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SHORT COMMUNICATION

Approximate bilateral symmetry in evaporation-induced polycrystalline structures from droplets of wheat grain leakages and fluctuating asymmetry as quality indicator

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Abstract The present paper reports on an observation that dendrite-like polycrystalline structures from evaporating droplets of wheat grain leakages exhibit bilateral symmetry. The exactness of this symmetry, measured by means of fluctuating asymmetry, varies depending on the cultivar and stress factor influence, and seems to correspond to the seed germination rate. In the bodies of plants, animals, and humans, the exactness of bilateral symmetry is known to reflect the environmental conditions of an organism's growth, its health, and its success in sexual selection. In polycrystalline structures, formed under the same conditions, the symmetry exactness depends on the properties of the crystallizing solution such as the composition and viscosity; however, it has never been associated with sample quality. We hypothesize here that, as in living nature, the exactness of approximate bilateral symmetry might be considered a quality indicator also in crystallographic methods applied to food quality analysis.

Keywords Droplet evaporation method · Biocrystallization method · Bilateral symmetry · Fluctuating asymmetry · Polycrystalline structures · Arsenic stress

Introduction

In physics, symmetry concepts play a central role describing different types of regularities present in the structure of systems (Naviliat-Cuncic 2005). Bilateral symmetry (BS) is one such regularity, omnipresent in the world around us. In the bodies of plants, animals, and humans, the exactness of this symmetry, i.e., the degree in which one side of the body corresponds to the other, measured by means of fluctuating asymmetry (FA), has been found to reflect an organism's developmental instability (Leung et al. 2000) and thus its ability to develop the appropriate species-specific character despite environmental perturbations (Gangestand and Thornhill 1999). FA serves, therefore, as an indicator of the environmental conditions of the organism's growth (Knierim et al. 2007), the organism's health (Cornelissen and Stiling 2005), its success in sexual selection (Moller and Eriksson 1994), and, in the case of humans, even its beauty and intelligence (Bates 2007). Although not as commonly as in living organisms, BS can also be observed in inanimate nature: for instance, in some minerals (Hazen 2004). It has been experimentally shown by Li et al. (2007) that in certain circumstances even polycrystalline structures can take surprisingly exact BS forms. As described by Granasy et al. (2003, 2004), the symmetric growth of polycrystalline structures is associated with the ordering capacities of the crystallizing species, whereas deviations from this optimal structure can be caused by the presence of impurities (Granasy et al. 2003) or by small rotational–translational mobility of particles (Granasy et al. 2004). The crystallization process thus becomes a "competition" between the

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ordering dendrite growth and the disordering influences of heterogeneities: impurities can deflect dendrite tips, whereas the small rotational–translational mobility of particles causes the particles to have difficulty in aligning with the parent crystal at the liquid–solid interface. Both cases lead to formation of a disordered, irregular large-scale structure containing "curly" dendrites (Lee and Losert, unpublished results in Granasy et al. 2003) and ramifications which do not match the initial orientation of the crystal. Interestingly, these features are known to also characterize structures deriving from the evaporation-induced crystallization of extracts from low-quality crops in the copper chloride crystallization or biocrystallization method (BCM; Huber et al. 2010).

The sensitivity of evaporation-induced crystallization methods, such as the droplet evaporation method (DEM; Yakhno et al. 2005), and the aforementioned BCM (Baumgartner et al. 2012), to the quality and health of the analyzed specimen, has been proved in many studies concerning food quality analysis (Yakhno et al. 2005; Kahl et al. 2009; Busscher et al. 2010; Kokornaczyk et al. 2011) and diagnostics (Shibata et al. 2000; Rapis 2002). However, until now there have been no explanations of the mechanisms involved in the pattern creation in terms of health and quality (Heaton 2001; Kokornaczyk et al. 2011).

In the present paper, we report on an observation that polycrystalline structures from evaporated droplets of seed leakages prepared out of seeds subjected to a stress treatment with arsenic trioxide (As_2O_3) exhibit higher FA with respect to the control. Varying degrees of FA were also detected when different wheat cultivars were tested. In the light of these results, we hypothesize that, as in living nature, the exactness of BS might reflect the sample quality in terms of environmental stress and intervarietal characteristics also in the case of polycrystalline structures. In order to support our hypothesis with traditional methods of seed quality analysis, we tested the samples by means of the germination test, which is known to characterize the seed viability (Milosevic et al. 2010): the germination rate corresponded to the FA values.

Materials and methods

Plant material and stress treatment

The analyzed wheat cultivars (Inalettabile, Gentil Rosso, Pandas, Benco, Nobel) belonged to the species *Triticum aestivum* L. and were grown in the same location at the experimental farm of the University of Bologna, Cadriano (latitude 44° 33 N and longitude 11° 21 E, 32 m above sea level), Italy, during the growing season 2007–2008. A part of the cv., Pandas seeds was stressed by immersion for 30 min in a 0.1 % As_2O_3 aqueous solution and by subsequent rinsing under running tap water for another 60 min. Seeds were allowed to dry in ambient air and stored in darkness until used in the experiments (Brizzi et al. 2011).

Droplet evaporation method

The experimental procedure is described in detail in Kokornaczyk et al. (2011). In short: whole seeds were washed, weighed, placed in test tubes, and watered with ultrapure water in *w*/*v* proportion 1:20. After 1 h, leakage drops were collected using a micropipette, placed on a clean microscope slide, and allowed to dry in a thermostat at 25 °C and UV light. The residues were then photographed under a dark field microscope MT4300H, MEIJI Techno, Saitama, Japan, with a connected CMOS Camera UK1175-C, EHD imaging GmbH, Damme, Germany, in QXGA (2,048× 1,536) resolution, and magnification 100×. The experiment was repeated four times with a total of 480 droplet residues (six samples, five droplet residues per replicate, four replicates, four experimentation days).

FA measurement

The FA measurement was performed blind on coded pattern images in magnification 100× by means of the software ImageJ (Collins 2007). The images were searched visually for the presence of BS structures (examples are depicted in Fig. 1a–f); images without BS structures were discarded. BS structures in remaining images were preselected on the basis of clearly defined end-points of the first-order ramifications. Subsequently, in up to three randomly chosen BS structures per image, the first-order ramification lengths were measured by means of the segmented line tool: L_1, length of the upper left branch; L_2, length of the lower left branch; L_1', length of the upper right branch; and L_2', length of the lower right branch (Fig. 1a). The method for FA calculation was adopted from Wilsey et al. (1998); the FA value was calculated for each structure, as follows:

$$FA = \frac{|L_1 - L_1'|}{L_1 + L_1'} + \frac{|L_2 - L_2'|}{L_2 + L_2'}$$

FA is a parameter inversely correlated to BS.

Germination test

The germination test (Cao et al. 2008) was conducted in three replicates, each consisting of 50 seeds placed in a Petri dish (120 mm diameter) containing wet sterile sand (50 g of sterile sand and 15 ml of ultrapure water). The seeds were incubated in a thermostat at 15 °C in dark conditions until all shoots reached ca. 5 mm in length (achieved after 5 days).

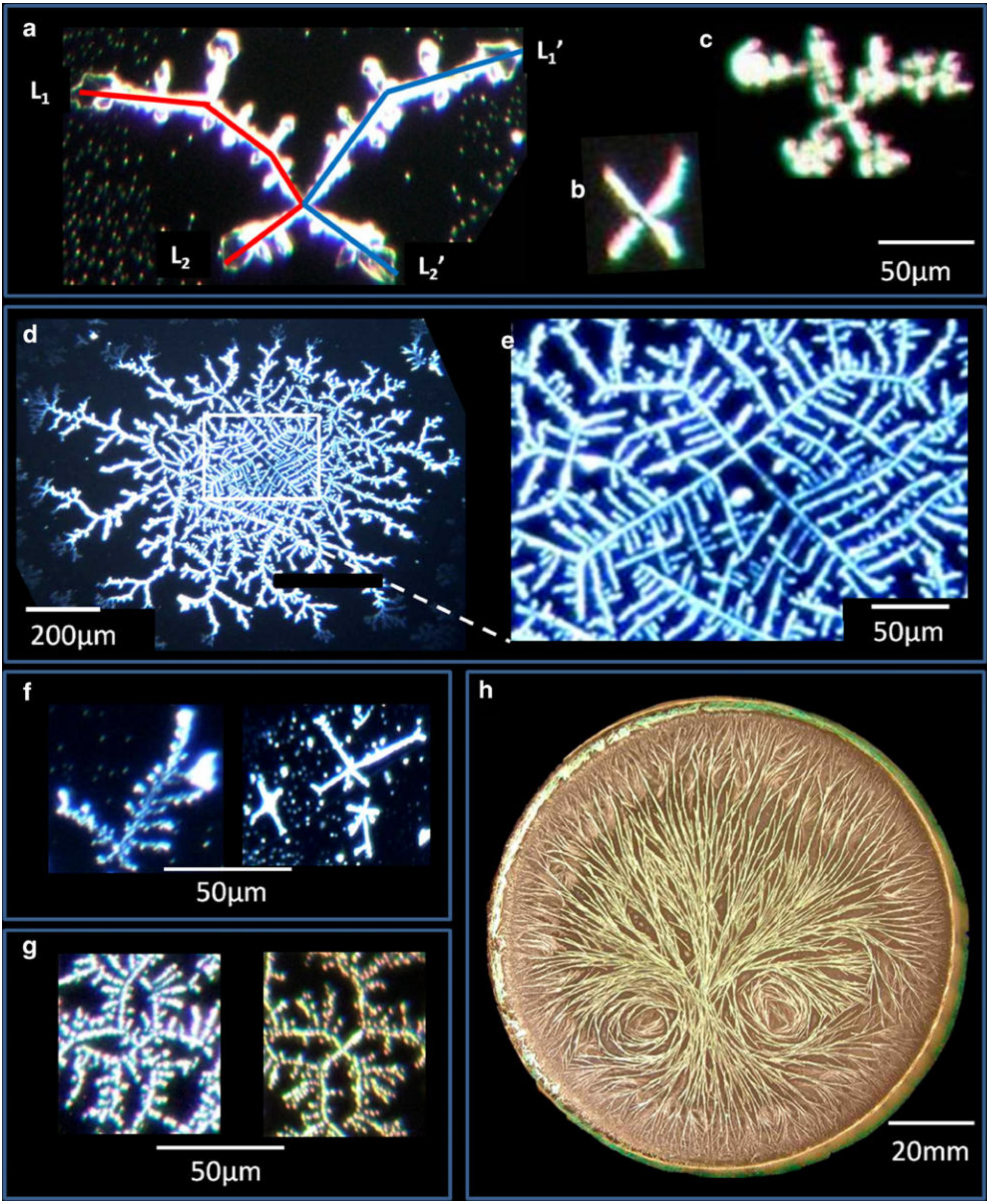

Fig. 1 Examples of BS in polycrystalline structures from evaporating droplets of wheat seed leakages (**a**–**g**) and BCM (**h**). **a** FA measurements: letters indicate the measured lengths of both symmetric branch pairs (L_1 and L_1', L_2 and L_2'). **b**, **c** Cross-like, separately placed BS structures. **d**, **e** Example of a whole polycrystalline structure (**d**) and a magnification of the BS structure (**e**). **e**, **f** Polycrystalline structures formed from nonstressed (**e**) and stressed (**f**) cv. Pandas seeds. **g** Branched structures exhibiting fourfold symmetry. **h** BS in a biocrystallization pattern

The number of germinated seeds was counted daily. The germination rate was expressed as the percentage of geminated seeds. The germination test was repeated twice.

Statistical analysis

All experiments were carried out according to a completely randomized design. The data were log transformed and analyzed by means of the Bartlett test and analysis of variance (CoStat, version 6.400). Multiple mean comparison was carried out by Turkey's honestly significant difference test. The Bartlett test for variance homogeneity was not significant; data are presented as not transformed.

Results and discussion

The crystalline structures, which can be observed inside the residues obtained from evaporated droplets of wheat seed leakages, contain, among other forms, cross-like crystals (Fig. 1b, c). These crystals serve as beginning points for further creation of branches (Fig. 1d, e) giving rise to structures exhibiting approximate BS. The exactness of this symmetry, measured by means of FA, seems to correspond to germination rate. In fact, as shown in Table 1, the stress treatment performed on cv. Pandas seeds induced both a significant increase of FA in the crystalline structures (i.e., less exact BS) with respect to the nonstressed control and a significant decrease of the germination rate. Figure 1 depicts examples of polycrystalline structures from nonstressed (d, e) and stressed (f) seeds. As regards intervarietal characteristics, the cultivars showing a higher germination rate created BS structures with significantly lower FA values (i.e., more symmetric structures) than those with lower germination rate (Table 2).

The DEM images obtained in the present experiment corresponded in their essential features to those reported in Kokornaczyk et al. (2011). The BS structures were present in 94.2 % of the 480 patterns (85.8 % of patterns was considered for the analysis, 8.4 % contained BS structures without clearly defined end-points of the first-order ramifications, and 5.8 % contained no BS structures). The creation of the BS forms seems to originate from fourfold symmetric forms, which via symmetry breaking (Naviliat-Cuncic 2005) mainly develop into BS structures. In a few cases (14 structures in 480 patterns), we observed branched, rosette-like structures exhibiting fourfold symmetry, where the above-lying lateral branches and lower branches seemed to resemble the BS forms (Fig. 1g).

Table 1 Changes in FA values of the polycrystalline structures from evaporating droplets of seed leakages and the germination rate (percent) of cv. Pandas induced by 0.1 % As_2O_3 stress vs. control seeds (unstressed)

Samples	*N*	FA	SE	*N*	Germination rate (%)	SE
Control	70	0.20 b	0.01	6	85.00 a	0.93
Stressed seeds	70	0.48 a	0.02	6	72.90 b	1.21

Different letters indicate significant differences at $p<0.05$

N number of samples, *SE* standard error

Table 2 Mean FA values of the polycrystalline structures from evaporating droplets of seed leakages and the germination rate (percent) for the five analyzed wheat cultivars

Wheat cultivars	*N*	FA	SE	*N*	Germination rate (%)	SE
Gentil Rosso	70	0.14 c	0.01	6	96.40 a	0.68
Inallettabile	70	0.15 c	0.01	6	95.80 a	1.82
Pandas	70	0.20 b	0.01	6	85.00 b	0.93
Benco	70	0.26 a	0.02	6	88.50 b	1.04
Nobel	70	0.27 a	0.02	6	78.10 b	1.90

Different letters indicate significant differences at $p<0.05$

N number of samples, *SE* standard error

Structures exhibiting BS can also be observed in BCM patterns. In a biocrystallogram, the crystalline structures are organized around one or more centers created from a lemniscate-like shape, from which midpoint branches depart towards the pattern's border; normally, the center is placed beneath the geometrical center of the pattern (Engqvist 1975), a trait which promotes BS rather than radial symmetry. An example of BS in a BCM pattern is shown in Fig. 1h (Kokornaczyk 2008).

Conclusions

Our results show that polycrystalline structures obtained from evaporating droplets of wheat seed leakages exhibit approximate bilateral symmetry. Furthermore, the FA values of these structures correspond to the seed germination rate and prove to be sensitive to both stress- and cultivar-related changes in seed viability. These first results suggest that, as in living nature, FA might serve as a quality indicator also in polycrystalline structures deriving from DEM. Moreover, the FA measurement could serve as an underpinning of the visual analysis of BCM patterns. BS features are normally sensed as harmonious and well-balanced, both desirable peculiarities in visual evaluation (for instance in the integrity criteria; Huber et al. 2010). Further investigations comparing the potential of FA measurement in DEM with other established methods applied to food quality analysis, like BCM, capillary dynamolysis (Zalecka et al. 2010), and animal food preference experiments (Woese et al. 1997) would be important to better explore the concept described here in relation to food quality.

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