



Proceedings of the 1st International Symposium

# BIOLOGICAL SHAPE ANALYSIS

**Pete E Lestrel**  
*editor*

 **World Scientific**

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**Proceedings of the 1st International Symposium**

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**Pete E Lestrel**

*University of California, Los Angeles, USA*

*editor*



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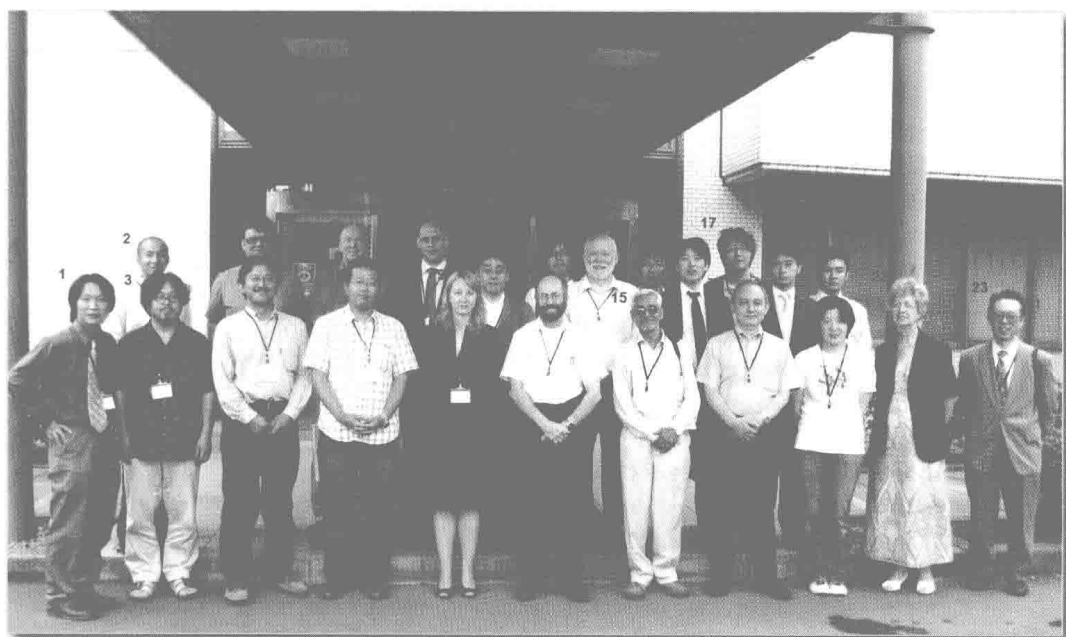
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# **BIOLOGICAL SHAPE ANALYSIS**

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## The First International Symposium of Biological Shape Analysis

June 3-5, 2009  
Tsukuba, Japan

**Organizers: H. Iwata, S. Ninomiya and P.E. Lestrel**

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## Preface

It is with great pleasure that I write this introduction to the Proceedings of the First International Symposium of Biological Shape Analysis (ISBSA). On the 25<sup>th</sup> of August, 2006, I was invited for a half-day workshop held at the Agricultural Center in Tsukuba, Japan. At that workshop I met a number of investigators in agricultural genetics with whom, it turned out, I shared common interests; namely, an understanding of the need for the application of more adequate models and techniques for the characterization of the biological form, whether of faunal or floral origin.

A discussion at dinner after the workshop that evening with Professor Seishi Ninomiya, head of the agricultural genetics section, led to the idea of eventually convening a conference to allow for a greater exchange of ideas dealing with the quantitative analysis of the biological form across a variety of disciplines. Professor Hiroyoshi Iwata was asked if he would consider being a co-organizer with us. He readily agreed. Unfortunately, two years passed due to other commitments before we could seriously begin the planning of the conference. Hiro and I began to work closely to bring the conference to fruition. Hiro took on the responsibility of local arrangements chair, for soliciting suitable potential participants from his area of agricultural genetics, and preparing the program brochure. I began the process of inviting participants in my area of human biology (physical anthropology), preparing the registration packet, and accepting abstracts.

Participants came from not only wide-ranging disciplines such as agricultural genetics, entomology, forensics, physical anthropology, as well as mathematics, but also from diverse geographical regions. These included Australia, the Czech Republic, India, Japan, Switzerland, the U.K. and the USA. Biological shape analysis can truly be considered a global enterprise.

We met June 3-6, 2009 under the auspices of the Agricultural Center in Tsukuba. The meetings were an enormous success, both on a professional as well as a personal level. It was decided to meet again at the Agricultural Center in Tsukuba and convene the second ISBSA on June 9-11, 2011.

These Proceedings from the first ISBSA, exhibit the widespread range of disciplines involved in the quantification of the biological form, comprised of size, shape, structure, as well as other attributes ([1], p. 10). Shape is defined here as the outline or boundary of an organism or part of an organism. This diversity of disciplines reflects the essential importance of form, and more specifically shape, across a wide spectrum of the biological sciences.

The measurement of form has had a long history and can, perhaps, be traced back to the work of Dutch anatomist Petrus Camper (1722-1789). He attempted to define differences in skull shape between ethnic groups in numerical terms. Using the *facial line* (drawn from the forehead to the upper lip) Camper constructed the *facial angle*, (formed by the intersection of the *facial line* with a line drawn from the ear aperture to the base of the nose. He then used this *facial angle* as a measure of

prognathism<sup>1</sup>. His work on the measurement of the face, published posthumously in 1792, had a major impact on physical anthropology and influenced the subsequent and widespread use, some 100 years later, of the conventional metrical approach (CMA) consisting of distances, angles and ratios ([2], p. 100).

However, if one is specifically interested in quantifying the *shape* of the largely irregular biological forms routinely encountered in the biological sciences, the widespread use of CMA becomes an inefficient model for the representation of shape. CMA presumes that the measurements used are a reasonable analog of the actual shape. For example, consider the commonly used shape index for teeth, Mesiodistal length<sup>2</sup> (MD) divided by Buccolingual width<sup>3</sup> (BL) used to characterize the shape of dentition. The MD/BL ratio assumes that teeth can be modelled as a rectangle; however, actual teeth are never rectangles ([3], p. 418), so the representation is imprecise and results drawn from such measurements may be misleading with respect to the actual shape of teeth.

As a consequence, alternative methods are needed if a significant amount of the biological information that is inherent in all biological morphologies is to be extracted. This has led, over the last three decades, to the application of a number of comparatively new approaches such as Fourier Descriptors (FDs), Thin Plate Splines (TPS), wavelets (CWTs), etc.

The use of FDs, in particular elliptical Fourier analysis (EFA) as a model or representation of form, largely resolves the above issue of representation with CMA; namely, by providing an acceptable correspondence or mapping of the model (EFA) with the actual shape. Nevertheless, EFA has the drawback of obscuring the location of landmarks and pseudo-landmarks of potential biological import; however, this limitation can be circumvented with the use of CWTs, either alone or in conjunction with EFA [4].

At this point, it is perhaps fruitful to ask: why is "Biological Shape Analysis", the title of these Proceedings, important? The answer to this question is based on a fundamentally important aspect of biology: namely, the elucidation of *process*. Process here refers to the identification of those factor(s) that determine the change of shape of the organism as a function of both development and evolution.

It is predicated that the initial step in the explication of process necessitates a focus on the phenotype, which in turn requires the use of measurement. Historically, the need for quantification of the biological form was initially rejected as unnecessary, in large part, because of the descriptive tradition in the biological sciences. For early biologists, the taking of measurements was viewed as an unnecessary and wasteful venture. As D'Arcy Thompson originally wrote in 1917:

*The introduction of mathematical concepts into natural science has seemed to many men no mere stumbling-block, but a very parting of the ways* [5].

Nonetheless, when visual differences are not readily describable in descriptive terms because of the irregularity of the shape, this necessitates quantification:

<sup>1</sup> Prognathism is an overly prominent projection of the positional relationship of the mandible and/or maxilla with respect to the face.

<sup>2</sup> Mesiodistal length refers to the length of a tooth measured along the tooth row.

<sup>3</sup> Buccolingual width refers to the width of a tooth measured from the cheek side to the tongue side.

*In contrast to the human capability of rapidly identifying and classifying the visual information present in the biological form, the mathematical description of the content of these visual images has been slow in forthcoming ([1], p. 4).*

Thus, during both development and evolution, the biological form (essentially its size and shape), is continually changing over time; raising the question of how the biological process determines the shape as seen in the biological organism. That is:

*Directly or indirectly, form is central to our understanding of biological processes. The form mediates between internal genetic information and external environment; it is the means by which genetic information is evaluated and acted on by natural selection ([3], p. 417).*

The biological process consists of three aspects: (1) the transmission of the hereditary material to the next generation, (2) the chemical structure making up the genotype (i.e., the DNA, mtDNA, mRNA, etc.), and (3) the mechanisms by which the genotype determines the phenotype. The first two aspects have now been largely verified starting with the work of Mendel (1865) and the Watson - Crick Model (1954). The third aspect however, remains to be fully resolved.

If one is to advance the explanation of the biological processes that are involved in, for example, from the development from the haploid gametes to the mature organism, whether plant or animal, then the pathway of process → shape remains one of the core problems of biology. Thus, questions dealing with process begin with and are ultimately dependent on measurements of the phenotype.

To make this clearer, consider the variability in beak morphology among the Galapagos finches. It has been recently shown that differences in beak morphology are not only due to ecological niche exploitation, but also a consequence of the expression of the growth factor, *bmp4*. That is, different beak shapes arise as a consequence of the timing in the expression of the protein *bmp4* during development. Ground finches, those with large beaks used for cracking seeds; also produce more *bmp4* at an earlier time than cactus finches that have smaller, more pointed beaks used for retrieving nectar. Thus, beak morphology, while under intense selection, has now been also shown to be directly linked to the genetic code [6].

However, while the finch research is certainly noteworthy, it is only part of the story. The concept of topobiology coined by Noble laureate Gerald Edelman [7], needs to be mentioned. Edelman argued that knowledge of the genetic code by itself is not sufficient to explain shape. While it is obvious that the genetic code acts as a blueprint for the organism's *bauplan*, it is the spatial interactions at the cellular level, the cellular adhesional properties, and the interaction at the cellular surface during morphogenesis that ultimately determines the phenotypic shape of organisms.

As a consequence, Edelman proposed the "morphoregulation" hypothesis:

*...that relates the control of regulatory genes in cascades with actual mechanochemical processes leading to form and pattern at each stage ([7], p. 43).*

Thus, process as it determines the biological shape, whether viewed in ontogenetic or phylogenetic terms, is both spatial in Edelman's sense as well as time dependent. Clearly, a full explanation of the process that determines the biological shape from conception to adult is not yet forthcoming and will continue remain a

challenge that will keep researchers in the biological sciences occupied well into the 21<sup>st</sup> century and beyond.

Returning to these Proceedings, many of the papers are, understandably, more focused on the quantitative assessment of shape, the first step in the agenda of elucidating process. However, a number of the papers in the Proceedings reflect “state of the art” issues in biology and focus more on process. That is, they attempt to connect aspects of the shape to their underlying genetic framework. For example, papers that use quantitative trait loci analysis (QTLs) or chromosome segment substitution lines (CSSLs), include Sherwood and McNulty (cranial shape), Iwata (crop organ shape), and Ohsawa and Minagawa (soybean plants).

Papers that applied EFA, (often in conjunction with PCA) to characterize the shape, include Yoshioka (flower shape), Zheng, *et al.* (citrus leaves), Suzuki and Hirata (rice), Tatsuta and Goka (beetles), Urbanová, Gore, *et al.* (both dealing with the eye orbit), Stephan, *et al.* (clavicle), Lestrel, *et al.* (cranial vault), and Morimoto, *et al.* (long bones).

Finally, the contributions of four participants also need to be mentioned because they typify the diversity that characterized the first ISBSA conference. These include Bhagwat, *et al.* (rice kernel shape), Kondo, *et al.* (human cranium), Thomas, *et al.* (3-D facial shape) and last and certainly not least, Gielis (geometry of nature).

Special thanks go to Seishi Ninomiya for suggesting the symposium, to Hiro Iwata for his co-organizing ability and for his friendship. Thanks also go to David Thomas and Charles Wolfe for their editing assistance. Finally, thanks are especially in order for Sook Lim, Scientific Editor at World Scientific, Singapore, for agreeing to publish these Proceedings of the ISBSA. Finally, I hope that these Proceedings will be useful to those interested in the quantitative analysis of the biological form and especially its shape.

Pete E. Lestrel  
Editor

Van Nuys, California, USA, December, 2010

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# A New Behavioral Experiment using Computerized Shape Analysis of Actual Flowers

YOSUKE YOSHIOKA<sup>1</sup>

## Abstract

Variation in corolla shape has been widely observed at inter- and intra-specific levels in entomophilous plants. Many ecologists have focused on this variation in the context of co-evolution of wild plants and their pollinators. The corolla shape is considered to be an important visual cue that attracts their pollinators. However, the ability of pollinators to discriminate among the corolla shapes under natural conditions has not yet been supported by a large body of evidence. This study demonstrates the efficacy of combining a traditional behavioral experiment with computerized shape analysis of actual flowers. Elliptic Fourier descriptors were used to describe these shapes by transforming coordinate information of the contours into coefficients. Principal components analysis then transformed these coefficients to a smaller number of variables called principal components. Using these methods, artificial flowers were created based on the natural diversity of petal shape in *Primula sieboldii* and *Brassica rapa*. Dual-choice tests were then performed to investigate the ability of bumblebees (*Bombus ignitus*) to detect differences in the aspect ratio of petals. The insects showed significant ability to detect differences in the aspect ratio when the morphological distance increased. Moreover, the bumblebees showed a significant preference for narrow petals in *P. sieboldii*. The insects also detected differences in this parameter of the *B. rapa* flowers at close range. The effectiveness of this behavioral experiment was demonstrated using the actual variation present in the corolla shapes of the two plant species. Whether pollinators can discriminate among flowers based on the variation in corolla shape and whether the variation affects pollinator behavior remain fundamental questions regarding the mechanisms responsible for the formation and maintenance of diversity in these characteristics of entomophilous plants. The method proposed here could be useful in answering these questions for many other plants.

## INTRODUCTION

Variation in corolla<sup>2</sup> shape has been widely observed at inter- and intra-specific levels in entomophilous plants<sup>3</sup>. Many ecologists have focused on this variation in the context of co-evolution of wild plants and their pollinators. The shape of flower corollas is considered to be an important visual cue that attracts their pollinators. Among the several visual cues (such as color, shape and pattern) many studies have investigated the discrimination ability of pollinators with respect to color, and

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<sup>2</sup> The corolla is defined as all the petals of a flower, forming a ring around the reproductive organs.

<sup>3</sup> Entomophilous plants are those flowering plants that are pollinated by insects.

revealed that pollinators such as bees, flies and butterflies show strong preferences for a particular color, and can discriminate among different colors. The ability of pollinators to discriminate differences in shapes and patterns has also been demonstrated with several behavioral studies. In these studies, many researchers have used artificial flowers to investigate the ability of insect pollinators to discriminate among flower shapes or patterns [1, 2, 3],

Artificial flowers confer an advantage in that they allow researchers to standardize the flower characteristics on other than the target characteristic (variable of interest). However, artificial flowers as used in these studies often bear little resemblance to real flowers (e.g., they may be squares, disks, or triangles), and the ability of pollinators to discriminate among the shapes of flower corollas under natural conditions has not yet been supported by a large body of evidence. Therefore, the results of previous behavioral experiments cannot be easily applied and may not model the true interaction between flower shape and insect behavior that is observed under natural conditions.

This study is intended as a demonstration of the effectiveness of combining a traditional behavioral experiment with computerized shape analysis of actual flowers as one way to resolve this problem.

## MATERIALS AND METHODS

Bumblebee *Bombus ignitus* was chosen as a model bee species because of ease in handling during experiments. Colonies of *B. ignitus* were obtained from Arysta LifeScience Corporation (Tokyo, Japan). Note that this species is not the primary pollinator of *P. sieboldii* and *B. rapa*. Colonies were provided with pollen on a daily basis and with a sucrose solution in feeder tubes when they were not being used in the experiments. The bumblebees had no experience foraging on any artificial flowers prior to the beginning of the experiments.

### Computerized shape analysis

Digital image analysis is a computerized technique that uses software to analyze the visual information stored in a computer [4]. Recent improvements in computer performance, combined with reductions in the cost of digital imaging hardware and software, have contributed to the increasingly widespread use of digital image processing in biological and agricultural research [5].

Several approaches have been used to quantify the variation in shapes of biological organs such as seeds, leaves, fruits, and roots. The traditional approach continues to use simple measurements such as length, width, area, and perimeter. These simple measures are perhaps adequate for comparatively simple structures; moreover, such measures have the advantage of simplicity. However, they cannot, either in terms of efficiency or sufficient detail, capture features such as the shape of more complex morphologies.

A more sophisticated approach captures the underlying factors that define a shape consisting of a set of parameters derived from Fourier descriptors. One of the more effective approaches involves the use of elliptic Fourier Descriptors (EFDs)