Fluctuating asymmetry: a viable indicator of stress on reef-building corals?

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ABSTRACT

Small, random deviations from perfect syrnmetry.(referred to as fluctuating asymmetry, or FA) are considered to be a result of minor developmental accidents, and have been shown to reflect the developmental stability of an organism and the influence of the environment upon it. FA theory has mostly been applied to bilaterally symmetrical animals and plants, with *only* a few published studies of radially symmetrical organisms. To test for FA in coral polyps, close-up photographic samples were taken of the scleractinian corals *Montastrea annularis* (columnar and massive morphs) *from* three locations around Utila Island, Honduras; and *Diploastrea heliopora* and *Pavia* speciosa from three sites around Singapore. Measurements were taken from projected images and analysed for symmetry. Although many scleractinian coral polyps appear to be radially symmetrical, the application of FA methods to the above species revealed that their polyps were antisymmetrical, and therefore not suitable subjects for this technique. The measures of symmetry used were novel and could be applied to other FA studies *of* radial organisms.

Keywords fluctuating asymmetry, Scleractinian corals, Photographic technique, Environmental stress, Honduras, Singapore.

Introduction

Fluctuating asymmetry (FA) refers to small, non-directional, deviations from perfect symmetry. These deviations are thought to be a result of minor developmental accidents (Van Valen 1962). Such accidents are caused by developmental noise, of either genetic or environmental origin, that has failed to be buffered by an organism's ability to correct for that noise, i.e. the organism's developmental stability. Thus, FA reflects the developmental stability of an organism (and therefore its genetic quality) and/or the effect of the environment.

FA has been measured in a wide biota including plants (Kozlov et at. 1996, Heard et al. 1998, Wilsey et al. 1998), insects (Clarke and McKenzie 1992, Kokko et al. 1996, Bjorksten et at. 2000), fish (Leary et al. 1985, Wiener 1987, Campbell and Emlen 1996), birds (Molter 1995, Teather 1996, Quek et al. 1999), mammals (Wauters et al. 1996, Lagesen and Folstad 1998, Leamy 1999) and humans (Waynforth 1998, Trivers et al. 1999). In all these examples, FA has been based on slight differences in size between a bilaterally symmetrical character, e.g. length of right wing minus length of left wing (usually referred to simply as 'right minus left' or R-L). The assumption is that both sides are under the infuence of identical genetic and environmental factors, therefore any fluctuation is an indication of developmental instability (Clarke 1992). Moller and Eriksson (1994) were the first to experiment with organisms that exhibit true radial symmetry (polysymmetry). In their study of flowers (and more recently, Moller 1996, Moller 1998, Moller and Sorci 1998), the longest and the shortest petals were selected, these were then treated as right and left sides in the calculations.

Many scleractinian corals have polyps that appear radially symmetrical, possessing even numbers of septa and seemingly circular body plans. This was the case for the scleractinian coral species studied here: *Diplosatrea heliopora,* Favia *speciosa* (Veron pers comm.) and the columnar and massive morphotypes of Mont agree, *annularis* (Van Veghel and Bak 1993). The species were chosen because of their relatively large and well-defined polyps, the symmetry of which could be measured using a non-destructive photographic technique (Todd et at. 2001a, Todd et al. 2001b). To take similar measurements from corals with smaller polyps, the tissue would need to be removed and the skeletal structure of each corallite examined under a microscope.

The sampling sites were selected to reflect hydraulic energy and sediment gradients as these are believed to influence coral growth. Hydraulic energy has been shown to affect skeletal bulk density (Scoffin et al. 1991) and colony growth form (Chappel 1980). Sedimentation is known to have deleterious effects on reefs (Rogers 1990) and can also affect coral morphology at both colony and polyp level (Lasker 1980, Dodge 1982).

FA has the potential to provide information on the developmental stability of an organism and the effects of the environment upon it, information that could be useful to coral research. The aim of this preliminary study was to use a photographic technique and novel symmetry measurements to determine the suitability of these three coral species as subjects for FA analysis.

Methods

Close-up photographic samples were taken of three coral species and the, resultant images analysed for symmetry. For each species, with the columnar and massive morphs of *M. annularis* separated, the samples from all sites were pooled to give a general indication of symmetry levels. As all species turned out to be

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antisymmetrical the study was not taken further, i.e. the data for each taxa were not divided into sites. [1]

Sampling sites

Deep and shallow *M. annularis* colonies were sampled from three locations around Utila, a small island off the north coast of Honduras, in July 1996.'The sites were Silver Gardens (016° 04.933' N, 086° 55.050' W) and Cabanas (016° 04.300' N, 086° 57.117' W) on the southern coast, and Turtle Harbour (016° 06.712' N, 086° 56.950' W) on the more exposed north side. These sites were originally selected for another study, but there were clear differences in wave action and thus -hydraulic energy between the more exposed northern site (prevalent NE winds) and the protected southern sites, especially in the shallower depths. The F. speciosa and *D. heliopora* samples were collected in August 2000, from three reefs off the south coast of Singapore: Cyrene Reef (01° 15.240' N, 103° 45.524' E), Pulau Hantu (01° 13.635' N, 103° 44.797' E) and Raffles Lighthouse (01° 0.477' N, 103° 44.495"E). These reefs represented a sediment gradient — Cyrene Reef being closest to shore (4 km) and most heavily impacted by sedimentation from land reclamation and dredging operations, while Raffles Lighthouse was furthest offshore (15 km) and the least affected by these activities' (Low and Chou 1994).

Sampling strategy

Around Utila Island, *M.' annularis* (columnar) was photographically sampled front a depth range of 3 m to 10 m, whereas *Al. annularis* (massive) was sampled from 20 m-30 m. Nine colonies of both morphs were sampled from each of the three sites around the island (27 colonies of each morph). From each of the three reefs off Singapore's southern coast, photographs of 10 colonies of F. speciosa were collected from a 3-6 m depth range (30 colonies in total); and four colonies of *D. 'heliopora* were sampled in depths of *less* than 5 m (12 colonies in total). Photographs of the coral polyps were taken with a Nikonos V underwater camera loaded with Fujichronie `Velvia' colour slide film (ASA '50). Ocean Optics (London) extension tubes and framers were attached to the camera to provide 2:1 images of F. *speciosa* and *D. heliopora* and I:1 images of, the smaller polyped, *M. annularis.* The corals were sampled between.10:00 and 15:00, when their polyps were fully deflated. Only the most central and seaward-facing area was.phdtographed to avoid the implications of any intracolonial variation in polyp morphology (Veron 1995).

Symmetry measures

Each image, representing one colony, was projected to 30 x *(M. annularis)* or 15 x (F. *speciosa* and *D. heliopora)* actual size, and five polyps were randomly selected for analysis. Therefore, 135 polyps were measured for each of the two *M. annularis* morphs, 150 polyps for F. *speciosa,* and 60 polyps for *D. heliopora.* The corallites of Al *annularis* had six distinct primary septa which, when the polyp was fully retracted, could still be clearly distinguished with the living tissue. attached. When the slide images of *M. annularis* were enlarged by projection, it was a simple process to trace around the six tissue-covered septa (from now on referred to as t-c septa) with a 0.3 mm pencil directly onto A2 plain paper. These outlines formed the basis of the symmetry measurements.

The t-c septa of F. *speciosa* and *D. heliopora* were also distinct and could be. outlined as above, but no primary septa were apparent when the tissue was still attached. To collect symmetry measures from these two species, in individual t-c septa was chosen at random, and its opposite number discerned by counting around the remaining t-c septa. Thus, if there were 24 t-c septa, I and 12 would be considered opposite. To find the pair of t-c septa that should be perpendicular to the first pair Of the polyp was truly symmetrical), t-c septa 6 and 18 were counted off. This approach meant that only polyps with t-c septa in multiples of four could be included in the analysis.

For all species, the centre of each t-c septa tip was established using an overhead projector sheet with concentric circles printed upon it. This was positioned over the outline of the t-c septa until one of the circles made a good match with the curve of the tip, a central dot was then marked and lines drawn between opposite pairs (Fig. la, c). A `centre of polyp' point was determined from the intersection of these lines (more lines would usually be included to help establish a reasonable approximation of the centre point). Measurements were then made, with vernier calipers, from each of the t-c septa tips to the polyp centre (Fig. lb, d).

Past studies of radial FA have chosen the most disparate segments of the organism, e.g. the longest and shortest petal of a flower, and treated these as right and left (Moller and Eriksson 1994, Moller 1996, Moller and Sorci 1998). This approach was considered for the present study, but there was some doubt as to whether or not the polyps being studied were truly polysyrnmetrical. Photographs reveal the shape of the mouth to be a slit rather than a perfect circle; suggesting that they might not be fully radial, but instead bi-radial, i.e. rather than having many lines of symmetry, they *only* had two lines perpendicular to each other. With the possibility that the polyps were bi-radial, the application of past protocols was considered inappropriate. Instead, a new approach was performed where t-c septa tip to polyp centre measurements were paired so differences between opposing t-c septa could be calculated. In a perfectly bi-radial organism, all opposite pairs of characters around the centre point should be equal. Furthermore, all measurements were included in the analysis, three pairs *for M. annularis* and two pairs for F. *speciosa* and *D. heliopora.* The average difference was calculated for the whole polyp and then adjusted for polyp size to give an FA index (relative FA). As there was *no* obvious right and left t-c septa 'the smallest distance was always subtracted from the largest, consequently there were no negative numbers in the results.

As it was possible for all pairs of distances from t-c septa tip to polyp centre to be equal, but for the polyp to still be antisymmetrical, the angles between intersecting lines were measured to 0.5^0 with a protractor. Three angles of *M. annularis* were available for measurement and two of *F. speciosa* and *D. heliopora* (Fig.1e, f). For each polyp, the smallest angle was subtracted from the greatest and the results also used in the FA analysis.

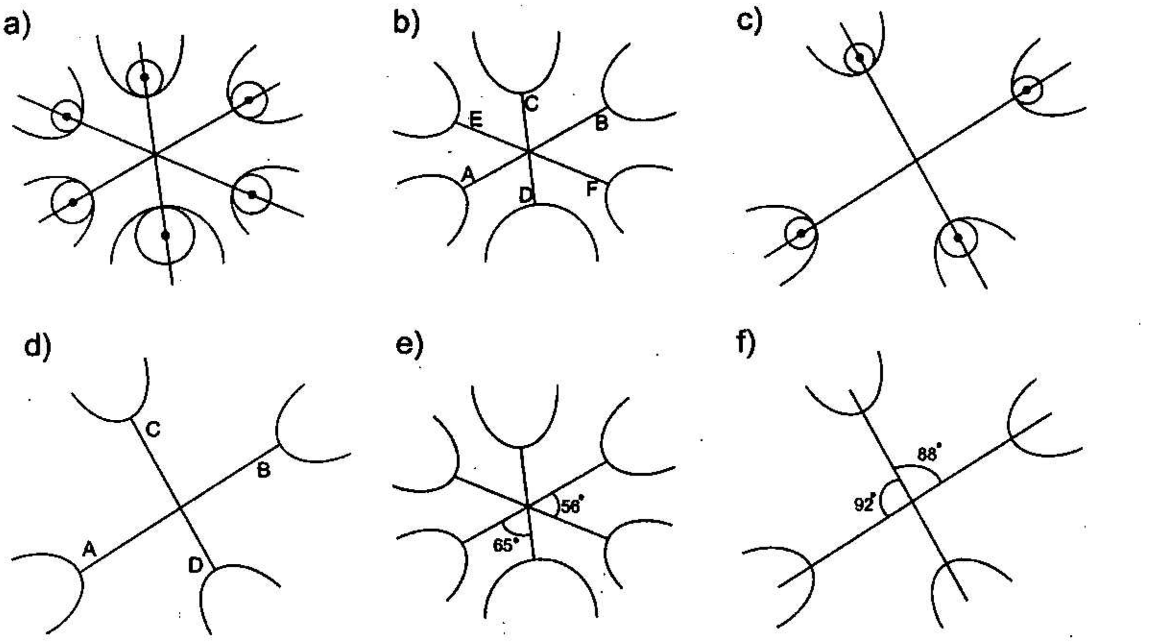

Fig. 1 The measurements on which the FA analysis was based; a) connecting lines between opposing pairs of t-c septa tip centres for *M. annularis*, b) t-c septa tip to polyp centre measurements for *M. annularis*, c) & d) are the same measurements as above for *F. speciosa* and *D. heliopora*, e) angle measures between intersecting lines for *M. annularis*, f) angle measures between intersecting lines for *F. speciosa* and *D. heliopora*.

Measurement error (ME) can produce false significant FA results or cause actual FA to appear as antisymmetry (Palmer and Strobeck 1986, Van Dongen 1999). Previous experiments, testing the precision of the photographic technique described here, demonstrated that the coefficient of variation for dimensional measurements of *F. speciosa* (repeated ten times) were in the range of 1.94 % - 2.06 % (Todd et al. 2001b). Such low ME could not distort true FA to the level of antisymmetry described in our findings. Polyps not perpendicular to the camera, if measured, could also distort results. Efforts were made to ensure that non-perpendicular polyps were excluded from the study, although this was essentially a subjective decision based upon lighting, shadow and focus.

Results

When looking at FA in a fundamentally symmetrical organism, for example a butterfly, the spread of signed R-L results (i.e. with positive and negative numbers) should be normally distributed around a mean of zero (Palmer and Strobeck 1992) (Fig. 2a). Or, in the case of unsigned R-L as here, where all data is shown on the positive side of the y-axis, the distribution should be half of a bell curve (Fig. 2b). Any significant departure from this distribution indicates a possible genetic basis for asymmetry, and therefore cannot be used to describe FA (Palmer and Strobeck 1992). Such departures usually fall into one of two categories: *directional asymmetry* (Fig. 2c, d) occurs when the mean size of one side of the body is usually significantly greater than the other; *antisymmetry* (Fig. 2e, f) also refers to a significant difference between sides, but it is unpredictable which side will be the more developed (Van Valen 1962).

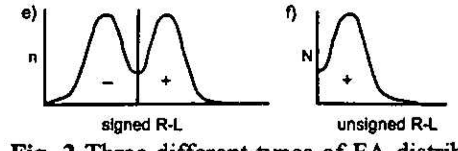

Fig. 2 Three different types of FA distributions; a) & b) true fluctuating asymmetry for signed and unsigned differences, c) & d) directional asymmetry for signed and unsigned differences, e) & f) antisymmetry for signed and unsigned differences (adapted from Palmer and Strobeck 1992). Note: the data for b), d) & f) are not the same as the data for a), c) & e); if the same data was used the shapes of the curves for b), d) & f) would retain the same pattern but be stretched upwards along the y-axis.

For every species, the data from all sampling sites were pooled to give a general impression of symmetry. Frequency distributions were plotted for the differences in distances from t-c septa tips to polyp centres and the greatest differences in angles between intersecting lines. The shapes of the resulting histograms for each species and the two morphs of *M. annularis* clearly indicate antisymmetry (Figs. 3, 4). They show that only rarely are there minimal differences in distances from t-c septa tip to polyp centre, or between angles, indicating that only rarely are the polyps truly symmetrical.

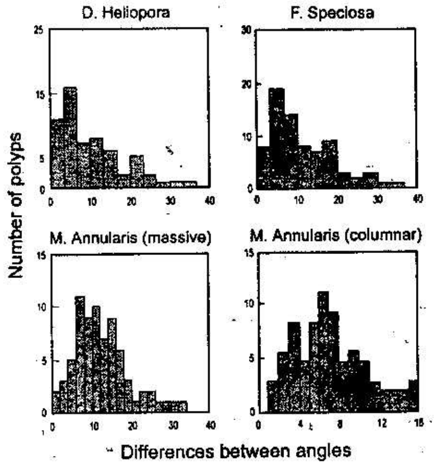

Fig. 3 Distribution of differences between distances of t-c septa tip to polyp centres for opposing pairs of t-c septa.

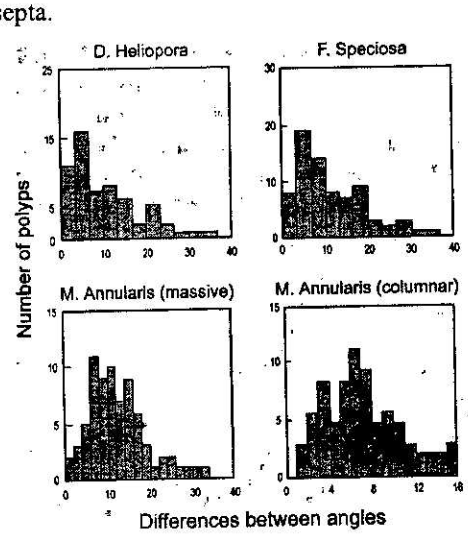

Fig. 4 Distribution of differences between angles for intersecting lines connecting t-c septa tip centres.

Discussion

The photographic technique enabled the efficient collection of numerous samples in a minimum of diving time. The resultant images were of high resolution and suitable for analysis when enlarged by projection. The measurements taken, distance from t-c septa tip to polyp centre and angles between intersecting lines, were novel as FA parameters but both were effective at discerning levels of symmetry. This was a preliminary project to determine if the study species were appropriate subjects for a more detailed FA investigation. The results clearly demonstrate that the polyps of these corals were antisymmetrical and therefore not suitable for further analysis. The species for this project were primarily chosen for their large and ostensibly circular polyps. Other species may have polyps that are truly symmetrical and thus more apposite for FA tests. As the photographic method is hampered by the limited number of corals to which it is applicable, studies of alternative species may need to be carried out on tissue-free skeletons. Measurements from cleaned skeletons would also provide greater accuracy necessary for a thorough FA investigation (Van Dongen 1999). There could be some environmentally correlated differences between colonies or populations in overall antisymmetry that may be worthwhile investigating in conjunction with other morphometric traits. This approach is a departure from FA theory and, due to the lack of *a priori* symmetry, it cannot be used as an indication of developmental stability (Palmer and Strobeck 1992).

Angle measures have not been used before to test for FA, however they yield valuable information about symmetry and should be considered for other FA studies on polysymmetrical organisms as simple distance or length measures can be misleading. Using angles also avoids the problems often associated with size scaling (Palmer and Strobeck 1986, Moller 1993, Sullivan et al. 1993), or with finding the true centre of an organism.

It is apparent that although the polyps of these species may look symmetrical, they are not. Perfect radial symmetry is possibly an ideal structure for a polyp as a circular form provides the best tissue mass to surface area ratio and offers equally balanced omnidirectional feeding (Ryland and Warner 1986). However, there are various physical constraints that can greatly affect polyp symmetry. Asexual budding is probably the most ubiquitous distorting effect, as both intra or extratentacular budding involves 'squeezing in' necessitating some adjustment by surrounding polyps. Colonies of encrusting species adhere and conform to the contour of the underlying substrate, and therefore, so must their polyps (Marfenin 1997). It is clear from casual observations that boring organisms have a notable deforming effect on surrounding polyps. Finally, micro-environmental factors such as sedimentation, water movement, food availability, and the direction of down-welling light may also have a significant influence (Hubbard and Pocock 1972, Marfenin 1997, Kim and Lasker 1998). All examples of the physical constraints mentioned above are primarily a result of the colonial nature of the corals sampled. As the majority of colonial corals are exposed to similar conditions, it is probable that most species will not possess polyps sufficiently symmetrical for FA studies.

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