

# Environmental and ontogenetic constraints on developmental stability in the spatangoid sea urchin *Echinocardium* (Echinoidea)

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Spatangoid irregular sea urchins are detritivorous benthic organisms particularly prone to variations of environment, and their mode of growth and plate morphology make them an appropriate model to assess the effects of environmental variations. Two populations of *Echinocardium flavescens* were sampled in two sites of the Norwegian coast characterized by contrasted environmental conditions. Different morphological descriptors (plate areas, interlandmarks distances, overall size, and shape of the posterior ambulacra) were used to appraise interindividual variations, and fluctuating asymmetry. The comparisons were carried out using classical fluctuating asymmetry (FA) methods, as well as Procrustean approaches. The population suspected to be less influenced by anthropic activities exhibits lower levels of FA for the size parameters (plate surfaces, interlandmarks distances, and centroid size) than the population located in a polluted area. Conversely, it shows higher FA values for the shape parameters (landmarks configuration). Interindividual variations appear to be correlated to FA. Variations are orientated according to the main growth axis of the ambulacra, and their intensity is stronger in the large posterior plates, which are also the youngest. These results are discussed with respect to architectural constraints involved in the sea urchin growth. © 2006 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2006, 88, 165–177.

**ADDITIONAL KEYWORDS:** Developmental instability – fluctuating asymmetry – geometric morphometrics – morphological variations – sea urchins.

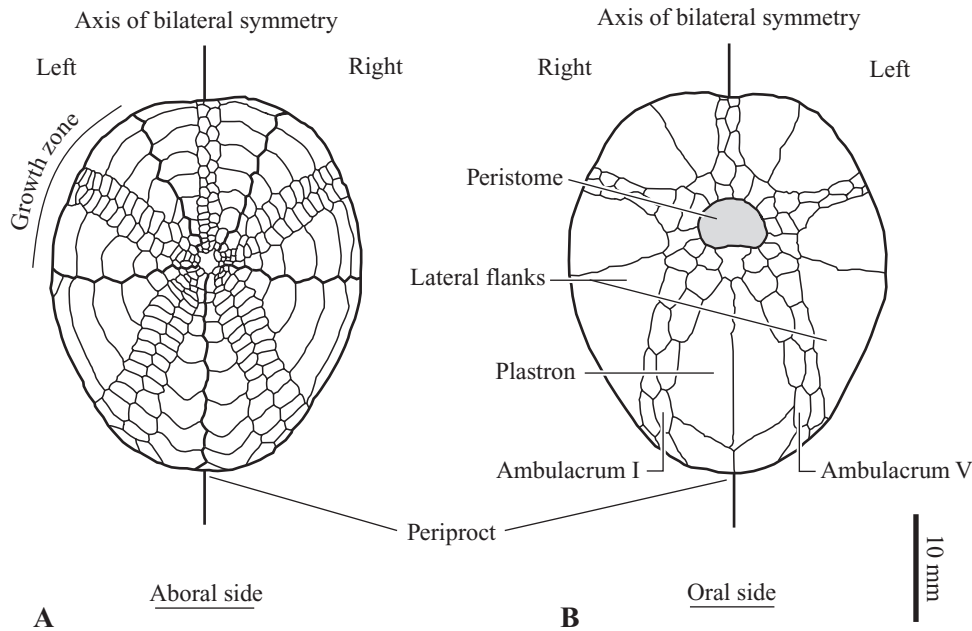
## INTRODUCTION

Soft sea bottoms provide a wide range of environments for benthic organisms. Correspondingly, benthic organisms living and developing in those environments are particularly prone to variations of the sediment: granulometry, organic content, degree of pollution (Dafni, 1980; Telford & Mooi, 1996; Linton & Taghon, 2000; Zulkosky, Ferguson & McElroy, 2002). This is particularly true for spatangoids that are detritivorous, generally endobenthic sea urchins with a rather reduced mobility (Nichols, 1959; Buchanan, 1966; Kanazawa, 1992). In such organisms, we may expect an important influence of stressing environmental conditions on several aspects of their phenotype, including morphology. The shape and

architecture of spatangoids tests should therefore provide an accurate record of substrate-related stresses experienced by the sea urchins during their postlarval development.

Modalities of the postlarval growth of sea urchins are now clearly understood in the framework of a new model: ‘Extraxial-Axial Theory’ (EAT) (David & Mooi, 1996; Mooi & David, 1997). The EAT distinguishes two principal regions in the skeleton of the developing sea urchin (axial vs. extraxial). The axial part grows by adding new elements according to a precise rule, the Ocular Plate Rule (OPR) (Mooi, David & Marchand, 1994). The OPR generates the precise order in which new plates appear, and is crucial in delineating homologies between the plates of the test. Most of the test of an adult is made of axial elements, organized into five growth zones. In regular sea urchins, the five growth zones are almost perfectly balanced in a radiating pattern. In irregular sea urchins, including spatangoids,

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**Figure 1.** The test of *Echinocardium flavescens* displaying the aboral side (A) and the oral side (B) where posterior ambulacra (ambulacra I and V) were studied.

a bilateral symmetry appears secondarily during the growth as a change in the initial balance between the five growth zones. The balance is stabilized in such a way that the test clearly displays a left and a right part, as well as an anterior–posterior axis, defined by the positions of periproct and peristome (Fig. 1A, B). Moreover, the irregularity is expressed in the shape and size of the plates of the test, and is clearly visible in the array of these plates.

Stress can disturb the balance between growth zones (i.e. it can impair developmental stability of the tests). Developmental stability is defined as the suite of processes through which organisms reduce phenotypic variation resulting from developmental accidents (Zakharov, 1989; Palmer, 1994). It is generally measured by evaluating fluctuating asymmetry (FA) levels, which correspond to deviations from perfect symmetry of normally symmetrical structures (Van Valen, 1962). Environmental stressors such as chemical pollutions (Pankakoski, Koivisto & Hyvärinen, 1992; Zakharov, Valetsky & Yablokov, 1997), temperature (Siegel & Doyle, 1975; Parsons, 1990; Leary, Allendorf & Knudsen, 1992) or food deprivation (Swaddle, Cuthill & Witter, 1994) have been shown to be significantly associated with higher levels of FA. Thus, FA has been proposed as a valuable biological indicator of environmental quality (Clarke, 1993). However, the use of FA is not without difficulties. A growing debate concerns the methodological appraisal

of FA [i.e. both the choice of morphological traits to consider (nature and number) and the statistical approach to use]. In such a context, developments in geometric morphometrics appear to be of a particular interest (Rohlf & Slice, 1990; Bookstein, 1991, 1996; Rohlf & Marcus, 1993). Such methods are appropriate to study interindividual variations, as well as developmental stability between left and right sides (Klingenberg & McIntyre, 1998; Auffray *et al.*, 1996; Smith, Crespi & Bookstein, 1997; Auffray, Debat & Alibert, 1999). The test of sea urchins displays a plate architecture particularly well suited for investigations by geometric morphometrics. Irregular sea urchins display a secondary bilateral symmetry that is strongly expressed in the general shape of the test, as well as in plate architecture. In the present study, we aimed to appraise and compare FA levels of two different populations of the spatangoid sea urchin *Echinocardium flavescens* (Spatangoida, Loveniidae) that were sampled in two different sites of the Norwegian coast, differing in environmental conditions. The questions addressed were: (1) could variations of FA levels be determined by differences in environmental conditions and (2) could these variations be related to the growth processes of sea urchins? More specifically, is there a gradual change of FA levels along the ambulacra according to the age of the plates, and/or are some parts of the test more developmentally variable and/or unstable than others (i.e. less constrained)?

## MATERIAL AND METHODS

### SPECIMEN COLLECTION

*Echinocardium flavescens* were collected in two localities of the Norwegian coasts in May 1997 (Fig. 2). The first sample was collected near the small town of Bodø (northern to the Polar circle) in a fjord largely free of anthropic pressure. The second was obtained from the Oslo fjord, 30 km south of Oslo (near Drøbak). Because of the important human activities occurring all around the fjord, particularly upstream of Oslo, it can be assumed that this site corresponds to a more stressful environment.

Thirty specimens were measured in each sample. Spines were gently brushed off the test after immersion in a sodium hypochlorite solution. Plate patterns were revealed by applying a solution of equal parts of absolute alcohol and glycerol, and drawings of the architecture of each specimen were made with a camera lucida.

### MEASUREMENTS AND ANALYSES

The study focused on the oral part of the posterior ambulacra (Fig. 1B) that constitute bilaterally symmetrical structures, the 'Oral-Posterior-Ambulacra' (OPA). They are inserted between two functional areas: the plastron and lateral interambulacra involved in locomotion and burrowing activities, respectively. Following Lovén's nomenclature (Lovén, 1874), the ambulacrum I on the right side is symmetrical to ambulacrum V on the left side (Fig. 1B).

The originality of the sea urchin biological model is that plate development occurs in two ways. Each individual plate of a sea urchin test displays its own size increments. Moreover, each plate is involved in the more general process of construction of its growth zone, controlled by the OPR, and its final shape is also constrained by the development of the surrounding plates. Hence, FA can be investigated for each plate independently, as well as for whole ambulacral structures, taking into account the architecture and all possible interactions between neighbouring plates.

Accordingly two descriptors were defined. First, nine individual plate surfaces were measured to assess FA between each homologous right and left plate independently (Fig. 3A). Second, 12 landmarks corresponding to triple junctions of plates (type 1 landmark *sensu* Bookstein, 1991) were defined on each ambulacrum (Fig. 3B). Left ambulacra drawings were mirrored for comparison with the right ones. From those descriptors, size and shape asymmetry could be envisaged separately. We used traditional morphometrics to analyse data relative to size: the nine plate surfaces and six independent interlandmarks distances, four measured transversally, and two meridionally



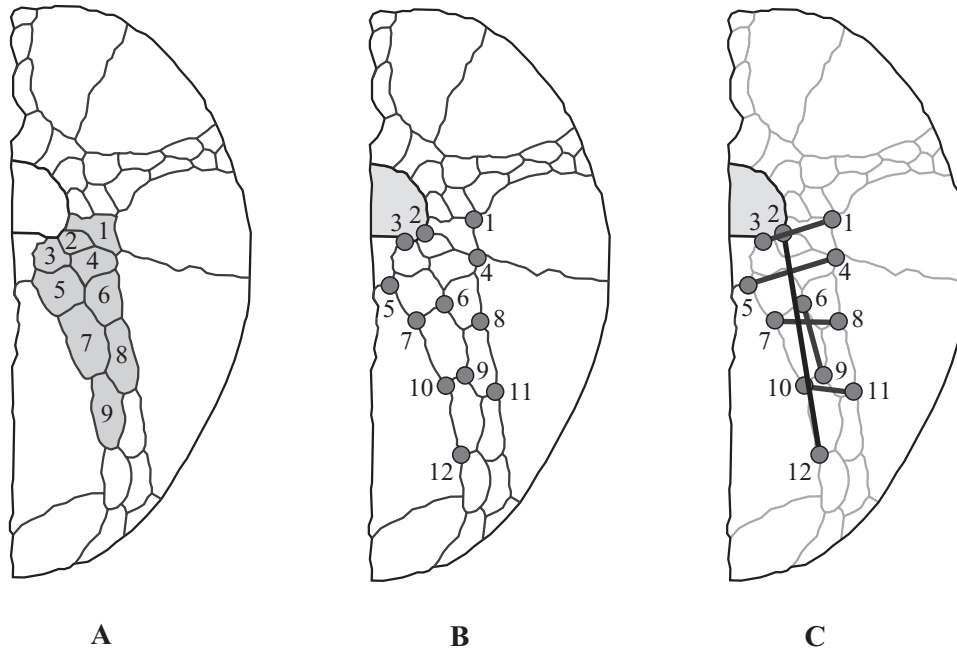
**Figure 2.** Geographical position of the two localities (black squares) where samples of *Echinocardium flavescens* were gathered.

(Fig. 3C). Procrustes superpositions of the 12 landmark configurations were performed to evaluate the whole OPA, shape asymmetry, and another aspect of size asymmetry based on centroid size. The centroid size is a proxy of size that corresponds to the square root of the sum of squared distances between each landmark to the geometric centre of the configuration (Slice *et al.*, 1996).

Data acquisition (including the making of drawings with replacement of specimens) was carried out twice, independently, to estimate and take any measurement error into account.

### INDIVIDUAL PLATE SURFACE ANALYSES

To detect size asymmetry on each of the nine plate surfaces, we performed a two-way (individual  $\times$  side) mixed-model analysis of variance (ANOVA) (Palmer & Strobeck, 1986; Palmer, 1994). After a series of preliminary tests, this procedure allows assessment of the



**Figure 3.** Location of the studied traits: the nine plates (A), the 12 landmarks (B) and the six distances (C) on a left half-test of *Echinocardium flavescens*.

significance of interindividual variations (individual effect), between side variations (side-effect, the so-called directional asymmetry or DA), non-directional asymmetry (i.e. FA in the absence of antisymmetry; interaction between individual and side-effects), and measurement error. For each variable and the two populations, we retained an index of interindividual variability (VAR) and an index of FA (FA10; calculated following Palmer, 1994) from the ANOVAs. Differences in VAR and FA10 between samples were assessed by *F*-tests.

#### ORAL–POSTERIOR–AMBULACRA ANALYSES

Size asymmetry of the six linear distances and of the centroid size were analysed according to the same ANOVA procedure as for individual plate surfaces. Shape asymmetry of the geometry of the 12 landmarks was quantified following the procedure of Klingenberg & McIntyre (1998). This procedure is an adaptation of the two-way mixed-model ANOVA (Palmer, 1994) to landmark geometry and Procrustes analyses. It was performed on Procrustes residuals to calculate the VAR and FA. A Procrustes fit corresponds to a geometric transformation that minimizes the sum of squared distances between corresponding landmarks of two configurations. It involves three basic steps: (1) a translation (decomposed according to  $x$  and  $y$ ); (2) a standardized scaling; and (3) a rotation (Rohlf & Slice, 1990). After Procrustes superimposi-

tion, each sea urchin was described by 24 residuals that are the  $x$  and  $y$  coordinates of the vectors connecting, at each of the 12 landmarks, every single specimen to an average (consensus) configuration. In Bodø and Drøback samples, separately, ANOVAs were calculated for each of the 24 residuals, and the sums of squares of every source of variation (individual, side, interaction, and error) were summed across the 24 variables to obtain the Procrustes sums of squares. These allow calculation of the equivalents of VAR and FA10 after division by the appropriate degrees of freedom (Klingenberg & McIntyre, 1998; Debat *et al.*, 2000). Next, it was necessary to visualize back how VAR, asymmetry (FA10), and eventually measurement error were expressed on the different landmarks of the configurations. For each sample, a two-way multivariate ANOVA (individual  $\times$  side) involving the 24 variables was performed, and two final variance-covariance matrices (VCV) were calculated: one related to the asymmetry calculated from the interaction between both effects, the other corresponding to the interindividual variation calculated from the individual effect. Then, principal components analyses (PCA) was carried out on VCV matrices for both sources of variation and for both samples. The contributions of the 24 variables to a given principal component can be depicted as vectors attached to each landmark. For each principal component of each analysis, this leads to a vector field allowing a visual interpretation that is connected with the ambulacral

plate architecture. In addition, we undertook comparisons of the two patterns, asymmetry and variation among specimens, as well as error, by testing the correlation between their respective VCV matrices within samples using permutation tests (the tests are suited so that the correspondence among  $x$  and  $y$  pairs of coordinates could be respected). Ten thousand permutations were realized. The above explanations concern comparisons within each sample, but correlation tests between VCV matrices were also carried out between samples for each source of variation (VAR, FA, and error). Further explanations of this technique are provided by Klingenberg & McIntyre (1998) and Debat *et al.* (2000).

## RESULTS

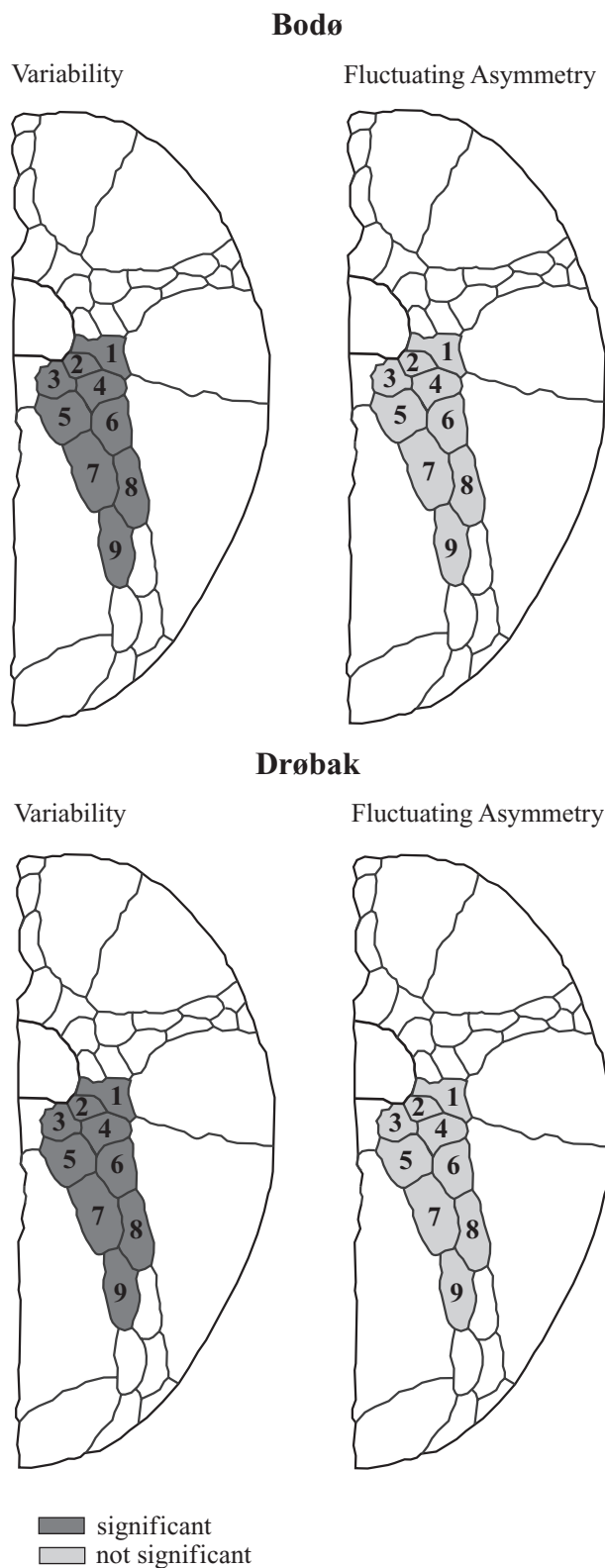
### INDIVIDUAL PLATE SURFACES

The results of the ANOVAs regarding the variability and the asymmetry of the nine selected plates in both samples are rather homogeneous (Fig. 4). After Bonferroni's correction of probability levels for multiple tests within each sample (Rice, 1989), the nine interindividual variabilities appear significant in Bodø and in Drøbak. By contrast, no FA is significant. Table 1 summarizes the values of VAR and FA10 for the nine plates in the two localities. Note that the side-effect is never significant, indicating the absence of DA.

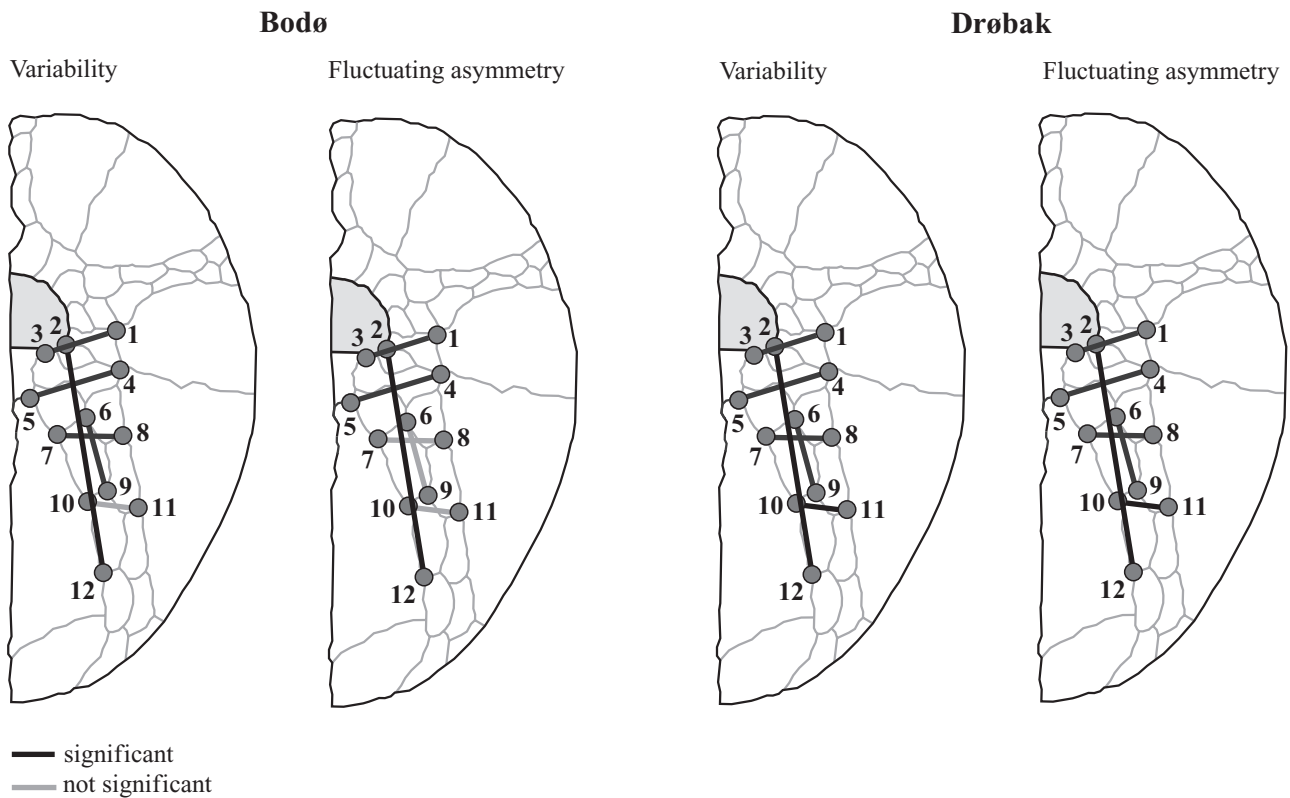
### ORAL–POSTERIOR–AMBULACRA SIZE

Regarding the six interlandmarks distances, the results appear to be fully consistent in Drøbak, with interindividual variability, as well as FA effect, being significant for all traits (Fig. 5). The *Echinocardium* display a more contrasted pattern in Bodø. The VAR of one distance (between landmarks 10–11) is not significant, and two distances (between landmarks 7–8 and 6–9) are not significant for FA (VAR and FA10 values are listed in Table 2). Out of the four possible comparisons between localities, one is significant and indicates that FA is higher in Drøbak than in Bodø ( $F = 5.9$ ,  $P < 0.001$ ). Concerning DA, only one (between landmarks 2–12 in Bodø) shows a significant side-effect after Bonferroni's correction. The greatest value of the 2–12 distance is on the right OPA.

Regarding the centroid size of the landmark configurations, both localities express significant interindividual variability and significant fluctuating asymmetry (Table 3). DA is significant in Bodø only, with a larger centroid size for the right OPA. Variance ratios between Bodø and Drøbak reveal no differences between VAR indices, and a significantly higher FA10 in Drøbak.



**Figure 4.** Results of Palmer's analysis of variance on plate surfaces for variability and fluctuating asymmetry.



**Figure 5.** Results of Palmer's analysis of variance on the six distances for variability and fluctuating asymmetry.

#### ORAL-POSTERIOR-AMBULACRA SHAPE

The OPA shape, appraised by the geometry of landmarks, shows that there is significant shape variation between sea urchins in Drøbak, but not in Bodø (Table 3), whereas FA is significant in both localities and higher in Bodø. For centroid size, a significant DA is detected in Bodø only. If we consider the possible relationship between the patterns of expression of FA and interindividual variability, the comparisons of VCV matrices by permutation tests show a significant strong correlation at Bodø, and a weaker correlation at Drøbak (Table 4). These correlations are clearly illustrated in Figure 6, which shows the contribution of the 24 variables ( $x$  and  $y$  coordinates of residuals) on the first PCA as 12 vectors. In Bodø, vectors of FA and VAR are parallel for most of landmarks (Fig. 6A, B), whereas few vectors show parallel directions in Drøbak (Fig. 6C, D). There is no general pattern observed concerning measurement error (Fig. 6E, F); measurement error is only correlated with VAR in Bodø, and with FA in Drøbak (Table 4).

Similarly, it is also possible to compare the patterns of expression of FA and VAR between samples of the two localities. Visual comparisons of the vector fields

between Bodø and Drøbak show a common pattern of size and orientation of the vectors for FA (Fig. 6B, D), but not for VAR (Fig. 6A, C). This observation is confirmed by permutation tests that reveal a highly significant correlation for FA ( $r = 0.51$ ;  $P < 0.0001$ ), but not for interindividual variability ( $r = 0.17$ ; not significant) and measurement error ( $r = 0.18$ ; not significant) (Table 5).

#### DISCUSSION

##### COULD VARIATIONS OF FA LEVELS BE DETERMINED BY DIFFERENCES IN ENVIRONMENTAL CONDITIONS?

All the OPA parameters exhibit significant levels of FA in Drøbak, whereas two distances are not significant in Bodø (Table 6). For parameters related to size (distances and centroid size), FA values are always higher in Drøbak than in Bodø. This appears to be consistent with the marked anthropic pressure present in the fjord of Drøbak and may be linked to the upstream proximity of Oslo, whereas the fjord of Bodø is far less affected by human activities. Conversely, shape asymmetry values are higher in Bodø than in Drøbak. Such a pattern may suggest two hypotheses: (1) either there

**Table 1.** Results of the plate surfaces analyses, showing interindividual, side and fluctuating asymmetry effects

	Plate 1		Plate 2		Plate 3		Plate 4		Plate 5		Plate 6		Plate 7		Plate 8		Plate 9	
	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak	Bodø	Dyrøbak
Mean size (mm <sup>2</sup> )	3.88	3.34	1.97	2.16	3.43	3.73	3.81	4.98	6.48	7.27	6.22	7.14	8.67	8.49	6.67	5.98	7.02	6.02
Size regression	0.041 ± 0.151 ± 0.265 ± 0.79	0.137 ± 0.054	0.265 ± 0.094	0.137 ± 0.054	0.265 ± 0.094	0.385 ± 0.095	0.328 ± 0.093	0.143 ± 0.069	0.25 ± 0.109	0.107 ± 0.049	8.49 ± 1.13	0.008 ± 0.038	0.075 ± 0.024	0.193 ± 0.073	0.195 ± 0.069	0.098 ± 0.05	0.114 ± 0.031	0.12 ± 0.06
F	0.77†	11.2**	11.3**	6.56**	7.9**	16.4***	12.3**	4.34*	5.3*	4.69*	56.6***	0.047†	9.5**	6.9*	7.98**	3.9†	13.6**	4.9*
Individual																		
d.f.	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
MS	2028.8	3388.9	2209.9	1833.2	2103.5	2445.7	2352.5	3606.7	3101.3	3619.3	3465	3017	3934.2	2957	2764.3	3575.8	3505.7	3250.3
F	1.95*	10.32***	3.1**	3.95**	4.99***	5.51***	5.07***	8.23***	12.69***	14.3***	7.29***	7.87***	9.37***	5.31***	5.29***	10.66***	10.69***	9.38***
Side																		
d.f.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
MS	326.7	102.7	0.3	800.3	346.8	73.6	512.5	1209.7	1.2	53.3	177.6	407	681.6	78.4	1598.7	192.5	1360.1	95.4
F	0.31†	0.31†	0.001†	1.29†	0.82†	0.17†	1.1†	2.76†	0.005†	0.21†	0.37†	1.06†	1.62†	0.14†	3.06†	0.57†	4.15†	0.28†
Interaction																		
d.f.	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
MS	1042	328.4	712.5	620.9	421.8	443.7	464.4	453.3	244.3	475.4	383.1	419.8	419.8	556.8	522.5	335.4	328	346.6
F	1.63†	0.91†	1.49†	1.69†	1.04†	0.84†	1.09†	0.77†	0.6†	0.93†	0.99†	0.85†	0.65†	1.06†	0.83†	1.17†	1.44†	0.68†
Error																		
d.f.	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
MS	638.2	359.6	476.9	367.6	406.4	526.2	424.3	565.8	409.2	272.3	480.7	450.4	642.8	526.2	629.1	287.2	227.4	507.9
VAR	246.7	765.1	374.3	303	420.4	500.5	472	792.1	714.2	841.6	747.4	658.5	878.6	600	560.4	810.1	794.4	725.9
FA10	201.9	-15.6	117.8	126.6	7.7	-82.5	20.05	-63.7	-82.4	-9.6	-2.65	-33.6	-111.5	15.3	-53.3	24.1	50.3	-80.6

The regression for size is a linear regression of  $IR - LI$  on  $(R + L)/2$ -values to test for the dependence between fluctuating asymmetry (FA) and size. MS, mean square; VAR, interindividual variability. The presentation follows the recommendations of Palmer (1994). \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; †Not significant.

**Table 2.** Results of the distances analyses, showing interindividual, side and fluctuating asymmetry effects

	Distance 1–3		Distance 4–5		Distance 7–8		Distance 10–11		Distance 6–9		Distance 2–12	
	Bodø	Drøbak	Bodø	Drøbak	Bodø	Drøbak	Bodø	Drøbak	Bodø	Drøbak	Bodø	Drøbak
Mean size (mm)	4.7	3.85	5.68	4.93	3.52	4.16	2.46	3.19	4.75	4.4	14.24	13.62
Size regression	0.251 ± 0.036 ± 0.093	0.036 ± 0.102	0.229 ± 0.093	0.167 ± 0.105	-0.063 ± 0.104	0.154 ± 0.049	-0.126 ± 0.221	0.223 ± 0.078	0.054 ± 0.038	0.135 ± 0.207	0.033 ± 0.043	0.185 ± 0.084
<i>F</i>	10.418***	4.863*	0.127†	6.066*	2.54†	0.37†	9.828**	0.326†	8.196**	2.044†	0.43†	0.565†
Individual												
d.f.	29	29	29	29	29	29	29	29	29	29	29	29
MS × 1000	996.623	551.466	998.829	766.98	600.029	700.137	372.904	687.294	1549.562	887.032	9553.137	6309.241
<i>F</i>	9.511***	6.952***	6.504***	7.205***	6.675***	15.529***	1.346†	9.825***	37.064***	8.443***	35.538***	18.225***
Side												
d.f.	1	1	1	1	1	1	1	1	1	1	1	1
MS × 1000	552.163	45.63	215.901	274.563	257.613	27	159.141	234.968	444.083	128.708	3534.941	237.63
<i>F</i>	5.269*	0.575†	1.406†	2.579†	2.866†	0.599†	0.574†	3.359†	0.622**	1.225†	13.373**	0.686†
Interaction												
d.f.	29	29	29	29	29	29	29	29	29	29	29	29
MS × 1000	104.786	79.32	153.582	106.451	89.894	45.086	277.044	69.954	41.807	105.056	268.817	346.18
<i>F</i>	2.262**	5.172***	2.434**	5.565***	1.445†	2.598***	2.110**	8.008***	1.684†	8.786***	3.605***	3.781***
Error												
d.f.	60	60	60	60	60	60	60	60	60	60	60	60
MS × 1000	46.322	15.335	63.107	19.13	62.22	17.355	131.299	8.736	24.833	11.956	74.566	91.558
VAR × 1000	223	118	211	165	128	164	24	154	377	196	2321	1491
FA10 × 1000	29	32	45	44	14	14	73	31	8	47	97	127

The regression for size is a linear regression of  $|R - L|$  on  $(R + L)/2$ -values to test for the dependence between fluctuating asymmetry (FA) and size. MS, mean square; VAR, interindividual variability. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; †Not significant.



**Table 3.** Results of the size and shape analyses, showing interindividual, side and FA effects

	Size		Shape	
	Bodø	Drøbak	Bodø	Drøbak
Mean centroid size	16.966	17.104	1	1
Size regression	0.110 ± 0.039	0.055 ± 0.035	-0.001 ± 0.001	0.001 ± 0.0004
<i>F</i>	7.885**	2.485†	0.282†	2.455†
Individual				
d.f.	29	29	580	580
MS × 1000	14348.34	10051.91	0.306	0.133
<i>F</i>	24.255***	25.651***	1.003†	3.964***
Side				
d.f.	1	1	20	20
MS × 1000	5971.94	429.6	0.636	0.044
<i>F</i>	10.095**	1.096†	2.086**	1.301†
Interaction				
d.f.	29	29	580	580
MS × 1000	591.56	391.88	0.305	0.034
<i>F</i>	11.077***	2.519**	16.495***	1.53***
Error				
d.f.	60	60	1200	1200
MS × 1000	534.01	155.59	0.018	0.022
VAR × 1000	3439.195	2415	0.00025	0.025
FA10 × 1000	28.775	168.145	0.143	0.006

The regression for size is a linear regression of  $|R - L|$  on  $(R + L)/2$  centroid size values to test for the dependence between size asymmetry and centroid size. The regression for shape is a regression of  $|R - L|$  Procrustes residuals values on  $(R + L)/2$  centroid size values to test for the dependence between shape asymmetry and size. MS, mean square; VAR, interindividual variability; FA, fluctuating asymmetry. †Not significant.

**Table 4.** Results of the permutation tests performed on variance-covariance (VCV) matrices: within sample analysis of the correlations between VCV matrices of individual, fluctuating asymmetry (FA) and measurement error effects

Samples	Effects	Correlation	<i>P</i>
Bodø	Individual/FA	-0.90	< 0.0001
	Individual/error	-0.36	0.01
	FA/error	0.36	0.0637
Drøbak	Individual/FA	-0.30	0.0393
	Individual/error	0.13	0.3241
	FA/error	-0.51	0.011

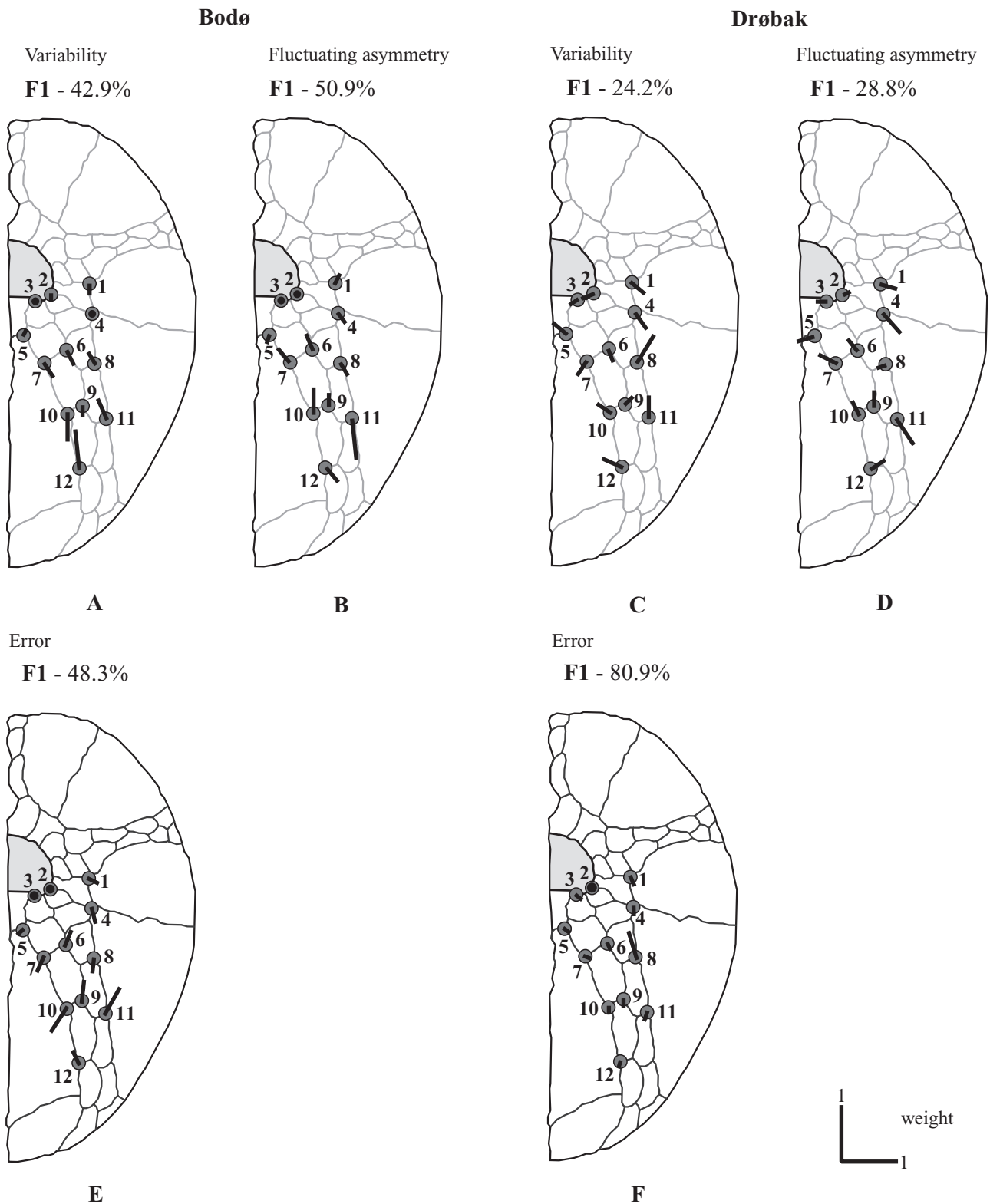
is no correlation between shape and size asymmetry whatever might be the mechanism explaining a stronger shape asymmetry in the less stressed environment or (2) shape asymmetry values in *E. flavescens* are not dependent upon environmental conditions, but may result from internal disruptions such as genetic (Auffray *et al.*, 1996) or epigenetic stressors (Evans & Marshall, 1996).

**Table 5.** Results of the permutation tests performed on variance-covariance (VCV) matrices: between sample analysis of the correlations between VCV matrices of individual, fluctuating asymmetry and measurement error effects

Effects	Sample	Correlation	<i>P</i>
Individual	Bodø/Drøbak	0.17	0.1227
Fluctuating asymmetry	Bodø/Drøbak	0.51	< 0.0001
Error	Bodø/Drøbak	0.18	0.3423

ARE SOME PARTS OF THE TEST MORE DEVELOPMENTALLY VARIABLE AND/OR UNSTABLE THAN OTHERS (I.E. ARE SOME PARTS LESS CONSTRAINED)?

FA was detected to be statistically significant for distances, centroid size and shape asymmetry in both samples, whereas it is statistically absent for individual plate surfaces. Thus, significant FA values for the entire OPA could suggest an additive effect of incon-



**Figure 6.** Factorial weights of Procrustes residuals on the first axis of the principal components analyses performed on variability, fluctuating asymmetry and error matrices in the two samples.

**Table 6.** Review of FA levels for the different descriptors and approaches

Descriptors	Bodø	Drøbak
Number of plate surfaces	0	0
Number of distances	4 (1–3, 4–5, 10–11, 2–12)	6
Size asymmetry	s	S
Shape asymmetry	S	s

s, lowest FA10 value; S, highest FA10 value.

spicuous asymmetries that are statistically non-significant at the plate level. The only two non-significant distances observed in Bodø correspond to short distances implying one or two plates. In addition, Table 2 shows higher FA10 and VAR values for distance 2–12 than for the other distances. This can be explained by the longer distance separating landmarks 2 and 12 compared to any of the other landmarks, in addition to distance 2–12 being positioned according to the growth axis of the OPA. This pattern is also consistent with the higher FA and VAR values presented by the anterior–posterior (*y* coordinates) than by the lateral–medial (*x* coordinates) directions of vectors in Figure 6. Accordingly, the length of the ambulacrum appears to be more sensitive to variability and developmental instability than the width. The elongated shape of posterior plates (as discussed above) certainly reinforces this hypothesis. FA expression could be particularly influenced by the antero-posterior stretching of ambulacral plates during growth. In other words, developmental instability could be more important when growth is locally more pronounced in a given direction.

Such results have to be considered with respect to the morphological descriptors used. Indeed, significant FA values were detected for distances and the Procrustes approach (i.e. for methods using landmarks as descriptors of plate boundaries). When the growth of a given plate is locally disrupted, the corresponding space is filled in by the adjacent plates in such a way that the overall OPA is not reduced in size. It works as a compensation phenomenon between plates that induces random fluctuations of plate boundaries and guarantees a certain robustness of the overall OPA shape. Local variations of plate boundaries may not necessarily be under any developmental control, but are only triggered by local and random disturbances of plate development. Hence, more than plate surfaces, plate boundaries appear to be the favoured place of FA expression, which means that methods using descriptors of plate boundaries (such as landmarks) may be more pertinent for assessing developmental instability in *E. flavescens*.

#### IS THERE A GRADUAL CHANGE ALONG THE AMBULACRA ACCORDING TO THE AGE OF THE PLATES?

Among the 12 vectors that depict the factorial weights of Procrustes residuals on the first axis of the PCA performed on the FA matrix (Fig. 6), a stronger weight (i.e. a greater length) characterizes the vectors of the posterior part of the OPA (vectors 6–12), in both localities. In other words, FA values increase from the anterior to the posterior part of the OPA. If FA results from the cumulative effect of numerous tiny developmental accidents during growth, a decrease of FA level from the anterior (first plates formed) to the posterior (younger plates) part of the OPA may be expected. The observed pattern contradicts the increase in developmental instability with growth, but it may be explained by the larger size and the more elongated shape of the posterior plates of the OPA. These posterior plates are slightly younger than the anterior ones; nevertheless, they grow more and undergo a stronger stretching and re-modelling of their shape during growth than the anterior plates. In other words, posterior plates are younger but undergo faster growth than older anterior ones, which may suggest that growth speed and growth increments are more auspicious than age with respect to cumulative departures from perfect symmetry.

#### FA AND INTERINDIVIDUAL VARIATIONS

The statistically significant correlations between FA covariance matrices of both samples, as well as between FA and VAR covariance matrices within each sample, suggest that, in *Echinocardium*, the expression of morphological variability is partly constrained and that variability shows similar patterns between specimens and between the sides of specimens. The idea of a constrained expression of FA is supported by the stronger FA values presented by anterior–posterior directions of vectors on the first PCA. This rather good correspondence between the patterns of FA and variation among individuals is in agreement with several previous studies (Leamy, 1993; Klingenberg & McIntyre, 1998; Klingenberg & Zaklan, 2000; Klingenberg *et al.*, 2001). After Klingenberg & McIntyre (1998), the congruence between the patterns of variation of FA and interindividual variability could be an indication that the same developmental processes are involved; but see also Klingenberg (2004) for a review.

These initial results concerning the developmental stability of a spatangoid sea urchin demonstrate that their plate architecture is a suitable characteristic for the calculation of FA. The results open promising avenues of research for using such benthic animals to assess the influence of environmental alteration

(pollution) on marine fauna, as well as for studying the ontogeny of FA.

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