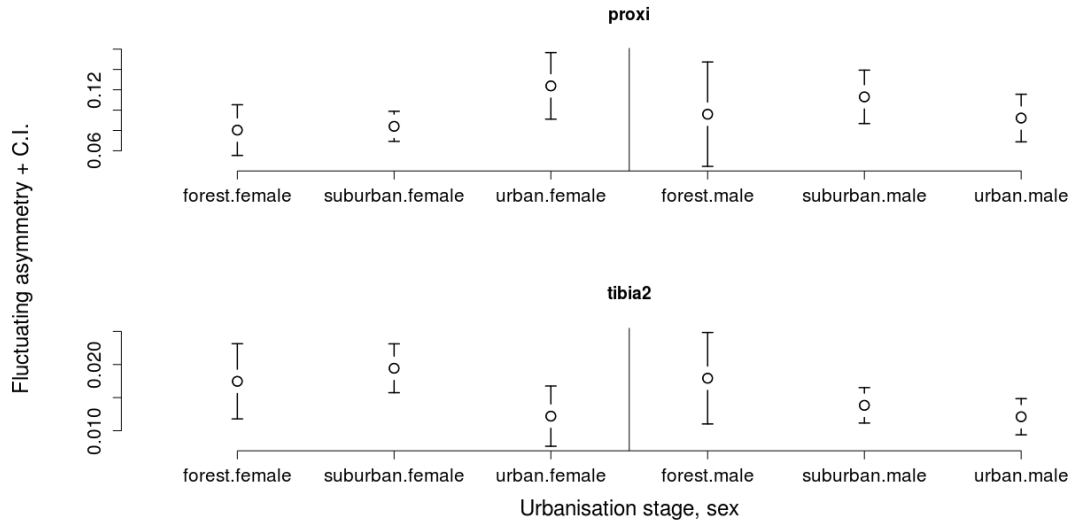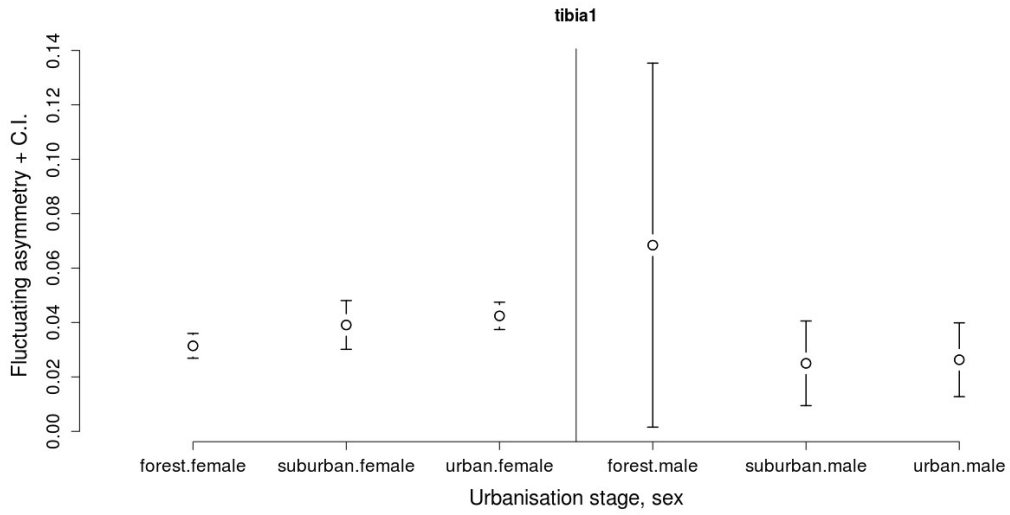459   Fig. 1. (top)

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

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461
462
463   Fig. 2. (top)
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466

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468   Fig. 3. (top)
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470

tibia1

Fluctuating asymmetry + C.I. — Urbanisation stage, sex

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473   Fig. 4. (top)

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Fluctuating asymmetry + C.I.

Urbanisation stage, sex

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