

Geographic and ontogenetic patterns of chaetotaxy variation in glacial relict *Saduria entomon* (L.) (Crustacea, Isopoda): inter-population, inter-individual and intra-individual variations (fluctuating asymmetry)

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We studied the variation in the number of chaetae of 15 characters in *Saduria entomon* (L.) (Crustacea, Isopoda) from the Baltic (2 samples), White, and south-eastern Barents Seas. The following parameters were analysed: (i) means, (ii) inter-individual variance, (iii) intra-individual or stochastic variance measured by fluctuating asymmetry, i.e. random deviations from perfect bilateral symmetry, which is a measure of developmental instability. The samples from the Baltic Sea were characterised by a greater number of chaetae than those from the White and Barents Seas in almost all characters. Inter-individual variance in the Barents Sea sample was lower than in the other samples, possibly because of low genetic variation. Fluctuating asymmetry was the least in the sample from the Baltic Sea (Gulf of Gdańsk), indicating that environmental conditions in this location are the most favourable. In juveniles the magnitude of the fluctuating asymmetry and its contribution to the total variance was higher than in adults.

Introduction

The distributional range of glacial relicts originates from a once continuous range, which was disrupted as a result of rapid climatic changes during the glacial period. Because of the young age of populations, differences between them have resulted mostly from adaptations to local environmental conditions since isolation and profound genetic differentiation is improbable.

Thus, geographically isolated populations of glacial relicts may represent a convenient model for microevolutionary studies (Sell 2003).

The isopod *Saduria entomon* (Linnaeus 1758) is one of the most common glacial relict animals in northern Europe. Its range of distribution includes the Baltic Sea, the White Sea (the Severnaya Dvina and Mezen' River estuaries), the Pechora River estuary, the coasts of northern Siberia as far as the Bering Strait, and then

southwards as far as the northern Sea of Japan. *S. entomon* also occurs along the coasts of North America from Alaska down to central California, and to the east as far as Labrador. It inhabits inland water bodies such as the Caspian Sea, Swedish relict lakes and Lake Ladoga (Grunner 1965, Kusakin 1979, Haahtela 1990).

Saduria entomon occurs at different depths, from 5 m (some coastal populations) down to 225 m in the Baltic Sea. Its maximum depth of occurrence in the Baltic Sea is limited by an oxygen concentration of at least 1 mg l^{-1} (Piechota 1973). The species lives in all kinds of bottoms that are not too hard for it to dig into, but prefers soft mud or sand. It can also swim effectively by beating its pleopods in an upside-down position. *S. entomon* is a predator, and its prey consists mainly of *Monoporeia affinis* (Amphipoda), also larvae of Chironomidae and other invertebrates, including its own species.

Saduria entomon is a stenothermic cold water species, but can survive for a short time at temperatures up to $15\text{--}20^\circ\text{C}$ (Haahtela 1990) and even 26°C (Kivivuori & Lagerspetz 1990). The salinity tolerance of *S. entomon* is quite high: even under the very constant low salinity (5‰–7‰) typical of the Baltic, it can survive for 12 days after sudden exposure within the whole salinity range 0‰–30‰, so long as the temperature is below 15°C (Hagerman & Szaniawska 1992). The most common salinities at which *S. entomon* occurs are 10‰–13‰ in the southwestern Baltic (Bogucki 1948) and 3‰–10‰ in the eastern parts of this sea (Järvekülg 1979).

Saduria entomon is therefore able to adapt to a broad spectrum of environmental conditions. Such adaptation should be reflected in the species' genetics, physiology and morphology. Morphological variation is expressed by variation in the mean values and variances of phenotypic characters. Mean values describe an "average individual" and are dependent on the population's gene pool and environmental conditions. The variance of individuals within a population is caused by several factors. Genotypic variation reflects the genetic differences among individuals. Macro-environmental variation is due to environmental variation between distinct environments. The micro-environmental component is due to relatively minor, and very

local, environmental variation. The ontogenetic component represents variation among different stages. These components of variation together apply to the heterogeneity of individuals within a population and can be referred to as the factorial component. This can, therefore, be used as an upper estimate of genotypic variation (Kozhara 1994, Lajus 2001, Lajus *et al.* 2003a).

There is, moreover, intra-individual variation, which is manifested in the differences between genetically identical structures that have developed under the same environmental conditions. This was first studied by Pearson (1901) and Astauroff (1930) and was called "stochastic variation" (Astauroff 1930). According to Astauroff, any process of formation in an organism has some self-dependent variation, which cannot be reduced either to genotypic differences, or to the direct effect of the environment (Astauroff 1930). Furthermore, Gärtner (1990) and Lajus *et al.* (2003a) argue that stochastic variation should be considered an independent component of phenotypic variation in the same way as the genotypic and environmental components.

At present, it is usually suggested that this kind of variation results from developmental instability, which reflects the incomplete ability of an organism to develop the same phenotype under the same environmental conditions. It is usually measured by fluctuating asymmetry, i.e. random deviations from perfect asymmetry (Graham *et al.* 2003). In this study we follow the many authors who, on the basis of experimental and observational data, argue that fluctuating asymmetry increases under genetic and environmental stress (Jones 1987, Leary & Allendorf 1989, Zakharov 1989, 1992, Parsons 1990, Graham *et al.* 1993, Leung & Forbes 1996, Møller & Swaddle 1997, Polak 2003). Genetic stress is a departure from genetic co-adaptation, that has resulted from inbreeding, outbreeding depression or other genetic effects. Environmental stress is caused by deviations from the environmental condition to which a population has adapted. But not all the authors agree with the utility of fluctuating asymmetry for stress assessment (Bjorsten *et al.* 2000a: see also comments on this paper by Møller 2000, Van Dongen & Lens 2000, and the reply by Bjorsten *et al.* 2000b). For contemporary reviews of the

problem, see Lens *et al.* (2002), Hoffman and Woods (2003), Zakharov (2003), and Leung *et al.* (2003).

Fluctuating asymmetry is necessary to distinguish from two other types of biological asymmetry: directional asymmetry — a pattern of variation of (*R-L*), where the variation is normally distributed about a mean that is significantly different from zero, and antisymmetry — a pattern of variation that is distributed about a mean of zero, but where the frequency distribution departs from normality in the direction of platykurtosis or bimodality (Palmer & Strobeck 2003).

Fluctuating asymmetry usually varies concordantly across characters (Soulé 1967, Zakharov 1987). However, in some cases, characters can show deviations from perfect concordance because of differences in the regression slope of fluctuating asymmetries on the means for different populations. Because of this, the use of many characters, allowing the parameters of variance-mean regression to be assessed, permits a more reliable judgement about a population's developmental stability (Lajus 1998, 2001).

The aim of this study is to describe phenotypic variation in *S. entomon* in relation to ontogenetic and geographical aspects. We selected chaetotaxy characters as a convenient system for this. The locations where we collected the samples differ considerably in their salinity and temperature regimes. We analyse how adaptation to these differences and isolation of populations during the post-glacial period is expressed in different parameters of phenotypic variation, such as fluctuating asymmetry (intra-individual variation), factorial variation (inter-individual variation) and means (inter-population variation).

Materials and methods

Environmental conditions in sampling locations

A brief description of sampling locations and conditions is given in Table 1. The living conditions of *S. entomon* in the Baltic Sea are quite well known, but the data on the Severnaya Dvina and Pechora river estuaries is very scant.

Table 1. Characteristics of environmental conditions in the sampling locations at the time of sampling and description of the samples based on Järvekülg 1979, Augustowski 1987, Majewski 1990, Maksimov 1997, Mikhailov 1997 (Gulf of Gdańsk and Gulf of Finland), Naumov and Fedijakov 1993 (Severnaya Dvina River estuary); and Hummel *et al.* 1999 (Pechora River estuary).

Location	Range of environmental conditions of usual occurrence			Conditions of sampling				Characteristics of samples								
	Depth (m)	<i>t</i> (°C)	<i>S</i> (%)	<i>S</i> (%)	Seasonal differences	Date	Depth (m)	<i>t</i> (°C)	<i>S</i> (%)	Sampling tool	Adult/ juveniles	<i>N</i>	Length (mm) (mean and ranges)	Sex ratio (♂:♀)	Mean length of ♂	Mean length of ♀
Gulf of Gdańsk	below 35	2–6	10–13	10–13	Small	Nov 1995	40	8	12	Dredge	Adults	30	4.76 (3.47–7.50)	8:22	5.97	4.32
Gulf of Finland	10–50	0–12	3–7	3–7	Moderate	Apr 1998	15	0	–	Gillnet	Adults	30	5.28 (3.94–6.39)	18:12	5.56	4.87
Severnaya Dvina River estuary	5–15	0–15	5–15	5–15	Considerable	Jul 1997	6	8	15	Excavator	Adults	15	4.60 (3.52–5.83)	8:7	5.28	3.74
Pechora River estuary	5–15	0–12	10–30	10–30	Considerable	Jul 1996	9	7	20	Dredge	Juveniles	30	2.03 (1.42–3.39)	10:20	2.36	1.87
											Adults	25	4.12 (3.22–6.89)	3:22	5.76	3.90
											Juveniles	25	2.37 (1.80–3.17)	10:15	2.45	2.32

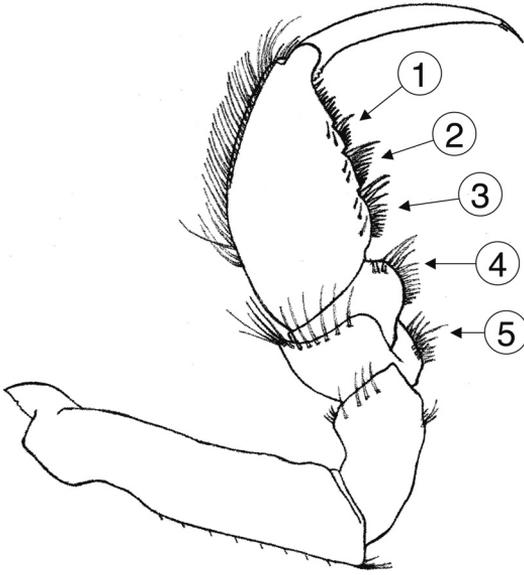


Fig. 1. The groups of chaetae analysed on *meropoditum*, *carpopoditum* and *propoditum*.

Size and sex determination

All samples were preserved in 70% ethanol. Lengths of individuals were measured from the middle of the anterior margin concavity on the head to the end of the *telson*. Sex was determined by the presence or absence of an *appendix masculina* on the second pair of pleopods and of genital papillae, characteristic of males (Haah-tela 1978).

Characters and counting

We analysed the numbers of chaetae on the inner margins of the *meropoditum*, *carpopoditum* and *propoditum* of the first three pairs of thoracic legs, whose function is locomotion (Fig. 1).

In all, 15 bilateral characters were counted. The list of characters counted on each leg is as follows:

1. Number of chaetae in section I of the *propoditum*.
2. Number of chaetae in section II of the *propoditum*.
3. Number of chaetae in section III of the *propoditum*.
4. Number of chaetae in the *carpopoditum*.
5. Number of chaetae in the *meropoditum*.

These characters were chosen after different groups of chaetae on various parts of the body had been examined. The selected groups of chaetae are aligned in straight rows. Such a clear structuring of chaetae groups and a clear separation of chaetae from one another allows them to be counted accurately and reduces the measurement error.

The numbers of chaetae were counted on the left and right sides of the body of the individual specimen with a stereomicroscope (magnification 8×7) (adults) and a microscope (12×5) (juveniles) by two operators (M.C. and M.M.). Each operator analysed half of each sample, except the sample from the Pechora River estuary, which was analysed by both operators.

Statistical analysis

For statistical comparisons of the samples by means, we carried out principal component analysis using the average of the left and right values. Predicted means were calculated for a length of 4 cm on the basis of linear regression of character values on length (after log-transformation).

Fluctuating asymmetry was expressed as a component of total variance based on the residuals of character total length regression (Lajus et al. 2003b). For size standardisation we used an individual's left and right observed values divided by the predicted value of the character, obtained from the character-total length regression (Lajus 2001).

To evaluate the measurement error we performed replicated measurements. Samples from the Gulf of Gdańsk, Gulf of Finland and the Severnaya Dvina River estuary (subsample of juveniles) were measured three times (October 1998, February 1999 and April 1999). The sample from the Pechora River estuary was measured twice — in February 1999 and in April 1999. On the basis of these replicates we found two evaluations of the measurement error. The first evaluation was based on a comparison of the first and the second replicates, the second evaluation on a comparison of the second and third

replicates. For the Pechora River estuary sample we obtained one assessment of the measurement error comparable with the second assessment of the other samples. The magnitude of the measurement error was estimated with the same formula as for the stochastic component (*see* above), but using X_1 instead of L and X_2 instead of R , where X_1 and X_2 are values from two consecutive replicate measurements (Lajus 2001). In all calculations we used fluctuating asymmetry after subtracting the measurement error.

Data for means and variations are based on measurements made in October 1999, when specimens for analysis were chosen at random from a mixed pooled sample in order to avoid the potential temporal effect of any differences in measurements.

The overall magnitude of sample variation was measured using a sum of ranks after ranking the variances of each character across samples (Zakharov 1989, Lajus *et al.* 2003b). For sample comparison we used the *t*-test, ANOVA and non-parametric statistics such as Kendall's coefficient of concordance W and the sign test (Lehmann 1975).

Results

Sizes and sexes

The animals in our study range in size from 1.42 to 7.50 cm (Table 1). Particular samples differ considerably in the average size of individuals and in the sex ratio — from 12% males in the subsample of adults from the Pechora River estuary up to 60% in the Gulf of Finland (Table 1). In all samples, males were larger than females. With respect to total length the average difference was 35% for the adult samples and 16% for the juvenile samples.

Tests for type of asymmetry

Since, apart from fluctuating asymmetry, there are also other types of asymmetry — directional asymmetry and antisymmetry (Van Valen 1962) — identification of the type of asymmetry is necessary. As a test for directional asymmetry,

we used the *t*-test of differences between the left and right values of the character. In all 90 cases (15 characters \times 6 samples) the differences between them were non-significant. For antisymmetry, our data were confined to a small number of discrete categories, which could have led to false positive results if the Kolmogorov-Smirnov test, recommended as a test for antisymmetry, had been applied (Palmer 1994). For the whole set of characters, the kurtosis of the $R-L$ distribution (i.e. the distribution of differences between the right and left values of a character), which should be negative in the case of antisymmetry, did not show any obvious tendency towards negativity (Tables 2 and 3); although the number of cases with negative kurtosis (platikurtic distribution) was higher than those with positive kurtosis (leptokurtic distribution) (53:37), the differences tested by the sign test were not significant for the whole set of characters (Table 3). We therefore regard the type of asymmetry in our study as fluctuating asymmetry.

Association of asymmetries with length and size of characters

Since the samples differ with respect to the size of characters and length of individuals, any dependence between asymmetries and these variables can lead to the appearance of false differences in asymmetry. Because of notable within-sample variation, such associations, if they exist, should appear in within-sample analyses. We tested the association of this fluctuating asymmetry index with parameters that differ in the samples: character size, total length of individual, and predicted values of characters for an individual's total length, and we found no significant association of fluctuating asymmetry with these variables (it was significant only in one case out of 18 (Table 4)).

Operator effect

Means

We found differences between the two operators in the counts of the number of chaetae. In adult

Table 2. Kurtosis and skewness of *R_L* (where the values of *R* and *L* have been pooled) and *R-L* (difference between right and left values) distributions of 15 characters (Fig. 1) in *S. entomon* from the Gulf of Gdansk (GG), Gulf of Finland (GF), Severnaya Dvina River estuary (SDE) and Pechora River estuary (PE). Subsamples of adults and juveniles are identified with A and J in subscript, respectively.

Character	Kurtosis of <i>R_L</i> distribution						Skewness of <i>R_L</i> distribution											
	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J
1(I)	0.42	-0.84	1.37	-0.27	-0.55	-0.35	-0.31	-0.21	-1.08	0.50	-0.19	0.09	0.23	0.96	-0.20	0.27	-0.57	0.45
2(I)	-0.08	-0.15	1.17	-0.46	0.11	0.40	0.27	-0.35	-0.95	0.20	0.26	0.32	-0.52	-0.15	0.14	-0.99	-0.55	0.41
3(I)	-0.42	-0.06	-0.05	0.19	0.00	-0.92	-0.18	0.50	-0.14	0.44	0.39	-0.19	0.42	-1.01	-1.14	1.39	-0.68	1.06
4(I)	-0.10	-0.60	0.33	-0.76	0.03	-0.44	0.15	0.17	0.40	0.15	-0.03	0.16	-0.13	-0.66	-0.33	0.32	5.02	1.38
5(I)	2.15	0.26	-0.93	-0.54	-0.18	-0.96	0.91	0.35	-0.02	0.31	-0.37	0.07	-0.65	1.80	-0.34	-0.22	-0.40	-0.29
1(II)	1.51	-0.86	-0.53	1.35	-0.68	0.72	0.44	0.14	0.31	0.74	0.14	-0.37	5.47	-0.19	-0.30	0.41	0.83	0.58
2(II)	0.05	-0.73	-0.24	0.29	-0.66	-0.21	0.18	0.21	0.61	-0.08	0.13	0.15	-0.02	-1.18	-1.27	-0.67	-0.31	-0.08
3(II)	-0.88	0.21	-0.04	0.45	-0.29	-0.50	-0.23	0.31	-0.47	0.65	0.17	-0.26	-1.17	0.57	3.71	0.12	-0.98	-0.42
4(II)	0.66	0.03	-0.79	0.21	0.13	-0.35	-0.12	-0.53	-0.33	0.29	0.00	0.24	2.08	0.45	0.65	0.16	-1.01	0.77
5(II)	-0.33	-0.03	-0.87	0.27	-0.18	-0.05	-0.09	0.15	0.26	0.65	-0.42	-0.16	0.21	-0.38	0.33	-0.62	0.26	-0.41
1(III)	0.06	-0.60	-0.45	-0.33	-0.95	0.15	-0.25	0.02	-0.39	0.16	0.05	-0.42	3.20	0.69	-0.68	-0.87	-0.26	-0.76
2(III)	0.94	-0.38	-0.80	-0.41	0.04	0.64	0.68	0.19	0.02	0.02	-0.03	0.60	-0.46	-0.90	-1.08	-0.24	-0.87	1.73
3(III)	-0.40	0.66	-0.19	-0.43	-0.10	0.12	-0.37	-0.61	-0.48	0.14	-0.31	0.07	-0.88	0.19	-1.22	-0.45	-0.05	-0.44
4(III)	-0.48	-0.53	-0.30	0.13	-0.06	-0.38	-0.03	0.04	0.69	0.36	-0.83	0.19	-0.93	0.44	-0.30	3.82	-0.19	0.04
5(III)	-0.05	0.10	-0.87	-0.66	-0.66	-0.35	0.08	0.12	-0.29	0.16	-0.06	0.27	-0.73	-0.73	-1.38	0.15	-0.61	0.20

animals, operator 1 produced higher counts than operator 2 with regard to all characters (average difference was 6%). The differences were statistically significant (*t*-test, *p* < 0.05) for 7/15 characters. In juveniles, where the other type of microscope was used, operator 2, by contrast, produced higher counts of all characters (the average difference was 11%; significant differences were obtained in 10/15 characters). It is interesting that in both cases the most significant differences, though with the opposite signs, were observed for character 5. This character also showed the highest measurement errors (*see* below) and was thus the most difficult to count. To avoid the effect of the operators on the results of sample comparison, we set the contribution of the operators to the final data equal.

Variations

On average, operator 1 showed higher estimates of asymmetry than operator 2: the average difference was 13%. These differences were more pronounced in adults, where the “operator” factor was significant (*p* < 0.05, one-way ANOVA) in 2/15 characters tested. Operator 1 showed higher magnitudes of asymmetry in 11/15 characters (NS, sign test). In juveniles, operator 1 again showed higher measures of asymmetries (5% on average), although the differences were not significant. No differences between operators were found when assessing factorial variation.

Differences between sexes

Differences between sexes with regard to the means were observed only in principal component 1; they are evidently caused by the size difference between males and females. We did not find any tendency towards higher asymmetry in either of the sexes for any given set of characters: asymmetry was higher in males in 7/15 characters.

Measurement error

In three samples where the measurement error was assessed twice, the first assessment was

significantly greater than the second (sign test, $p < 0.01$). Because the second assessment was closer in time to the final data analysis, we used this for further calculations. The magnitude of the measurement error in juveniles was usually 5–6 times higher than in adults and these differences were significant ($p < 0.01$, sign test for pooled samples, see Table 5). This appears to be due to the different size of the animals (the average length of adults is 4.69, that of juveniles is 2.20) and also because different microscopes were used. Different characters clearly diverge with respect to measurement error, the highest systematic estimates being obtained for character 5. Because of the absence of notable differences between samples within adults and juveniles, we used averaged estimates of the measurement error separately for adults and juveniles. On average, the contribution of the measurement error to fluctuating asymmetry was quite low — about 1% for adults and 3% for juveniles.

Patterns of chaetotaxy variation among characters

Means

The number of chaetae was highest in the first section of the *protopoditum* (character 1), and lowest in the second (2); it rose again to the previous level in the third section of the *protopoditum* (3) and in the *carpopoditum* (4), but fell a little in the *meropoditum* (5). The differences between legs were very slight (Table 6 and Fig. 2).

Variances

The correlation coefficients between means and stochastic variances varied from $r = -0.43$ (Pechora River estuary) to $r = -0.74$ (Gulf of Finland) (Fig. 2). Whereas character 2 had a minimal number of chaetae, it had maximal stochastic variance, two–three fold greater than that

Table 3. Number of positive values of kurtosis (K) and skewness (S). The total number of cases analysed is 15 for single samples and 90 (15×6) for the pooled sample. Significance was tested by the sign-test.

Location	$K_{R,L}$	$S_{R,L}$	K_{R-L}	S_{R-L}
Gulf of Gdańsk	7	7	6	12 ($p < 0.05$)
Gulf of Finland	5	11	7	6
Severnaya Dvina River estuary (adults)	3	6	4	7
Severnaya Dvina River estuary (juveniles)	6	14 ($p < 0.01$)	8	8
Pechora River estuary (adults)	4	6	3	9
Pechora River estuary (juveniles)	5	10	9	9
Total	30 ($p < 0.01$)	54	37	51

Table 4. Number of positive correlations of the fluctuating asymmetry index $|(L - \text{pred})/\text{pred} - (R - \text{pred})/\text{pred}|$ with character size (expressed as $(R + L)/2$), length of individual and predicted character value for an individual's length (calculated from regression of character value on body length). The number of cases analysed is 15 for single samples and 90 (15×6) for the pooled sample.

Sample	Correlations, r of FA index with		
	Character size	Total length	Predicted character value
Gulf of Gdańsk	Adults	14 ($p < 0.01$)	11
Gulf of Finland	Adults	9	10
Severnaya Dvina River estuary	Adults	6	4
Severnaya Dvina River estuary	Juveniles	8	10
Pechora River estuary	Adults	7	6
Pechora River estuary	Juveniles	8	10
Total		52	50

of the other characters. Factorial variances did not appear to be associated with either means or stochastic components (Fig. 2). The contribution of the stochastic variation to the total variance varied from 39% in character 5 to 61% in character 2 (Table 6).

Kurtosis and skewness

In general, the kurtosis and skewness of R,L distributions (where the values of R and L are put together and considered as independent values) displayed some deviations from normality (Table 3). Distributions tended to be platykurtic and right-skewed. In single samples these differences were not significant according to the sign test except in one case (Table 3), but when all the samples were pooled, differences were significant ($p < 0.01$). Skewness (R,L) was more often positive, but dominance of positive cases was not significant. Distributions of asymmetry ($R-L$) also display a similar tendency, i.e. kurtosis was more often negative (platykurtic distributions) and skewness was more often positive, but this tendency was not significant when the sign tests were applied to the pooled sample (Table 3).

Ontogenetic changes

Means

An increase in the number of chaetae in adults in comparison with juveniles was observed in all characters (Fig. 3). The increase was greatest in the *meropoditum*, where the difference between juveniles and adults from the Severnaya Dvina River estuary was 47%–69%, whereas in the *propopoditum*, where growth was minimal, it was 20%–34% (Fig. 3). There were differences between legs: in the third leg the increase in the number of chaetae was notably greater than in the first one. The patterns were similar but not so clear in the Pechora River estuary sample, probably because of the smaller size difference between adults and juveniles. The average increase in the number of chaetae in adults in comparison with juveniles was 37% for the Severnaya Dvina River estuary and 27% for the Pechora River estuary sample. Within all samples there was also a positive association between the number of chaetae and body length: the average coefficient of correlation r between them within a sample averaged for all samples and for all characters was 0.35.

Table 5. Magnitude of the measurement error (ME). The first assessment is based on a comparison of the measurements made in October 1998 and February 1999, the second, on a comparison of the measurements made in February 1999 and April 1999. GG = Gulf of Gdańsk, GF = Gulf of Finland, SDE = Severnaya Dvina Estuary and PE = Pechora Estuary. Subsamples of adults and juveniles are identified with A and J in subscript, respectively.

Character/ leg	GG		GF		SDE _A		PE _A	PE _J
	ME(1)	ME(2)	ME(1)	ME(2)	ME(1)	ME(2)	ME(2)	ME(2)
1(I)	0.000422	0.000089	0.000600	0.000112	0.000209	0.000000	0.000000	0.000096
2(I)	0.000273	0.000000	0.000269	0.000000	0.000988	0.000000	0.000000	0.000000
3(I)	0.000122	0.000000	0.000420	0.000000	0.000250	0.000412	0.000000	0.000000
4(I)	0.000136	0.000136	0.000701	0.000000	0.000256	0.000000	0.000000	0.000312
5(I)	0.001408	0.000317	0.000714	0.000172	0.002708	0.000799	0.000271	0.000841
1(II)	0.000079	0.000000	0.000055	0.000000	0.000115	0.000000	0.000088	0.000000
2(II)	0.000227	0.000000	0.000238	0.000000	0.000000	0.000000	0.000000	0.000000
3(II)	0.000190	0.000000	0.000342	0.000000	0.000000	0.000000	0.000000	0.000000
4(II)	0.001142	0.000000	0.000080	0.000000	0.001241	0.000000	0.000144	0.000255
5(II)	0.003872	0.000158	0.000311	0.000153	0.006059	0.001165	0.000205	0.000690
1(III)	0.000217	0.000000	0.000266	0.000000	0.000689	0.000000	0.000094	0.000104
2(III)	0.000000	0.000000	0.000000	0.000000	0.000665	0.000000	0.000000	0.000000
3(III)	0.000545	0.000000	0.000205	0.000000	0.000388	0.000000	0.000000	0.000000
4(III)	0.000000	0.000000	0.000000	0.000000	0.000228	0.001965	0.000176	0.000000
5(III)	0.001873	0.000456	0.000246	0.000278	0.005394	0.001711	0.000000	0.000354
Average	0.000700	0.000077	0.000296	0.000048	0.001279	0.000403	0.000065	0.000177

Table 6. Standardised means (predicted values for the total length 4.0 cm), stochastic variance (after partitioning out measurement errors), factorial variance, relative stochastic variance (contribution of stochastic variance in the total variance) and measurement error of 15 characters (Fig. 1) in *S. entomon* from the Gulf of Gdańsk (GG), Gulf of Finland (GF), Severnaya Dvina River estuary (SDE) and Pechora River estuary (PE). Subsamples of adults and juveniles are identified with A and J in subscript, respectively.

Char- acter/ leg	Standardised means					Stochastic variance					Factorial variance					Relative stochastic variance					Measurement error					
	GG _A	GF _A	SDE _A	PE _A	PE _J	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J	GG _A	GF _A	SDE _A	SDE _J	PE _A	PE _J	Adults	Juveniles	
1(I)	16.06	18.09	15.76	14.54	17.35	16.65	0.00499	0.00620	0.00638	0.00683	0.00403	0.00606	0.00302	0.00652	0.00529	0.01268	0.00567	0.00409	0.62	0.49	0.55	0.35	0.42	0.60	0.00007	0.00005
2(I)	9.05	8.45	7.91	7.90	8.30	7.71	0.00917	0.00889	0.00920	0.01290	0.01138	0.02695	0.00374	0.00819	0.00953	0.00698	0.00093	0.00282	0.71	0.52	0.49	0.63	0.92	0.91	0.00000	0.00000
3(I)	13.49	13.90	10.23	10.47	11.03	9.60	0.00331	0.00379	0.01050	0.01083	0.00434	0.01431	0.00548	0.00885	0.00655	0.02206	0.00140	0.00235	0.38	0.30	0.62	0.33	0.76	0.86	0.00000	0.00021
4(I)	12.03	12.63	10.79	10.49	11.12	10.92	0.00309	0.00533	0.00401	0.00549	0.00339	0.01303	0.00686	0.00075	0.00445	0.00346	0.00416	-0.00058	0.26	0.88	0.47	0.61	0.45	1.05	0.00005	0.00016
5(I)	10.42	11.94	9.56	10.39	11.19	9.14	0.00244	0.00604	0.00423	0.01146	0.00420	0.01798	0.01264	0.00421	0.01333	0.01334	0.00278	0.00516	0.16	0.59	0.24	0.46	0.60	0.78	0.00025	0.00082
1(II)	15.74	17.35	14.95	13.58	17.01	16.47	0.00362	0.00508	0.00299	0.00962	0.00506	0.00732	0.00476	0.00574	0.00900	0.00513	0.00796	0.00299	0.43	0.47	0.25	0.65	0.39	0.71	0.00003	0.00000
2(II)	9.04	9.07	8.51	7.82	8.64	7.88	0.00706	0.01185	0.01153	0.01184	0.01029	0.01694	0.00725	0.00559	0.00447	0.00350	0.00180	0.00465	0.49	0.68	0.72	0.77	0.85	0.78	0.00000	0.00000
3(II)	13.92	13.38	11.29	10.99	11.72	9.65	0.00207	0.00384	0.00478	0.01779	0.00411	0.01037	0.00674	0.00394	0.00585	0.01698	0.00174	0.00406	0.24	0.49	0.45	0.51	0.70	0.72	0.00000	0.00000
4(II)	13.30	14.09	11.70	11.28	12.75	11.87	0.00337	0.00483	0.00285	0.01041	0.00689	0.01271	0.01203	0.00616	0.00482	0.01024	0.00118	0.00341	0.22	0.44	0.37	0.50	0.85	0.79	0.00005	0.00013
5(II)	10.83	11.29	10.29	9.11	11.20	9.18	0.00207	0.00740	0.00512	0.01514	0.00671	0.01841	0.01189	0.00684	0.01573	0.02007	0.00194	0.00634	0.15	0.52	0.25	0.43	0.78	0.74	0.00017	0.00093
1(III)	15.00	16.28	13.23	13.25	16.12	15.25	0.00296	0.00446	0.00291	0.00907	0.00293	0.00989	0.00722	0.00536	0.01022	0.00518	0.00310	0.00211	0.29	0.45	0.22	0.64	0.49	0.82	0.00003	0.00005
2(III)	8.80	8.80	7.96	7.51	7.97	7.20	0.00476	0.01057	0.00649	0.00715	0.00676	0.02045	0.01251	0.01009	0.00312	0.01093	0.00322	0.00325	0.28	0.51	0.68	0.40	0.68	0.86	0.00000	0.00000
3(III)	14.24	14.18	10.40	10.25	11.50	8.86	0.00312	0.00455	0.00788	0.00922	0.00343	0.01045	0.01005	0.00559	0.00940	0.01199	0.00323	0.00322	0.24	0.45	0.46	0.43	0.51	0.76	0.00000	0.00000
4(III)	13.73	14.04	11.28	11.09	13.04	12.49	0.00275	0.00597	0.00633	0.01100	0.00360	0.01386	0.01240	0.00543	0.00388	0.00637	0.00432	0.00211	0.18	0.52	0.62	0.57	0.45	0.87	0.00006	0.00098
5(III)	11.32	12.42	9.61	10.27	11.84	10.29	0.00437	0.00638	0.00310	0.01214	0.00414	0.01663	0.01049	0.00637	0.01542	0.02363	0.00272	0.01108	0.29	0.50	0.17	0.34	0.60	0.60	0.00024	0.00103

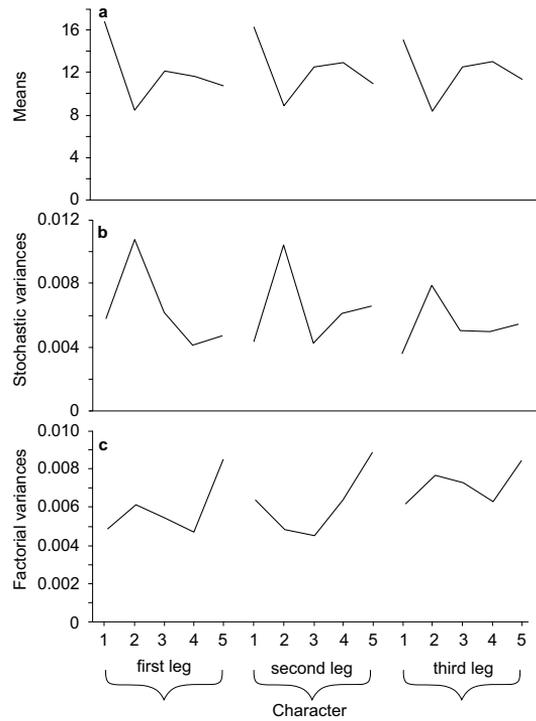


Fig. 2. Variation of (a) means, (b) stochastic and (c) factorial variances in different characters (averaged for all samples of adults).

Variations

Stochastic variance decreased with growth. In juveniles from the Severnaya Dvina River estuary it was higher than in adults for all characters (Fig. 4 and Table 6). A minimal decrease (a few percent) was observed in character 3, whereas in the other characters it was usually 2–5 fold. In the Pechora River estuary sample, differences were again smaller, but the stochastic variances also tended to be greater in juveniles. The factorial variance did not show evident changes with growth; in the Severnaya Dvina River estuary sample it was greater in 10/15 in juveniles, and in the Pechora River estuary sample in 8/15 cases.

Mean-variance associations

Changes in the stochastic variances correlated negatively with changes in means for the sample

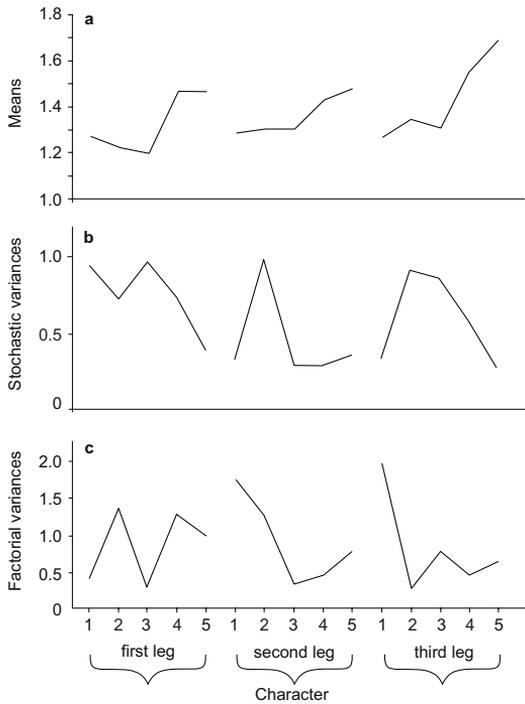


Fig. 3. Ratio of (a) means, (b) stochastic and (c) factorial variances between adults and juveniles from the Severnaya Dvina River estuary.

from the Severnaya Dvina River estuary (Pearson $r = -0.44$ ($p < 0.1$); Spearman $r = -0.52$, ($p < 0.05$)) (Fig. 4). For the Pechora River estuary sample $r = -0.21$ (NS). Thus, the stochastic variation of characters growing more intensively tends to decrease faster. At the same time, there was no correlation between changes in the factorial variance and changes in means or the stochastic variance. This resulted in a decrease in the contribution of the stochastic component to the total variance with growth. Non-equal changes in the level of stochastic variance in different characters resulted in differences in the slope of the means-stochastic variances regression in adults and juveniles. In adults in the sample from Severnaya Dvina River estuary the slope was -1.14 , in juveniles -0.36 (log-transformed values were used because they give better linearity between variances and means (Lajus 2001)). In the Pechora River estuary sample in adults the slope was 1.13 and in juveniles -0.58 . A statistical comparison of the regression lines of stochastic variances-means

(Sokal & Rohlf 1981) showed that these differences were not significant.

Kurtosis and skewness

Ontogenetic changes in the kurtosis and skewness of a character's distribution were quite similar for the samples from the Pechora and Severnaya Dvina River estuaries. Changes were quite obvious with respect to R,L skewness, which tended to be positive in juveniles, but more often became negative in adults. At the same time, no changes were observed in the $R-L$ skewness. This means that the changes in R,L skewness describing the combined distribution between and within individuals were caused mostly by changes in the characteristics of the distributions between individuals, but not within individuals. Some of the observed changes in the kurtosis of the R,L distribution were mostly the result of changes in the kurtosis of the $R-L$ distribution, while R,L kurtosis and $R-L$ kurtosis displayed similar patterns of ontogenetic changes, from normal distributions in juveniles to platykurtic distributions in adults.

Changes in the skewness and kurtosis of a character's distributions can affect the magnitude of variances to some extent. In particular, the tendency for $R-L$ distributions to be more often platykurtic can mask a tendency for the stochastic variance to decrease. Were there no changes in the nature of the distribution, changes in the level of stochastic variance would most likely be even more pronounced. At the same time, the tendency for the factorial variation to decrease with growth may be explained, at least to some extent, by changes in the skewness of R,L distributions from right-skewed (and therefore increasing variance) in juveniles to normal in adults.

Differences between populations

Means

To compare the samples by means, we performed principal component analysis based on the average between left and right values. PC1 explained 80.3% of the total variance. The loadings of all

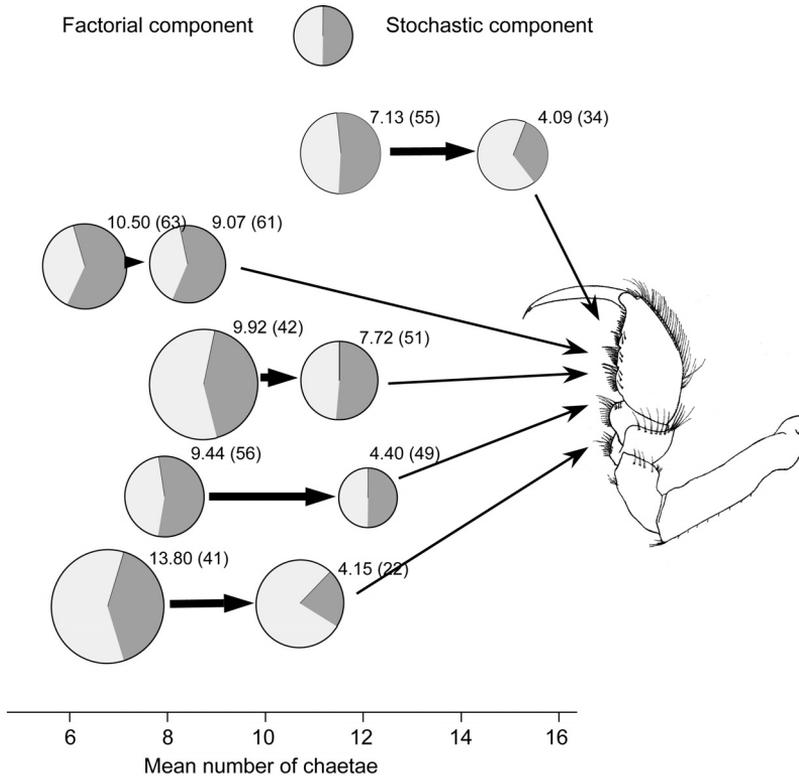


Fig. 4. Magnitude of phenotypic variance and contribution of its different components for five characters (the upper one is first): factorial and stochastic for adults (left circle of each pair) and juveniles of *Saduria entomon* from the Severnaya Dvina River estuary. Each character is the average for three legs. The area of the circles is proportional to the total variance. The figures indicate the absolute value of the stochastic component ($\times 1000$) and its percentage in the total variance (in parentheses).

characters on this principal component were positive and high (ranging between 0.81 and 0.95). Thus, we interpreted this principal component as the overall number of chaetae, i.e. proportional changes in different characters without changes of ratio between them. Because the number of chaetae correlated positively with the size of individuals, the PC1 mostly reflected variation in size. The correlation coefficient between the length of individuals and PC1 was $r = 0.88$ ($p < 0.001$). Other principal components did not correlate with length. PC2 explained 5.5% of the total variation, PC3 — 3.3%, PC4 — 2.0% and PC5 — 1.8%. These principal components showed significant ($p < 0.05$) variation among the samples, tested by one-way ANOVA. They reflect the non-proportional changes between characters that lead to changes of ratio between them. The other principal components did not

reveal significant differences among the samples.

There are no evident patterns in the position of samples along the principal component axes (Fig. 5a and b). Samples are not subdivided into distinct groups, and there is no association with geographical location. Moreover, there is no concordance among different principal components across the samples (tested by Kendall's coefficient of concordance W). One can see that subsamples of adults and juveniles from SD and PE do not cluster together and differences between them do not display a similar direction. This means that to some extent the differences between the samples from different locations may have been caused by their allometric growth. At the same time, because the differences in size between the samples of adults are noticeably less than those between subsamples

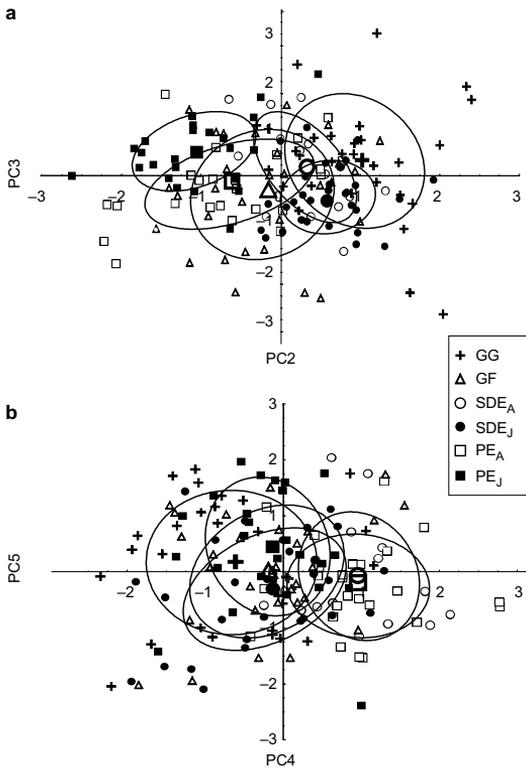


Fig. 5. Distribution of individuals in coordinates of (a) PC2–PC3 and (b) PC4–PC5. Ellipses including 50% of individuals are shown. Centres of distributions are marked with larger symbols. GG = Gulf of Gdańsk; GF = Gulf of Finland; SDE = Severnaya Dvina River estuary; PE = Pechora River estuary. Subsamples of adults and juveniles are identified with A and J in subscript, respectively.

of adults and juveniles (Table 1), this effect cannot be large.

The overall number of chaetae, i.e. that explained by PC1, has different causes: firstly, variation in length among the individuals; and secondly, the systematic difference between samples in the number of chaetae even at the same predicted length (Table 6). Kendall's concordance W of predicted means across the samples was as high as 0.81 ($p < 0.001$). If we take the number of chaetae in the Severnaya Dvina River estuary sample to be 1, for the Pechora River estuary sample it is equal to 1.10, for the Gulf of Gdańsk it is 1.15, and for the Gulf of Finland sample the figure is 1.20 (Fig. 6).

The relationships between the samples are similar for different legs (Fig. 6); it is therefore

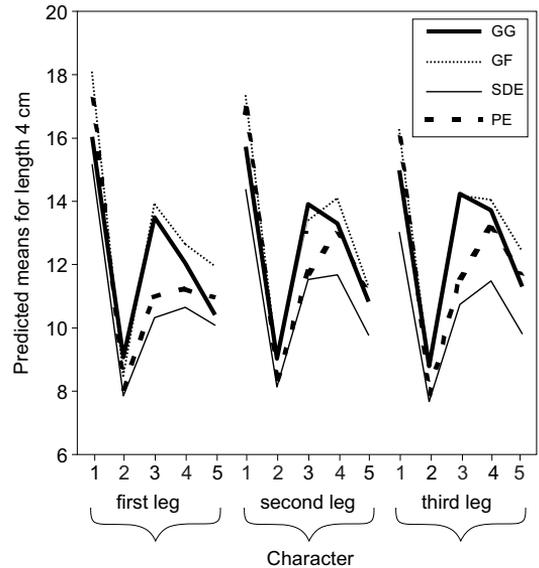


Fig. 6. Number of chaetae (predicted value for 4.0 cm) in different populations. GG = Gulf of Gdańsk, GF = Gulf of Finland, SDE = Severnaya Dvina River estuary, PE = Pechora River estuary. Subsamples of juveniles and adults are pooled.

possible to consider averaged patterns for all three legs. If we assume the number of chaetae in character 3 to be equal to 1, the values of the characters will be as shown in Fig. 7. Subsamples of juveniles from the Severnaya Dvina River estuary show quite similar patterns with adults from the same location, though juveniles from the Pechora River estuary sample differed greatly from the adults. In general, one can state that the profiles presented here reflect patterns of chaetotaxy variation specific to the local population and which cannot be explained by allometric growth.

Variations

Since there are examples when slopes of variance-mean regression were different, which resulted in discordance of variances across characters (Lajus 1998, 2001), we investigated the slopes of variance-slope regressions prior to comparing the samples by magnitude of variances. Regression slopes of stochastic components against means were negative in

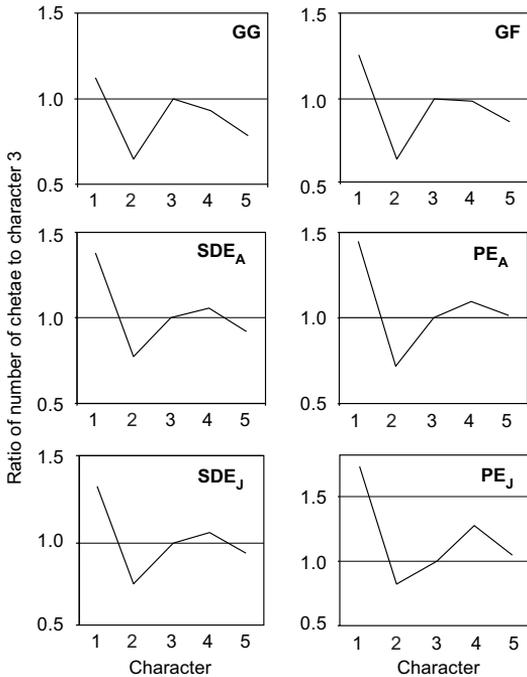


Fig. 7. Ratio of the mean number of chaetae in characters 1–5 to the number of chaetae in character 3 (number of chaetae on the third section of the *protopoditum*) averaged for three legs: GG = Gulf of Gdańsk, GF = Gulf of Finland, SDE = Severnaya Dvina River estuary, PE = Pechora River estuary. Subsamples of adults and juveniles are identified with A and J in subscript, respectively.

all cases and did not change very much among the samples: from -0.93 in the Gulf of Gdańsk sample to -1.14 in the Severnaya Dvina River estuary sample (the differences were not significant, according to the test in Sokal and Rohlf (1981)). The Kendall coefficient of concordance W was equal to 0.678 ($p < 0.001$). In factorial variances the differences in slopes were not significant either, but the range of slopes varied noticeably among the samples: from -0.67 in the Gulf of Gdańsk sample to 0.38 in the Pechora River estuary sample. Kendall's concordance $W = 0.116$ (NS). In all cases the slopes of the factorial component against means were not as steep as those for the stochastic component of the same sample (considering absolute values).

As long as the slopes are homogenous, the samples can be compared with respect to the average magnitude of variances. We ranked the samples for each character (the maximum rank

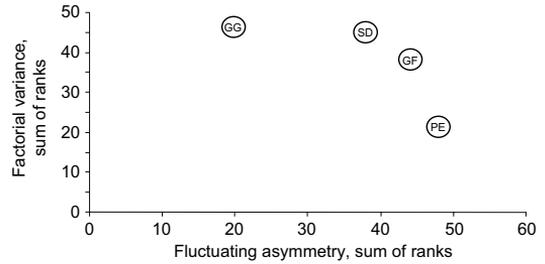


Fig. 8. Sum of ranks for (a) stochastic and (b) factorial components. GG = Gulf of Gdańsk, GF = Gulf of Finland, SDE = Severnaya Dvina River estuary, PE = Pechora River estuary.

has the highest variance) and found the sum of ranks (Fig. 8). Minimal fluctuating asymmetry was found in the Gulf of Gdańsk sample. The differences between this sample and the other samples, apart from the Severnaya Dvina River estuary sample, were significant ($p < 0.01$, sign test). The factorial variance was the least in the sample from the Pechora River estuary (Fig. 8). The differences between this sample and all other samples were significant ($p < 0.01$).

Discussion

Characters

Our study was based on the chaetotaxy patterns of *S. entomon*. Chaetotaxy is frequently used in morphological studies on arthropods due to its deep morphogenetic canalization (Beklemishev 1964). Chaetae like ones under consideration are touch sensoric organs and proprioceptors.

Although diverse characters can vary to different extents when fluctuating asymmetry is studied (see for example Parsons 1990, Eggert & Sakaluk 1994, Palmer 1994, Woods *et al.* 1999, Lajus 2001), there is no way of predicting which character will be more sensitive to differences in developmental stability between populations. This was why, when we chose the characters for the present study, we selected a convenient measuring system encumbered by only minimal measurement error (see Material and methods).

Methodological problems of measuring morphological variation

All the measurements in this study were performed by two operators. Because their contribution was equal, the results were not affected by differences between the operators. At the same time, our results showed that, even if they are properly trained and work together, different operators can obtain different results, especially with regard to means and fluctuating asymmetry: in both cases, differences were systematically greater for one character (character 5) than for the others.

When assessing the measurement error, i.e. the repeatability of the measurements, we found that assessments made at different times tended to be lower in later trials. We assume that this can be explained by the improvement in accuracy of measurements as the operators gained in experience. In a study on copepods (Lajus & Alekseev 2000), the measurement error in sequential measurements also decreased, and in that paper it was explained by the gradual stabilisation of the position of specimens in balsam. However, as regards the present data it is quite possible that in the work on copepods the differences between sequential assessments of measurement error may have involved not only the stabilisation of the position of specimens but also the operator's greater experience. The contribution of the measurement error to fluctuating asymmetry in the present study was 1%–3%, i.e. a figure considerably lower than that reported for other species (Palmer & Strobeck 1992, Merilä & Bjorklund 1995, Lajus & Alekseev 2000).

Our results therefore show that data have to be treated with caution if they have been obtained by different operators or at different times. If the analysis cannot be performed by a single operator, the contribution of different operators should be equal. To avoid the effect of temporal differences in measurements, it is important to analyse samples not one after another in sequence, but by taking specimens from the pooled sample at random.

Ontogenetic changes in chaetotaxy variation

The number of chaetae in all characters increased with size, demonstrating allometric growth, i.e.

that the increase was not proportional in different characters. We also observed changes in fluctuating asymmetry: size-standardised values of fluctuating asymmetry decreased with growth. The decrease of fluctuating asymmetry with growth is known from the literature (Møller & Swaddle 1997, Swaddle & Witter 1997, Tompkins 1999). As for the total standardised variance, measured by coefficient of variation (CV), it also, as a rule, decreases during growth (Yablokov 1974). From the data presented here, we may conclude that with growth the greatest decrease occurs in the stochastic component of total variance, but not in the factorial one. Because of this, the contribution of the stochastic component to the total variance decreases in older animals. Though a decrease in stochastic variance was manifested in all characters, this was more evident in chaetae groups growing more intensively.

Association of parameters of chaetotaxy variation across characters

A negative correlation of the stochastic components with means across characters were found in this study as well as in other species (Soulé 1982, Lajus 1998, 2001, Lajus & Alekseev 2000, Lajus *et al.* 2003b). However, we did not find either the negative correlation between the factorial components and the means, or the positive correlation between factorial and stochastic components reported in other studies (Lajus 1998, 2001, Lajus *et al.* 2003b). The reason for this is probably the relatively small difference in means between the least and greatest characters. In the present study this difference was only two-fold, whereas in other studies it was several-fold greater. This made it more difficult to observe trends characteristic of the relationships between variances and means.

Differences between the samples

Means

In the means, two types of differences between samples were found. The first was the difference in the average number of chaetae — 'coefficient

of chaetotaxy' (Fig. 6), the second was the difference in the ratio between different characters (Fig. 7). The direct application of principal component analysis, as is commonly thought, would not have displayed the latter type of variation because it was not separated from variation caused by differences in length of individuals (because the number of chaetae is also correlated with length). To analyse this type of variation of means is nevertheless possible by the analysis of predicted values of character-length regression. We obtained the increase in the 'coefficient of chaetotaxy' in the order Severnaya Dvina River estuary–Pechora River estuary–Gulf of Gdańsk–Gulf of Finland. The means of meristic characters are known to depend on many factors, including temperature and salinity. One example of the direct effect of salinity on aquatic animals is the study of the brine shrimp *Artemia salina*. It was demonstrated that the number of caudal bristles decreases from 16 to 12 when the salinity increases from 50% to 100% sea water (Michinomae & Kaji 1976). However, there is no consistent rule which would enable one to predict how a particular character would vary under given environmental conditions. Hence, we will not attempt to explain which factors caused the observed differences between the *S. entomon* populations and to what extent; we shall merely presume that the differences in the average number of chaetae are due to the direct effect of environmental conditions.

The residual differences in means among samples (after removal of the differences in the average number of chaetae) were not clearly associated either with the environmental conditions in the sampling locations or with the geographical distances between the locations. One of the reasons for these differences could be genetic divergence after isolation of the populations. This suggestion is in accordance with the results of analysis of allozyme variation of samples collected in the same location as our samples (except the sample from Gulf of Finland, which was taken not near the Neva River, but off Tvärminne, Finland) (J. Sell & T. Sywula unpubl. data). These results displayed a similarity of genetic relationship among the samples with those based on analysis of residual variation of means.

Among-individual variation (factorial variance)

Heterogeneity of individuals is due to two sources: diversity of environmental and genotypic variation; however, the contribution of these components to factorial variation is unknown. We may suppose that the factorial component in estuarine populations should be higher than in Baltic populations, reflecting the high diversity of environmental factors in estuarine habitats (Table 1). However, it should be noted that *S. entomon* is an active animal (Shurin 1961) and thus able to select the most favourable conditions. This may considerably reduce the effect of environmental diversity to phenotypic variation because different animals are known to be able to select similar conditions at the same time.

Therefore, we believe that a more important part in differences between samples in factorial variation is played by genotypic variation. From this point of view, the low factorial variation of the sample from the Pechora River estuary (Fig. 8) can be explained by the loss of genetic variation due either to specialisation via stabilising selection or to the particular conditions of this estuary. Within an isolated population existing for several thousand years, inbreeding is also highly probable at any stage of its existence, which may be another reason for the loss of genotypic variation.

Within-individual variation (fluctuating asymmetry, stochastic variance)

When one considers the factors potentially capable of bringing about differences in developmental instability between different populations of *S. entomon*, one can take into account such environmental characters as temperature, salinity, food availability, oxygen concentration or chemical pollution. Unfortunately, not all these parameters can be analysed for all the locations under study: especially scant are data on the estuaries of the Severnaya Dvina and Pechora Rivers (*see* Table 1).

Data on fluctuating asymmetry suggest that the population of *S. entomon* from the Gulf of

Gdańsk possesses a higher developmental stability than the other populations under study. This is in quite good agreement with environmental characteristics in different locations. Environmental conditions in the Gulf of Gdańsk are probably quite close to favourable for *S. entomon*. Salinity varies in the Gulf of Gdańsk within quite narrow limits between 7.2‰ to 12.5‰ (Piechota 1973). The temperature depends on depth and, because here there are no barriers preventing the animals from migrating, they can select the most favourable conditions. In the Gulf of Gdańsk *S. entomon* live at a temperature from 2 to 12 °C and the highest biomass is observed at 4–5.6 °C (Piechota 1973). Note that the highest biomass is not necessarily an indicator of preferable abiotic conditions, since the distribution of its main prey *Pontoporeia affinis* (Amphipoda) is of great importance for the distribution of *S. entomon* (Ankar 1977, Järvekülg 1979). In the Gulf of Finland it was found that distributions of these species are highly correlated (Maksimov 1997).

Other habitats are characterised by less favourable conditions. In the Gulf of Finland, and in practice, the estuary of the river Neva, *S. entomon* occurs in waters of reduced salinity; this is due to the outflow of the Neva. Moreover, the Neva discharges a substantial quantity of chemicals from the St. Petersburg area (pop. five million inhabitants) into the Gulf, which makes it the most polluted part of the Baltic Sea (Savchuk & Skakalskii 1997).

Estuarine environments are characterised by a very considerable variation in hydrological parameters depending on season, tide, waves, river runoff etc. Thus, *S. entomon* evidently occurs under suboptimal conditions more often than in the stable and deep environments of the Baltic Sea. For instance, in the Pechora River estuary *S. entomon* sometimes can be found at salinities up to 30‰ (Hummel *et al.* 1999). This is probably because the temperature of the less saline waters coming from the river is too high in summer. In contrast, colder waters are associated with the sea and consequently with high salinities. There is no environment where both temperature and salinity would be close to the optimal. Very likely, estuarine *S. entomon* have to accommodate constantly to changes in environmental conditions and also to follow

their prey. At the same time, the combination of available conditions must be quite rich to have ensured the survival of the species over several thousand years. It is quite possible, however, that all this time *S. entomon* lived in these areas under suboptimal conditions, which was reflected in elevated fluctuating asymmetry.

The differences in fluctuating asymmetry found between the populations may be explained not only by the direct effect of environmental differences, but also by genetic differences between the populations. The number of populations in estuaries is quite limited, and is probably subject to large fluctuations owing to the unstable environment. This may cause loss of genetic variation (bottle-neck effect) and result in disruption of genomic co-adaptation and in reduced developmental stability (*see for instance* Leary & Allendorf (1989), Clarke (1993), Markow (1995) and recent reviews in Polak (2003)). In large populations living under more stable conditions such effects are less likely.

This hypothesis accords with the data on factorial variation, discussed above (section ‘Among-individual variation (factorial variance)’). There is a tendency for estuarine populations to exhibit lower factorial variation than Baltic populations. In general, moreover, there is a tendency towards negative association between factorial variation and fluctuating asymmetry, which may just be different manifestations of loss of genetic variation. On the one hand this results in a decrease in factorial variation, but on the other to inbred depression, which causes an increase in asymmetry. An association between factorial variation and fluctuating asymmetry was also reported in studies of populations of the cyprinid fish *Abramis brama* (Kozhara 1994) and on the White Sea herring *Clupea pallasii* (Lajus 2001).

Conclusion

In this paper, we have described changes in morphological variation in *Saduria entomon* related to ontogenesis and geographic variation. The study is based on 15 characters of chaetotaxy, which we have tried to treat as a whole system. The main methodological approach we fol-

lowed was the decomposition of phenotypic variation into its elements with further separate analysis of them. We believe that we have found quite a convenient system of characters for the morphological analysis of *S. entomon*, because they enabled us to observe evident ontogenetic and geographic differences in this species, and because all the characters manifest generally similar patterns of variation.

In this study, we analysed not only the biological sources of variation, but also non-biological ones, such as departures from perfect repeatability of the measurements and differences between operators. In particular, different operators, even if they train and work together, reveal some differences in measuring means and fluctuating asymmetry; this factor should be taken into consideration when comparing the results obtained by different operators.

In the chaetae we observed a decrease in the standardised fluctuating asymmetry and in the standardised total variance during ontogenesis. These effects had been known earlier, but the approach we applied showed that the decrease in the total variation in the number of chaetae in *S. entomon* is due to the decrease in fluctuating asymmetry (a stochastic component of total variance) because the other component of total variance, namely factorial one, does not change.

The differences among populations with regard to means can be divided into (i) changes in the overall number of chaetae in all characters ('coefficient of chaetotaxy'), which could be a direct effect of the environment, and (ii) residual variation, which manifests itself in differences in the ratio of the number of chaetae in different characters. Because of the similarity of sample clustering based on residual variation and on genetic characteristics (J. Sell & T. Sywula unpubl. data), we suggest that the residual variation reflects genetic differences between populations.

Among-population differences in variances (fluctuating asymmetry and factorial variance) can be explained quite well by the environmental conditions under which populations occur. Estuarine populations are exposed to a much higher variability of hydrological characteristics than populations from the Baltic Sea, where they live in a deeper and more stable environment. This,

together with the comparatively small population size, may lead to a decrease in genetic variability (bottle-neck effect) and therefore to a decrease in factorial variation, and an increase in fluctuating asymmetry. The stressful and unstable environmental conditions in estuaries probably also exert a direct effect on the development of *S. entomon* and lead to an increase in fluctuating asymmetry.

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References

- Ankar, S. 1977: The soft bottom ecosystems of the northern Baltic Proper with special reference to the macrofauna. — *Contributions from the Asko Laboratory, University of Stockholm* 19: 1–62.
- Astauroff, B. L. 1930: Analyse der erblichen Störungsfälle der bilateralen Symmetrie im Zusammenhang mit der selbstständigen Variabilität ähnlicher Strukturen. — *Zeitschrift für induktive Abstammungs- und Vererbungslehre* 55: 183–262.
- Augustowski, A. 1987: *Bałytk potudniowy*. — Ossolineum, Wrocław.
- Beklemishev, V. N. [Беклемишев, В. Н.] 1964: [*Basis of comparative anatomy of invertebrates, vol. 2. Organology*]. — Nauka, Moscow.
- Bjorksten, T. A., Fowler, K. & Pomiankowski, A. 2000a: What does sexual trait FA tell us about stress? — *Trends Ecol. Evol.* 15: 163–166.
- Bjorksten, T. A., Fowler, K. & Pomiankowski, A. 2000b: Reply from T. Bjorksten, K. Fowler and A. Pomiankowski. — *Trends Ecol. Evol.* 15: 331.
- Bogucki, M. 1948: O rozrodzie podwoja (*Mesidotea entomon* L.) w Bałtyku. — *Biuletyn Morskiego Laboratorium Rybackiego w Gdyni* 4: 143–154.
- Clarke, G. M. 1993: The genetic basis of developmental stability. I. relationships between stability, heterozygosity and genomic coadaptation. — *Genetica* 89: 15–23.
- Eggert, A. K. & Sakaluk, S. K. 1994: Fluctuating asymmetry and variation in the size of courtship food gifts in decorated crickets. — *American Naturalist* 144: 708–716.
- Graham, J. H., Freeman, D. C. & Emlen, J. M. 1993: Developmental stability: a sensitive indicator of populations under stress. — In: Landis, W. G., Hughes, J. S. & Lewis, M. A. (eds.), *Environmental toxicology and risk assessment, ASTM STP 1179*: 136–158. American Society for Testing and Materials, Philadelphia.
- Graham, J. H., Shimizu, K., Emlen, J. M., Freeman, C. D. & Merkel, J. 2003: Growth models and expected distribution of fluctuating asymmetry. — *Biological Journal of*

- the Linnean Society. [In press].
- Grunner, H.-E. 1965/1966: Krebstiere oder Crustacea. V. Isopoda. — *Die Tierwelt Deutschlands* 51. VEB Gustav Fischer Verlag, Jena.
- Gärtner, K. 1990: A third component causing random variability besides environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? — *Laboratory animals* 24: 71–77.
- Haahtela, I. 1978: Morphology as evidence of maturity in Isopod Crustacea, as exemplified by *Mesidotea entomon* (L.). — *Ann. Zool. Fennici* 15: 186–190.
- Haahtela, I. 1990: What do Baltic studies tell us about the isopod *Saduria entomon* (L.). — *Ann. Zool. Fennici* 27: 269–278.
- Hagerman, L. & Szaniawska, A. 1992: *Saduria entomon*, ecophysiological adaptations for survival in the Baltic. — In: Bjørnstad, E., Hagerman, L. & Jensen, K. (eds.), *Proceedings of the 12th Baltic Marine Symposium: 71–76*. Olsen & Olsen, Fredensborg, Denmark.
- Hoffman, A. A. & Woods, R. E. 2003: Associating environmental stress with developmental stability: problems and patterns. — In: Polak, M. (ed.), *Developmental instability: causes and consequences: 387–401*. Oxford University Press, Oxford.
- Hummel, H., Strelkov, P. P., Sukhotin, A. A., Naumov, A. D., Berger, V. J., Koukina, S. E., Bek, T. A., Novikov, G. G., Lubin, P. A., Denisenko, S. G., Larroux, C., Amiard-Triquet, C., Calafat-Frau, A. M., Regoli, F., Orlando, E. & Palerud, R. 1999: Biodiversity and adaptation strategies of Arctic coastal marine benthos. Final report of INTAS project 94-391. — *Netherlands Institute of Ecology, NIOO Report* 1999-1: 1–98.
- Järvekülg, A. [Ярвекюльг, А.] 1979: [Bottom fauna of Eastern part of the Baltic Sea]. — Valgus, Tallinn. [In Russian].
- Jones, J. S. 1987: An asymmetrical view of fitness — *Nature* 325: 298–299.
- Kivivuori, L. & Lagerspetz, K. Y. H. 1990: Thermal resistance and behaviour of the isopod *Saduria entomon*. — *Ann. Zool. Fennici* 27: 287–290.
- Kozhara, A. V. 1994: Phenotypic variance of bilateral characters as an indicator of genetic and environmental conditions in bream *Abramis brama* (L.) (Pisces, Cyprinidae) population. — *Journal of Applied Ichthyology* 10: 167–181.
- Kusakin, O. G. [Кусакин, О. Г.] 1979: [Marine and brackish-water Isopoda of cold and temporal waters of the Northern hemisphere]. — “Nauka”, Leningrad. [In Russian].
- Lajus, D. L. 1998: Two-dimensional model of phenotypic variation. — *Proceedings of Zoological Institute RAS* 267: 107–114.
- Lajus, D. L. & Alekseev, V. R. 2000: Components of morphological variation in baikalian endemic cyclopoid *Acanthocyclops signifer* complex from different localities. — *Hydrobiologia* 417: 25–35.
- Lajus, D. L. 2001: Variation patterns of bilateral characters: variation among characters and among populations in the White Sea herring (*Clupea pallasii marisalbi*). — *Biological Journal of the Linnean Society* 74: 237–253.
- Lajus, D. L., Graham, J. H. & Kozhara, A. V. 2003a: Developmental instability and the stochastic component of total phenotypic variance. — In: Polak, M. (ed.), *Developmental instability: causes and consequences: 343–363*. Oxford University Press, Oxford.
- Lajus, D. L., Knust, R. & Brix, O. 2003b: Fluctuating asymmetry and other parameters of morphological variation of eelpout *Zoarces viviparus* (Zoarcidae, Teleostei) from different parts of its distributional range. — *Sarsia*. [In press].
- Leary, R. F. & Allendorf, F. W. 1989: Fluctuating asymmetry as an indicator of stress: implications for conservation biology. — *Trends Ecol. Evol.* 4: 214–217.
- Lehmann, E. L. 1975: *Nonparametrics*. — Holden-Day, Inc. McGraw-Hill International Book Company.
- Lens, L., Van Dongen, S., Kark, S. & Matthysen, E. 2002: Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? — *Biological Reviews* 77: 27–38.
- Leung, B. & Forbes, M. 1996: Fluctuating asymmetry in relation to stress and fitness: Effect of trait type as revealed by meta-analysis. — *Ecoscience* 3: 400–413.
- Leung, B., Knopper, L. & Mineau, P. 2003: A critical assesment of the utility of fluctuating asymmetry as a biomarker of anthropogenic stress. — In: Polak, M. (ed.), *Developmental instability: causes and consequences: 415–426*. — Oxford University Press, Oxford.
- Majewski, A. 1990: *Zatoka Gdańska*. — Wydawnictwo Geologiczne, Warszawa.
- Maksimov, A. A. [Максимов, А. А.] 1997: [Macrozoobenthos of the Eastern part of the Gulf of Finland]. — In: Davidian, I. N. & Savchuk, O. P. [Давидян, И. Н. & Савчук, О. П.] (eds.), [Ecosystem models. Assessment of current status of the Gulf of Finland. Part II. Hydrometeorological, hydroclimatic, hydrobiological, geological history and water dynamics of the Gulf of Finland]: 405–416. Gidrometeoizdat, St. Petersburg. [In Russian].
- Markow, T. A. 1995: Evolutionary ecology and developmental instability. — *Annual Review of Entomology* 40: 105–120.
- Merilä, J. & Bjorklund, M. 1995: Fluctuating asymmetry and measurement error. — *Systematic Biology* 44: 97–101.
- Mikhailov, A. E. [Михайлов, А. Е.] 1997: [Water temperature and salinity]. — In: Davidian, I. N. & Savchuk, O. P. [Давидян, И. Н. & Савчук, О. П.] (eds.), [Ecosystem models. Assessment of current status of the Gulf of Finland. Part II. Hydrometeorological, hydroclimatic, hydrobiological, geological history and water dynamics of the Gulf of Finland]: 225–235. Gidrometeoizdat, St. Petersburg. [In Russian].
- Michinomae, M. & Kaji, S. 1976: Effect of acetamide on the bristle formation in the brine shrimp *Artemia salina*. — *Annotationes Zoologicae Japonenses* 49: 149–156.
- Møller, A. P. 2000: Symmetry, size and stress. — *Trends Ecol. Evol.* 15: 330.
- Møller, A. P. & Swaddle, J. P. 1997: *Asymmetry, developmental stability and evolution*. — Oxford University Press, Oxford.
- Naumov, A. D. & Fedijakov, V. V. [Наумов, А. Д. & Федяков, В. В.] 1993: [The eternally alive White Sea].

- Izd. Sankt Petersburgskogo gorodskogo dvortza tvorchestva iunykh, St. Petersburg. [In Russian].
- Palmer, A. R. 1994: Fluctuating asymmetry analysis: — In: Markow, T. A. (ed.), *Developmental instability: its origins and evolutionary implications*: 335–364. Kluwer Dordrecht.
- Palmer, A. R. & Strobeck, C. 1992: Fluctuating asymmetry as a measure of developmental stability: *Implications of non-normal distributions and power of statistical tests*. — *Acta Zool. Fennica* 191: 57–72.
- Palmer, A. R. & Strobeck, C. 2003: Fluctuating asymmetry analysis revisited. — In: Polak, M. (ed.), *Developmental instability: causes and consequences*: 279–319. — Oxford University Press, Oxford.
- Parsons, P. A. 1990: Fluctuating asymmetry: An epigenetic measure of stress. — *Biological Reviews* 65: 131–145.
- Pearson, K. 1901: Mathematical contributions to the theory of evolution. IX. On the Principle of Homotypis and its relation to heredity, to the variability of the individual and to that of Race. Part 1. Homotypis in the vegetable Kingdom. — *Phil. Trans. R. Soc. Lond. A* 197: 285–379.
- Piechota, W. 1973: Biologia i ekologia skorupiaków dennych Bałtyku południowego. — *Zesz. Nauk. Wyd. BiNoZ UG, Oceanografia* 2: 91–107.
- Polak, M. (ed.) 2003: *Developmental instability: causes and consequences*. — Oxford University Press, Oxford.
- Savchuk, O. P. & Skakalskii, B. G. [Савчук, О. П. & Скакальский, Б. Г.] 1997: [Pollution loadings and water-cleaning actions]. — In: Davidian, I. N. & Savchuk, O. P. [Давидян, И. Н. & Савчук, О. П.] (eds.), [*Ecosystem models. Assessment of current status of the Gulf of Finland. Part II. Hydrometeorological, hydroclimatic, hydrobiological, geological history and water dynamics of the Gulf of Finland*]: 261–268. Gidrometeoizdat, St. Petersburg. [In Russian].
- Sell, J. 2003: Haplotype frequency distribution in north-eastern European *Saduria entomon* (Crustacea: Isopoda) populations. A phylogeographic approach. — *International Review of Hydrobiology*. [In press].
- Shurin, A. T. [Шурин, А. Т.] 1961: [Agglomerations of bottom fauna of the Gulf of Riga]. — *Trudy Nauchno-issledovatel'skogo instituta rybnogo khozjajstva SNKh Latvii SSR* 3: 343–368. [In Russian].
- Soulé, M. E. 1967: Phenetics of natural populations. II: Asymmetry and evolution in a lizard. — *American Naturalist* 101: 141–160.
- Soulé, M. E. 1982: Allomeric variation. I. The theory and some consequences. — *American Naturalist* 120: 751–764.
- Sokal, R. R. & Rohlf, F. J. 1981: *Biometry*. — W.H. Freeman and company, New York.
- Swaddle, J. P. & Witter, M. C. 1997: On the ontogeny of developmental stability in a stabilised trait. — *Proc. R. Soc. Lond. B* 264: 329–334.
- Tompkins, J. L. 1999: The ontogeny of asymmetry in earwig forceps. — *Evolution* 53: 157–163.
- Van Dongen, S. & Lens, L. 2000: Symmetry, size and stress. — *Trends Ecol. Evol.* 15: 330.
- Van Valen, L. 1962: A study of fluctuating asymmetry. — *Evolution* 16: 125–142.
- Woods, R. E., Sgro, C. M., Hercus, M. J. & Hoffman, A. A. 1999: The association between fluctuating asymmetry, trait variability, trait heritability and stress: a multiply-replicated experiment on combined stresses in *Drosophila melanogaster*. — *Evolution* 53: 493–505.
- Yablokov, A. V. 1974: *Variability of mammals*. — Amerind Publishing Co. New Delhi.
- Zakharov, V. M. [Захаров, В. М.] 1987: [*Animal asymmetry: population-phenogenetic approach*]. — Nauka Press, Moscow. [In Russian].
- Zakharov, V. M. 1989: Future prospects for population phenogenetics. — *Soviet Scientific Reviews Section F, Physiology and General Biology Reviews* 4: 1–79.
- Zakharov, V. M. 1992: Population phenogenetics: Analysis of developmental stability in natural populations. — *Acta Zool. Fennica* 191: 7–30.
- Zakharov, V. M. 2003: Linking developmental stability and environmental stress: a whole organism approach. — In: Polak, M. (ed.), *Developmental instability: causes and consequences*: 402–414. Oxford University Press, Oxford.