Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Soil Biology 41 (2005) 85–90
EUROPEAN JOURNAL OF SOIL BIOLOGY
www.elsevier.com/locate/ejsobi

Original article

Measuring fluctuating asymmetry of the terrestrial isopod *Trachelipus rathkii* (Crustacea: Isopoda, Oniscidea)

Ferenc Vilisics *, Péter Sólymos, Elisabeth Hornung

Department of Ecology, Institute for Zoology, Faculty of Veterinary Science, Szent István University, H-1400 Budapest, P.O. Box 2, 1400 Budapest, Hungary

Available online 11 October 2005

Abstract

Studies on fluctuating asymmetry (FA) in soil invertebrates, particularly in isopods, are almost absent so far. As a result, methodology of measuring FA in isopods is little known. However, the ecological importance of these animals in decomposition processes and the fact that FA in soil invertebrates might serve as an indicator of environmental stress, e.g. soil pollution, make the topic worth for investigation. Our results revealed that woodlice are potentially able to signal environmental stresses via FA. We compared six traits (length of three segments of antennae, two segments of the seventh legs and the number of ocelli) between two populations (Hungary: Hajós, Blaskovics-puszta) and genders of the species *Trachelipus rathkii* living under different environmental stresses (i.e. changing humidity). Asymmetry was significantly higher in the Hajós population, where the soil moisture has changed dramatically for the last years. Asymmetry was lower in Blaskovics-puszta, where the habitat has remained constant during the last centuries and the population had time to adapt to seasonally changing environments. Gender did not have an effect on FA. Traits differed significantly from each other, thus it is advisable to use several traits simultaneously to detect FA. FA was higher in the a1 segment of the antennal flagellae than in the ischium of the pereiopod and ocelli. We also studied the effect of regeneration of lost body parts on the FA values. Asymmetry in the treated groups was significantly higher than in the control ones. This calls the attention to the problem of outliers, which may be caused by physical harm and can lead to artificial results.

Keywords: Trachelipus rathkii; Environmental stress; Asymmetry; Sodic grassland; Gallery forest; Hungary

1. Introduction

Fluctuating asymmetry (FA) quantifies the random and non-directional deviation from symmetry. Despite controversial discussion (e.g. [12,17]), it is widely used as a measure of developmental stability of bilateral organisms [18,28] because of the apparent ease with which it can be measured. It either shows no change or it increases with increasing extrinsic (environmental) or intrinsic (mainly genetic) stress [5,20,21]. Chemical or biological pollutants, changes in microclimate, soil parameters, diseases, lack of food and often human perturbation proved to be factors of stress that led to a higher level of FA [3,4,6,22]. High levels of environmental stress result in more reliable relationships between FA and the presumed developmental stability than low levels. Thus, "lower quality" individuals have to face the difficulty of sufficient energy allocation to maintain the necessary levels of developmental stability and that may occur in higher levels of FA [12].

Contrary to the increasing body of studies of FA, only few works have been done on isopods (i.e. L. Tuerlinckx, unpublished [22]). Peters et al. [22] investigated FA in the number of ocelli of isopod populations living on heavy metal contaminated soils. He concluded that FA was higher in populations of higher heavy metal concentrations. These results indicate that isopods might be good subjects for such studies. These saprophagous ground dwelling arthropods are poten-

* Corresponding author. Fax: +36 1 478 4232.
E-mail address: Vilisics.Ferenc@aotk.szie.hu (F. Vilisics).

1164-5563/$ - see front matter
doi:10.1016/j.ejsobi.2005.09.003

tially able to signal environmental stresses (e.g. contamination) via FA.

We aimed to examine the reliability of measuring FA in isopods based on several metrical (length of antennal and leg segments) and meristic (number of ocelli) traits. We evaluated the congruence of the FA in these traits. We analysed the FA values in two populations of the isopod species *Trachelipus rathkii* (Brandt, 1833) living under different environmental stresses. We tested whether FA varies among habitats and sexes. We also studied the regeneration of lost body parts to evaluate the influence of possible predation/injury effects on FA values.

2. Materials and methods

2.1. Study area and sampling

The target species, *T. rathkii*, is common in Hungary [7]. It occurs mainly in river valleys, and moderately humid habitats [26]. There are data on the species' persistence in synanthropic environment as well [8,11]. We sampled *T. rathkii* specimens by pitfall traps filled with diluted ethylene-glycol in the year 1998, in two habitats.

The first habitat was a drying riparian forest near Hajós village (UTM grid-cell: XP71) in the Szigetköz region (North-West Hungary). It had been one of the more extended intact areas of Szigetköz, where the seasonally flood of the Danube played a major role in the maintenance of natural plant associations. The studied gallery forest (*Carduo crispi–Populetum nigrae* and *Leucojo aestivi–Salicetum albae*) is considered an edaphic plant association, and the general structure is determined by soil quality and groundwater level. Natural vegetation has changed radically, and ruderal vegetation has emerged since the drainage caused by the barrage system built on the Danube in 1992 [9,10]. As a result, our sampling site is now characterised by dry upper soil layer with vanishing arboreal vegetation.

The second habitat was a sodic grassland (*Artemisio-Festucetum pseudovinae*) near Blaskovics-puszta (UTM grid-cell: DS61) (authority of the Kőrös-Maros National Park). It is primary alkaline grassland on solonetz-type soil located at the south-eastern part of the Great Hungarian Plain. Soil moisture shows extreme fluctuations due to seasonal weather changes. The climate is mainly continental with wet winters and springs, followed by warm and dry summers. Draining of soil during the arid seasons causes secondary alkalinisation. The plant association is dominated by generalist monocots (e.g. *Festuca pseudovina*). The sampling area has remained almost intact for the last 200 years [13,14].

2.2. Hypotheses

We hypothesised that the stress appearing as radical and rather fast irreversible environmental change at Hajós, caused a higher level of FA in isopods compared with the stress operating in an intact and constantly harsh environment at Blaskovics-puszta.

We presumed higher FA in isopods of the gallery forest suffering from the direct and indirect effects of radical and fast changes in groundwater level. Therefore, woodlice living in the sodic grassland should exhibit low levels of FA, as a result of presumable adaptation to low pH and seasonal extremities of rainfall and soil moisture.

2.3. Data acquisition and measurement

For measurement we used specimens that were collected from the two sampling sites in May and June 1998. Codes of antennal segments were given according to Schmölzer [25]. In our preliminary analysis, we measured the length of the exo- and endopodites of uropods, ischiopodites and meropodites of seventh pair of pereiopods, the antennulae, the aIII, aIV and aV segments of antennae, a1 and a2 segments of flagellae to find the most reliable traits for detecting FA in isopods. We also measured the width of the head capsule as an independent measure of body size to test size dependence in the data. We found that the measurement of the lengths of aI and aII antennal segments, the antennulae, the basipodits and carpopodits were problematic, due to their size and shape. Thus, these traits were excluded from further analyses.

The width of the head capsule, the length of aV, a1 and a2 antennal segments (Fig. 1a), ischiopodits and the meropodits (Fig. 1b), and the number of ocelli (Fig. 1c) proved to be repeatable, and we used these traits in the present analysis. Because the measurement of the a2 flagellal segment includes the apical seta as well, care must be taken not to break it during dissection. The arrangement of ocelli is quite regular (Fig. 1c), thus counting them can be done with high precision. After dissection we took pictures of the objects with a Nikon Coolpix 4500 digital camera assembled to a Nikon SMZ800 stereomicroscope. To maintain the original length and shape of the object during handling we always kept the material wet. The measurement of the body parts was performed by the ImageJ morphometrical software [23].

2.4. Data analysis

During data analysis, we followed the work of Palmer [16]. The measured specimens were grouped according to habitat (Hajós versus Blaskovics-puszta) and gender. Right (R) and left (L) values of each trait of 117 individuals were measured. The number of individuals varied from 21 to 43 among the groups (habitat × gender). This sample size was adequate to test FA differences based on our preliminary results. We tested for directional asymmetry by one-sample t-test. The departure of the mean ($R - L$) from an expected mean of zero was not significant (alpha = 0.05) in either character, except for the a1 antennal segment in the Blaskovicspuszta—female group ($P = 0.038$). The distribution of the ($R - L$) values fol-

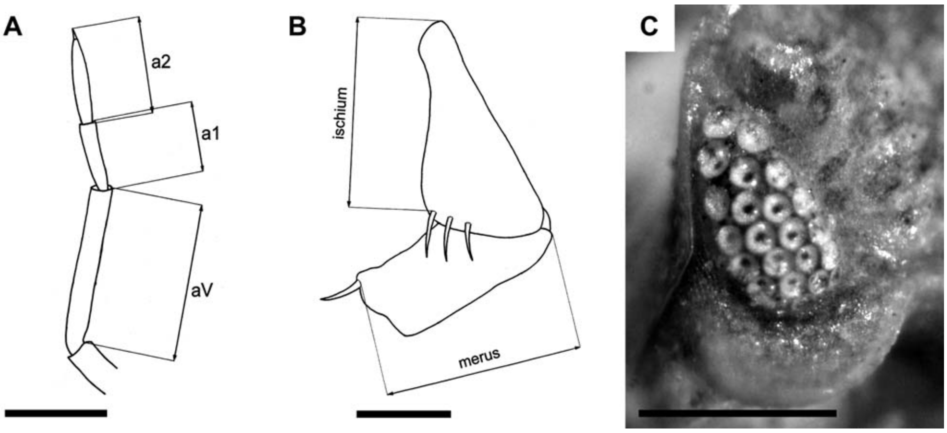

Fig. 1. Measurement of the trait values of which FA was calculated in the species *T. rathkii*: (a) lengths of antennal segments, (b) lengths of leg segments, (c) number of ocelli. Scales bar indicates 0.5 mm.

lowed roughly normal distribution. Outliers of the field data set were excluded from the analyses due to possible effects of injuries (see Section 2.5 for explanation).

We tested the size dependence of FA within samples by linear regression of the absolute deviation of $R - L$ ($|R - L|$) values against the head width as an independent measure of body size. Only the number of ocelli exhibited significant dependence on head width (slope of regression = 0.406, $r^2 = 0.165$, $F = 4.345$, df = 1, 22, $P < 0.05$). We detected significant size differences among samples (head width, one-way ANOVA, $F = 62.5$, df = 3, 153, $P < 0.001$) and the size dependence among samples was significant according to the log [variance($R - L$)] vs. mean [($R + L$)/2] plots. Based on these results we calculated size corrected and unsigned FA index (FA = $|R - L|$/mean [$(R + L)/2$]) as suggested by Palmer [16], where the unsigned difference is divided by the sample mean of the average trait size.

We tested differences in FA by general linear model (GLM) with repeated measure structure treating traits as repeated measures, and habitat and gender as fixed factors. This multivariate model reflects the number of individuals rather than the number of traits by individuals, thus pseudoreplication is avoided [12]. For traits we used estimated marginal means from GLM for pairwise multiple comparisons, and significance levels were corrected by the false discovery rate (FDR, the expected proportion of false discoveries among discoveries) method, proved to better compromise between type-I and -II errors [1,2].

2.5. Regeneration experiment

The full or partial loss of certain body parts due to physical harm (i.e. predation) influences the asymmetry in the individuals. We tested experimentally the rate of asymmetry in a lab population of *T. rathkii* after regeneration of fully lost body parts. In two treated groups (10 females and 10 males, each), we removed the right antenna or the right seventh pereiopod, while members of the control groups (10 females and 10 males, each) remained intact. The animals were kept under tempered conditions (temperature: 20 °C, relative humidity: 95%) until the subsequent moult. We compared unsigned asymmetry values ($|R - L|$) in the four groups by GLM with repeated measure structure, treating traits as repeated measures and gender and treatment as fixed factors. For traits we used estimated marginal means from GLM for pairwise multiple comparisons, and significance levels were corrected by the FDR method.

3. Results

3.1. Field populations

We found significant differences in the FA values between the two habitats (Table 1 and Fig. 2a), the estimated marginal mean of FA was significantly higher in the Hajós population

Table 1
Results of the GLM analysis of FA in the studied isopod field populations, treating traits as repeated measures, and habitat and gender as fixed factors[a]

Source of variation	df	SS	MS	*F*
Between-subject effects				
Intercept	1	1.6207	1.6207	182.37***
Habitat (H)	1	0.0393	0.0393	4.43*
Gender (G)	1	0.0025	0.0025	0.29 ns
Interaction: H × G	1	0.0052	0.0052	0.59 ns
Error	113	1.0042	0.0089	
Within-subject effects				
Trait (T)	1	0.1418	0.1418	4.38*
Interaction: T × H	1	0.0298	0.0298	0.92 ns
Interaction: T × G	1	0.0089	0.0089	0.27 ns
Interaction: T × H × G	1	0.0213	0.0213	0.66 ns
Error (T)	113	3.6540	0.0323	

***: $P < 0.001$, *: $P < 0.05$, ns: not significant.

[a] FA = $|R - L|$/mean[$(R + L)/2$], where the unsigned difference is divided by the sample mean of the average trait size.

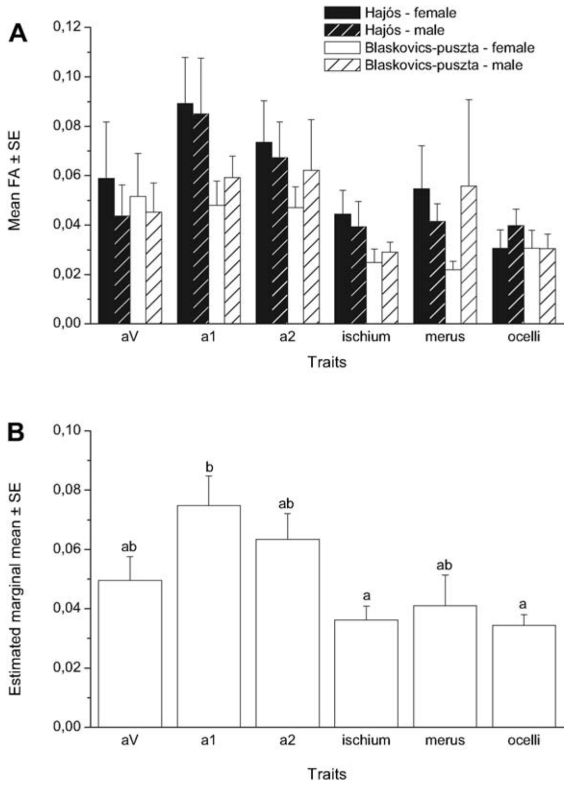

Fig. 2. Between (a) and within (b) subject differences of the size corrected unsigned asymmetry (FA = |*R* – *L*|/mean[(*R* + *L*)/2]) values in the measured traits (aV: fifth antennal segment; a1: first segment of flagellum; a2: second segment of flagellum) of the field populations. Marginal means are estimated from GLM. Different letters indicate significant (*P* < 0.05) differences, original *P*-values were corrected by FDR correction due to multiple comparisons.

(0.058 ± 0.005 S.E.) than in the Blaskovics-puszta population (0.042 ± 0.005 S.E.). Differences were not significant concerning gender and interaction of habitat and gender (Table 1). The a1 antennal segment possessed significantly higher FA than ischium and ocelli (Fig. 2b).

3.2. Regeneration experiment

In the regeneration experiment, differences were not significant among sexes, and the effect of treatment was significant (Table 2 and Figs. 3a and 4). Estimated marginal mean of FA was significantly higher in the treated group (0.203 ± 0.01 S.E.) than in the control (0.036 ± 0.01 S.E.). FA owing to the removal of body parts resulted in significantly higher asymmetry in the trait aV than in other traits, and FA were significantly lower in ischium and merus compared with other traits (Figs. 3b and 4).

4. Discussion

We measured six traits in two Hungarian populations (Hajós and Blaskovics-puszta) of the isopod species *T. rathkii* living under different environmental stress. Our results revealed that the a1, a2 and aV segment of the antennal flagellum, and the meropodits and ischiopodits of the seventh legs were suitable for measuring FA. All the analysed traits

Table 2
Results of the GLM analysis of FA (FA = |*R* – *L*|) in the regeneration experiment, treating traits as repeated measures, and gender and treatment as fixed factors

Source of variation	df	SS	MS	*F*
Between-subject effects				
Intercept	1	2.8608	2.8608	268.43***
Gender (G)	1	0.0038	0.0038	0.36 ns
Treatment (M)	1	1.3978	1.3978	131.16***
Interaction: G × M	1	0.0056	0.0056	0.53 ns
Error	36	0.3837	0.0107	
Within-subject effects				
Trait (T)	1	0.6422	0.6422	25.55***
Interaction: T × G	1	0.0161	0.0161	0.64 ns
Interaction: T × M	1	0.5510	0.5510	21.92***
Interaction: T × G × M	1	0.0204	0.0204	0.81 ns
Error (T)	36	0.9048	0.0251	

***: *P* < 0.001, *: *P* < 0.05, ns: not significant.

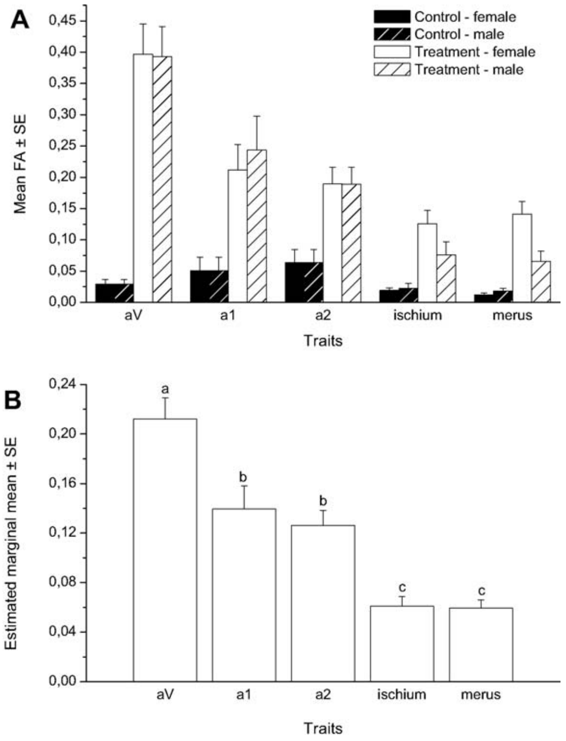

Fig. 3. Between (a) and within (b) subject differences of the unsigned asymmetry values (|*R* – *L*|) in the measured traits (aV: fifth antennal segment; a1: first segment of flagellum; a2: second segment of flagellum) of the regeneration experiment. Marginal means are estimated from GLM. Different letters indicate significant (*P* < 0.05) differences, original *P*-values were corrected by FDR correction due to multiple comparisons.

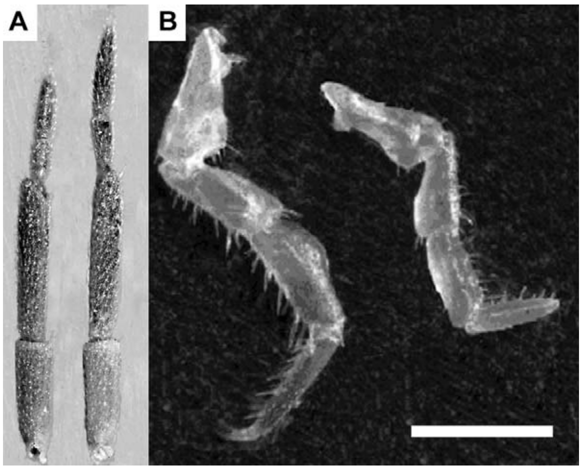

Fig. 4. Asymmetry in the (a) antennae and the (b) seventh pair leg of individuals of *T. rathkii* after regeneration of the removed body parts (smaller body parts were previously removed, other body parts were intact). Scale bar indicates 1 mm.

showed consistent results in terms of differences in FA within the two habitats. Although traits exhibited different levels of FA: a1 proved to be the most asymmetric, and ischium and number of ocelli showed the lowest FA values. Thus it is advisable to use several traits simultaneously to detect FA. Contrary to Peters et al. [22], we can not suggest the number of ocelli as a unique measure of FA.

The results supported our hypothesis that isopods of the draining gallery forest suffered from a higher environmental stress than those of the sodic grassland. This may be due to the direct and indirect effects of the rapidly sinking ground water level. Although we have no knowledge of isopod food preferences in ruderal environment, we assumed that changes in vegetation have led to negative changes in food supplies as well.

The sodic grassland in Blaskovics-puszta also provided harsh environmental conditions to isopods, however, similar vegetation has been existing in the area since the 18th century [13]. Isopod populations could have been adapted to these conditions resulting in a lower level of FA.

Research both on invertebrates and vertebrates proved that asymmetries of bilateral body parts or basically symmetrical traits play an important role in mate choice [15,17,24,27]. Antennae, as the most important sensory organ of woodlice and the seventh pair of legs may have function in mate choice in isopods. Thus, the level of their FA may influence isopod populations through sexual interactions.

The regeneration study of lost body parts revealed that asymmetry in the treated group was significantly higher than in the control one. Physical harm can lead to increased FA values appearing as outliers in the data. Since environmental stress has general impact on the whole organism, asymmetries can occur in all bilateral traits. Contrary, predatory injury may cause significant asymmetry in a single body part. The effect of physical harm on isopods and the relationship between moulting and regeneration needs further investigation.

Because terrestrial isopods comprise a significant part of soil dwelling faunas [19], we suggest that the study of FA in field populations can serve as a reliable indicator of environmental stress and pollution.

Acknowledgements

Two anonymous referees are thanked for their valuable comments and suggestions on an earlier version of the manuscript. We are grateful to H. Read (London, UK) for language revision. This research was supported by the grant of the Hungarian Scientific Research Fund (OTKA T 043508).

References

[1] Y. Benjamini, D. Drai, G. Elmer, N. Kafkafi, I. Golani, Controlling the false discovery rate in behavior genetics research, Behav. Brain Res. 125 (2001) 279–284.

[2] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. Roy. Statist. Soc. Ser. B. Methodological 27 (1995) 289–300.

[3] E.A.J. Bleeker, A.L. Heather, D. Groenendijk, M. Plans, W. Admiraal, Effects of exposure to azaarenes on emergence and mouthpart development in the midge *Chironomus riparius* (Diptera: Chironomidae), Environ. Toxicol. Chem. 18 (1999) 1829–1834.

[4] G.M. Clarke, Fluctuating asymmetry of invertebrate populations as a biological indicator of environmental quality, Environ. Pollut. 82 (1997) 207–211.

[5] P. David, A. Hingle, K. Fowler, A. Pomiankowsky, Commentary-measurement bias and fluctuating asymmetry estimates, Anim. Behav. 57 (1999) 251–253.

[6] D. Groenendijk, L.W.M. Zeinstra, J.F. Postma, Fluctuating asymmetry and mentum gaps in populations of the midge, (Diptera: Chironomidae) from a metal-contaminated river, Environ. Toxicol. Chem. 17 (1998) 1999–2005.

[7] S. Farkas, Population dynamics, spatial distribution, and sex ratio of *Trachelipus rathkei* Brandt (Isopoda: Oniscidea) in a wetland forest by the Drava river, Isr. J. Zool. 44 (1998) 323–331.

[8] L. Forró, S. Farkas, Checklist, preliminary distribution maps, and bibliography of woodlice in Hungary (Isopoda: Oniscidea), Miscellanea Zoologica Hungarica 12 (1998) 21–44.

[9] B. Kevey, A Duna szlovákiai elterelésének hatása a Szigetköz növényvilágára. Die wasserwirtschaftlichen Probleme des Szigetköz vornehmlich mit Rücksicht auf die Waldvegetation, Nord-West-Ungarn, Moson Megyei Műhely 2 (2) (1999) 75–95.

[10] B. Kevey, A Duna szlovákiai elterelésének hatása a Felső-Szigetköz fehér nyárligeteire (*Senecioni sarracenici–Populetum albae* Kevey in Borhidi–Kevey 1996). Wirkung der slowakischen Donau-Umleitung auf die Weißpappel-Auen (*Senecioni sarracenici-Populetum albae*) in Felső-Szigetköz (in der Oberen-Schüttinsel), Süd-West-Ungarn, Kanitzia 12 (2004) 177–195.

[11] Z. Korsós, E. Hornung, K. Szlávecz, J. Kontschán, Isopoda and Diplopoda of urban habitats: new data to the fauna of Budapest, Ann. hist.-nat. Mus. natn. hung 94 (2002) 193–208.

[12] L. Lens, S. Van Dongen, K. Salit, E. Matthysen, Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between the studies? Biol. Rev. 77 (2002) 27–38.

[13] Z. Molnár, A Pitvarosi-puszták növénytakarója, különös tekintettel a löszpusztagyepekre, Bot. Közlem. 79 (1992) 19–27.

[14] Z. Molnár, A Pitvarosi-puszták és környékük vegetáció–és tájtörténete a középkortól napjainkig, Natura Bekesiensis 2 (1996) 65–97.

[15] A.P. Møller, C. Zamora-Muñoz, Antennal symmetry and sexual selection in a cerambycid beetle, Anim. Behav. 54 (1997) 1509–1515.

[16] A.R. Palmer, Fluctuating asymmetry analysis: A primer, in: T.A. Markow (Ed.), Developmental Instability, Its Origins and Evolutionary Implications, Kluwer, Dordrecht, Netherlands, 1994, pp. 335–364.

[17] A.R. Palmer, Notes and comments, detecting publication bias in meta-analyses: a case study of fluctuating asymmetry and sexual selection, Am. Nat. 154 (1999) 220–233.

[18] A.R. Palmer, C. Strobeck, Fluctuating asymmetry and developmental stability: heritability of observable variation vs. heritability of inferred cause, J. Evol. Biol. 10 (1997) 39–49.

[19] M.G. Paoletti, M. Hassall, Woodlice (Isopoda, Oniscidea): their potential for assessing sustainability and use as bioindicators, Agric. Ecosyst. Environ. 74 (1999) 157–165.

[20] P.A. Parsons, Fluctuating asymmetry: an epigenetic measure of stress, Biol. Rev. 65 (1990) 131–145.

[21] P.A. Parsons, Fluctuating asymmetry: a biological monitor of environmental and genomic stress, Heredity 68 (1992) 361–364.

[22] E.L. Peters, C.H. Jagoe, T.J. Bell, Effects of accumulation of hazardous metals on terrestrial isopod (*Armadillidium vulgare*) in urban environments. Abstracts of the 86. Annual meeting of the Ecological Society of America, Madison, WI (2001), pp. 329.

[23] W. Rasband, ImageJ—Image processing and analysis in Java. National Institutes of Health, USA, http://rsb.info.nih.gov/ij/ (accessed: 12.10.2003).

[24] P.A. Rohde, T. Amundsen, P. Fiske, Fluctuating asymmetry, mate choice and experimental designs, Anim. Behav. 54 (1997) 1030–1033.

[25] K. Schmölzer, in: Ordnung Isopoda, Lieferung 4 Akademie-Verlag, Berlin, 1965, pp. 12 (Landasseln).

[26] R. Snider, H. Shaddy, The ecobiology of *Trachelipus rathkii* (Isopoda), Pedobiologia (Jena) 20 (1980) 394–410.

[27] J.P. Swaddle, Experimental design and the signaling properties of fluctuating asymmetry, Anim. Behav. 54 (1997) 1034–1037.

[28] L. van Valen, A study of fluctuating asymmetry, Evolution 16 (1962) 125–142.