

CHAPTER 25

Contributions of Morphometrics to Medical Entomology

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“J’ai besoin de savoir que tout n’est pas confondu. (I need to know that everything is not confounded)”

—Jean Tardieu, in *La Part de l’Ombre*

25.1 INTRODUCTION

Morphometrics quantitatively describes the morphological variation of objects. When applied to biological forms, it is a particular field of biometrics. In medical entomology, where a major interest is the biology of insects in their natural environment, morphometrics might be considered as a tool for quantifying the phenotypic variation of an organism. Morphometrics focuses on variation, its parameterization, and relation to extrinsic factors. As long as phenotypic variation has environmental and/or genetic causes, morphometrics can help detect local adaptations and genetic divergence among populations. Morphometric characters are related to growth and development, and they are usually continuous. Traditionally, they were estimates of distances between anatomical points called landmarks. More recently, they have come to be the coordinates of these landmarks in a given system of orthogonal axes. We will present here some concepts and statistical analyses related to the use of these data, insisting on their biological relevance, with some examples of applications in medical entomology. Both traditional and geometric approaches will be presented. Special attention is given to applications involving Triatominae (Hemiptera: Reduviidae), the vectors of Chagas disease in Latin America (see chapter in this book) and Phlebotominae, the vectors of leishmaniasis. Finally, some information will be given about morphometric software.

25.1.1 From Dimensions to Biology

In the absence of artifactual variation, a distance between two anatomical landmarks or their relative position to other such points (see Figs. 25.1 and 25.2), depend on the morphological development of the organism under study; their variation with geography is arguably an effect of both environmental influence and adaptive changes; and their changes from one species to another reflect the process of natural evolution. When properly analyzed, metric-trait variation allows one to read some biological and evolutionary information embedded in the morphology [79,81,91]. One of the earliest morphometric studies is illustrative. After a severe storm in February 1898, among the moribund sparrows taken to the laboratory by Bumpus, some survived, others died. Examining a few measurements of their skeleton, Bumpus showed that “the birds which perished, perished not through accident, but because they were physically disqualified,” and “the birds which survived, survived because they possessed certain physical characters” [64]. Thus, a simple set of measurements was able to illustrate the Darwinian concept of selection for the most fit. Morphometrics has this ability to make visible to us many aspects of the biology of an organism, such as its physiology, its pathology, and its phenotypic or genetic evolution.

25.1.2 Tradition and Modernity

The virtues of traditional morphometrics are today improved by the introduction of geometric techniques [83]. Morphometrics is often presented as “traditional,” making

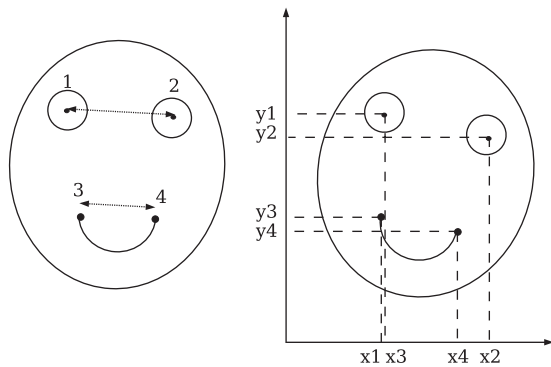


Fig. 25.1. Distances between anatomical landmarks (left) are advantageously replaced by coordinates of these landmarks in a given system of orthogonal axes (right).

use of limited sets of measurements, or “modern” (or “geometric”), making use of total geometric information (see Figs. 25.1 and 25.2). This difference, which derives mainly from the kind of metric data (distances versus coordinates), has generated a “revolution” [3,83]. Improvements or novelties exist indeed at various levels, the most important one being the direct description of shape itself. After some mathematical processing, the geometric figures represented by the landmarks are compared as different point sets between

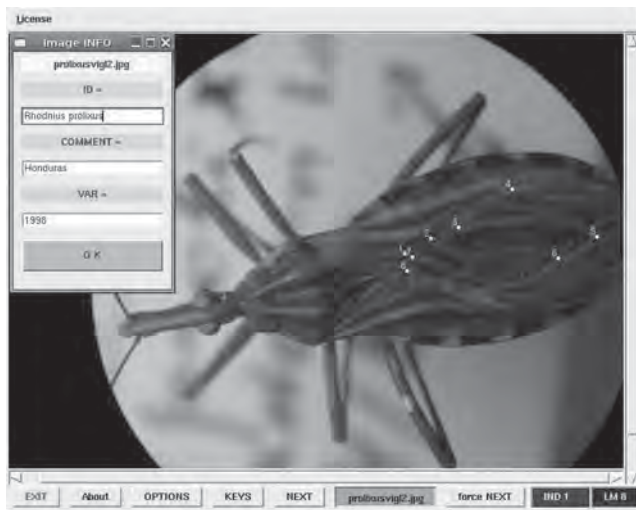


Fig. 25.2. Screenshot of a landmark collection session under the COO program (<http://www.mpl.ird.fr/morphometrics>). Top left window figures a small database gathering relevant informations. Yellow dots on the insect are the landmarks of the wing, labeled in the order of collection. The bug is an undissected, dry pinned *Rhodnius prolixus*. It is a South and Central American species of the subfamily Triatominae (Hemiptera, Reduviidae). After *Triatoma infestans* (see Fig. 25.12), it is the main vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. The collection of landmarks is performed on a digital picture, using the “mouse-click” with a dedicated program (here COO, see <http://www.mpl.ird.fr/morphometrics>; a more versatile and frequently used program is TPSdig, see <http://life.bio.sunysb.edu/morph/>).

individuals or populations. Shape can also be studied by other direct techniques exploring outlines, textures, surface patterning, or even internal configuration of a form: These techniques will not be discussed in this chapter.

In spite of these advantages and the attractiveness of modern morphometrics, it has yet to gain popularity in medical entomology, where traditions may be hard to move.

25.2 CAUSES OF METRIC VARIATION?

Morphometric variation is under the influence of physiological (or pathological) status, adaptive changes, and genetic differences. Whereas different molecular markers applied to an insect will be differently affected by the environment—and some could be completely neutral—the metric characters are generally supposed to be of both environmental and genetic origin. The drawback is that there is no magic science to make the correct partition between these ultimate causes of metric variation: The genetic make-up of a population or/and its environment. The cause of morphometric variation cannot be found in the metric variation itself, it has to be searched by other methods and may become the object of an inquiry, itself. It is, however, possible to obtain from the data some helpful insight. As a first step to remove heterogeneous environmental influences and focus more on genetic differences, one could rear a complete generation of various samples under the same laboratory conditions [16,19]. It is however important to take into account possible genetic drift effects (number of founders) or even microenvironmental influences within laboratory conditions. A more speculative approach to tentatively partition causes of metric variation is the separate analysis of size and shape, based on the idea that shape would have less environmental variance [32]. If the main interest is to focus on environmentally induced changes, the study of fluctuating asymmetry of bilateral characters is an elegant, but challenging, approach (see Fig. 25.12). Adapted methodology (the study of bilateral structures) and accurate statistical techniques exist to reveal these environmentally induced changes [74], which are now applied to geometric morphometrics [55,66,89].

25.2.1 Physiological Causes

The main cause of metric differences related to physiology is obviously differential growth, when this growth heterogeneity is of environmental origin. Depending on more or less favorable environmental conditions, and on aging in vertebrates, individuals may be more or less developed. For conspecific individuals, traditional morphometrics proposes a set of statistical methods to remove this effect of age or growth from their metric variation. Scaling for size is interesting when one wants to remove the effects of physiological differences and concentrate on other causes of intraspecific variation. In that case, the size estimator—the one that’s effect

will be removed from the metric variation—should be constructed from the dimensions of the anatomical structure under study, not from an external indicator (weight, etc.). An external indicator of size is acceptable when the objective is to study the meaning of size variation itself. A complete review of these methods for traditional morphometrics may be found in Ref. [54].

25.2.2 Pathological Causes

Some mutation or toxin may affect the morphogenesis of some individuals. Morphometrics is not always required to detect such changes, as they generally produce obvious, visible deformations. Many times, pathological causes produce extreme individuals (“outliers”). They may be removed from the dataset or included provided more robust statistical techniques are used [75]. However, when the environmental aggression is directed at populations instead of individuals, and moderate in degree (insecticides at nonlethal doses), the morphological change may be more subtle. It may become visible when examining the range of variation at some characters, or the level of fluctuating asymmetry for bilateral traits [69].

25.2.3 Adaptive Causes

Adaptation to a different ecotope, or simply geographic adaptation, are the likely causes of phenetic changes as observed within a single species. Adaptive causes are of a genetic nature, but we make them distinct from genetic causes (see next section) by their trigger mechanisms. Although genetic causes are random mutations or even speciation events, adaptive causes are within-species differentiation induced by the interaction of genotypes with various environments. In each environment the best-suited phenotypes are selected. With time, the genotypes corresponding to the best-adapted phenotype are selected, creating genetic differentiation in the corresponding populations (“genetic assimilation,” [95]). Beside this collection of genotypes induced by phenotypic preference, adaptive differences are supposed to be produced also by direct selection on genotypes. These kinds of metric differences observed between conspecific, geographic populations disappear slowly, or sometimes do not disappear at all even after many generations in laboratory [50]. Note that under the same laboratory environment, new differences may appear which were hidden by environmental compensations, or due to genetic drift occurring in laboratory after many generations [28]. Rearing the insects is not necessary to detect adaptive causes, they are suspected also when it is possible to observe residual, significant differences after correction for differential growth. Specialized statistical procedures may show that even after removing the effect attributable to growth heterogeneity, significant differences remain. These “allometry-free” shape differences (see Section 25.3.2.1) are not the signature of species differences, they may also be found among conspecific populations living in different eco-geographical regions [32]. However, the partition of shape and size may be

regarded as an analytical process increasing our capacity to interpret metric variation.

25.2.4 Genetic Causes

Interspecific metric differences most probably have a genetic origin. The nature of these genetic differences is not within the scope of this introduction, but their effects on metric traits deserve some discussion here. Although there is no special metric feature marking the difference between species, this topic is influenced by common ideas found in the literature, not completely true and not completely false.

25.2.4.1 *The amount of differences* The level of interspecific differences is generally (much) higher than the corresponding intraspecific variation, even across geographic populations. This led some authors to look for “how much” species differ in general [93], but this needs to be addressed separately for each group [32,33].

25.2.4.2 *The nature of differences* As shape is supposed to have a larger genetic variance than size, it is generally believed that species differ not only by size but also, and probably more, by shape [5]. The same approach as for adaptive causes may be applied here, that is, the partition of size and shape. There is, however, no rigorous way to attribute residual metric differences (i.e., shape) to the speciation process instead of to locally adaptive causes, and again, other methods are needed to clarify the source of variation.

25.2.4.3 *The localization of differences* When metric differences are found in organs responsible for mechanical reproductive barriers, they may be given more evolutionary importance than differences located elsewhere. It is well known that sandfly species generally have different dimensions or shape in at least one piece of their complex genitalia [96].

25.2.4.4 *The circumstances of the differences* When groups compared are sympatric, size or shape differences are a good indicator, although not a proof of speciation [14,19,35].

25.3 SIZE AND SHAPE

The characters provided by morphometric analyses, either distances between landmarks (traditional morphometrics) or their coordinates (geometric morphometry), contain information on both size and shape. Imagine you describe two triangles by the length of the three sides, say 3,3,3 units for the first one, and 30,10,30 similar units for the second one: These values are immediately describing different sizes, small (3,3,3) and large (30,10,30), and altogether different shapes, an equilateral (3,3,3) and an isosceles (30,10,30) triangle For live organisms, the question has been: Can we compare just shape, thus removing the size differences, and alternatively, can we compare just size—by constructing

a size estimator (like here the sum of the sides of each triangle, for instance). Morphometricians not only want to find a general character of size (global size) to better focus on size variation but also to remove its effects from the metric variation so that residual variation then represents shape variation. In either case, it is necessary to construct an acceptable estimator of size.

25.3.1 The Search for a Global Estimator of Size

In morphometrics, one single individual may be described by many characters of different anatomical parts, so that the question arises how to construct a relevant measure for the study of global size variation.

25.3.1.1 Dimensionality To avoid the complexity of working with and, especially, interpreting many characters (multidimensionality), one dimension of the organism could be adopted as representing its “global size.” For instance, it is generally considered that wing length in mosquito is an acceptable index of body size [61]. This could, however, suffer some exceptions or it could be different for other insects. To avoid never-ending discussions about size representativity of one particular character, one could include each measured character as a partial estimate of global size. The many dimensions would then be combined into one summary of size variation, that is, one variable. According to the way this combination is done, the global size variation that is captured includes (allometry) or not (isometry) the unique variation at the level of individual characters.

25.3.1.2 Isometric change of size The isometric estimator of global size describes changes of size that do not modify the proportions of the object. From one object to another, every character of the object is multiplied by the same value (Fig. 25.3). Isometric change is described by a single coefficient. It could be compared to the amplification or reduction made by a quality photocopier: The proportions of the object are intact, its global size is changed. Figure 25.3 shows an isometric change applied to an equilateral triangle, which remains geometrically an equilateral triangle. An example of isometric size variation is the one used to construct “log-shape ratios” [22] from distance measurements: This estimation of global size is simply the average of all the log-transformed measurements of one individual. Another example of such estimation, used in

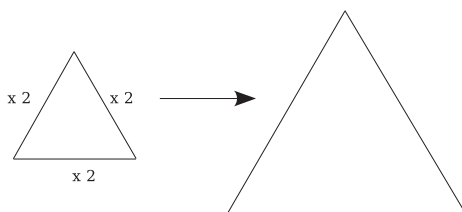


Fig. 25.3. An isometric change of size: proportions were left unchanged.

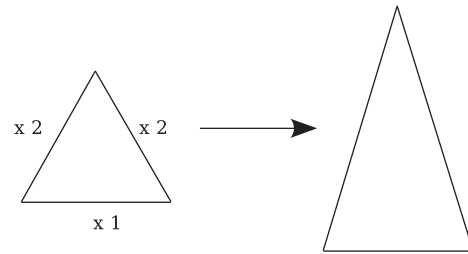


Fig. 25.4. An allometric effect: size and proportions have changed.

geometric morphometrics, is called the *centroid size* [9], “centroid” because its computation uses the geometric center of the configuration of landmarks. It is the square root of the sum of the squared distances (SS) of each landmark to the centroid (see Gower, 1971 in 80). In the case of small, circular variation at each landmark, this estimator of isometric change of size is not correlated to shape variation [9].

25.3.1.3 Allometric change of size With allometry, each body dimension has its own rate of growth. The allometric estimator of size variation takes this heterogeneity into account. Obviously, such complex change can alter the initial proportions (allometric effects). Figure 25.4 shows a simple allometric change applied to an equilateral triangle, which then became an isosceles triangle. The corresponding mathematical descriptor may use as many coefficients as characters. Various statistical techniques based on principal component analysis (PCA) are proposed to capture the allometric change as represented by the first principal component; in his review, Klingenberg [54] recommends the use of the first common principal component [4,44]. This approach is only valid when allometry is the cause of most variation in the sample, which is what the first principal component actually expresses. Allometric changes of size could be the effect of differential growth and age-related development, or, in case of various species, simply the effect of complex morphological evolution.

25.3.2 Shape As Size-Free Variation

The search for a good estimator of global size, either isometric or allometric, has two objectives: The first one is the study of size variation itself and the second is its removal from the metric variation in order to produce residual variables representing shape. Many statistical techniques have been proposed to produce variables independent of size variation, that is, size-free (shape) variables [54]. Because size has to be “removed” to represent shape, the accuracy of shape capture depends on the size descriptor, itself depending also on the characters used and their units, either the distances between anatomical landmarks (traditional morphometrics) or the coordinates of these landmarks (geometric morphometry). In both geometric and traditional morphometrics, the same concepts of shape construction are valid.

25.3.2.1 Allometry-free variables When it is the allometric component of size changes that is tentatively

removed, the residual variation should be called “allometry-free” shape variation. Statistical techniques to perform such scaling are often based on principal component analyses where each character is generally a log-transformed distance between anatomical points (traditional morphometrics) or coordinates (geometric morphometrics). Or, they may rely on techniques of regression or on additional information (for a review, see [54]).

25.3.2.2 Isometry-free variables When removing size variation focuses on the isometric changes, residual variation should be called “isometry-free” Proportions are preserved (see Fig. 25.3), so that “isometry-free” variation may be used to study data for which size has not been documented (see Figs. 25.5 and 25.6). The most common technique of size removal, including that used in modern geometric techniques, is to do just that: Factor out an isometric component of size from the metric variables producing, then, “isometry-free” variation.

25.3.2.2.1 Log-shape ratios In traditional morphometrics, log-transformed data are used [52] so that isometry-free variables are also called “log-shape ratios” [22]. Due to the loss of one degree of freedom, these variables still need an additional transformation through a simple principal component analysis, so that they can be subjected to standard statistical analyses [22].

25.3.2.2.2 Procrustes residuals The Procrustes¹ superposition refers to steps allowing the construction of size and shape variables from landmark coordinates for their use in morphometric studies. The reader interested in the full definition of the many specialized terms used in geometric morphometry will find a complete glossary at <http://life.bio.sunysb.edu/morph/>, which is an updated version of Slice et al. [88]. In the following steps, the homologous landmarks of various wings are superimposed on those of a consensus wing² so as to optimize some measure of goodness of fit—the minimum sum of squared distances of landmarks to their consensus homolog.

Translation First, each configuration of points is translated to the same centroid (Fig. 25.5)—a SS-minimizing step.

Scaling Second, each configuration is divided by its own centroid size, so that each configuration has a centroid size of

¹Procrustes, whose name means “he who stretches,” was a thief in Greek mythology (the myth of Theseus). He preyed on travelers along the road to Athens. He offered his victims hospitality on a magical bed that would fit any guest. As soon as the guest lay down Procrustes went to work upon him, either stretching the guest or cutting off his limbs to make him fit perfectly into the bed (Grose Educational Media, 1997–1998).

²In the most common case, Generalized Procrustes Analysis, the consensus is constructed using the average coordinates of individual landmarks in a sample. An initial specimen is chosen as a first approximation and the consensus is iteratively recomputed after subsequent fittings of the sample.

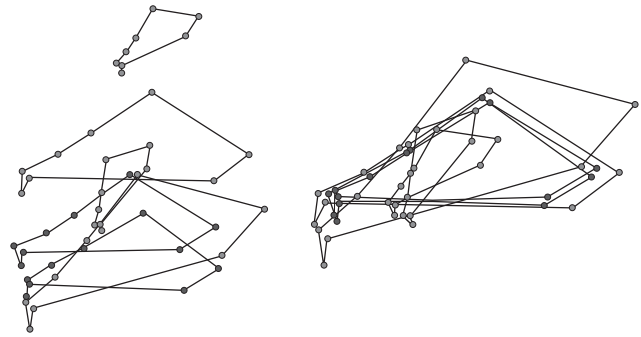


Fig. 25.5. This figure shows the effect of the translation step (left → right) for six configurations of eight wing landmarks (see Fig. 25.2) of the genus *Rhodnius* (Hemiptera, Reduviidae). The landmarks have been joined by line segments to aid visualization. Four wings belong to *R. robustus* (blue polygons, large ones from Venezuela, small ones from French Guyana), two wings are Venezuelan *R. prolixus* (brown polygons). The striking size differences apparent for the two *robustus* wings of Guyana are mainly due to different microscope magnifications. *Left:* Configurations of raw landmarks as they were captured on the computer screen: there are artifactual differences due to position (corrected by translation, see right part of the figure), magnification (later corrected by size scaling, see Fig. 25.6) and orientation (corrected after rotation, see Fig. 25.6). *Right:* One of the first steps in a Procrustes analysis is usually the translation of the configurations to a common centroid. MOG software, version 0.67 (Dujardin). See color plates.

one (Fig. 25.6). Size (isometric, see Section 25.3.1) variation is thus removed, or rather sequestered in a separate variable, centroid size, for additional study. This is not a SS-minimizing step, but results in all configurations being of a standard size [46,84].

Rotation Then, each configuration is rotated to fit the corresponding homologous landmarks of the consensus configuration (Fig. 25.6) using rotation parameters to minimize SS differences. Nonparametric techniques of fitting also exist, which might be more accurate when configurations are similar except at very few landmarks [80].

Residual Coordinates After Procrustes superimposition is completed, coordinates have lost four degrees of freedom (for coordinates in a two-dimensional space) [53]. They could be used in the same way “log-shape ratios” are used, by performing a PCA on it and removing the noncontributive components. However, because they have a non-Euclidian metric (Procrustes distance), these residuals should, technically, not be used as input to perform multivariate analyses [87]. They can be used, however, in nonparametric tests as implemented in the Morpheus software (<http://life.bio.sunysb.edu/morph/>), see an example in Ref. [47].

25.3.3 Shape As Geometry

The specific contribution of geometric morphometrics is the construction of variables that describe the geometry of a configuration and can be used to describe differences

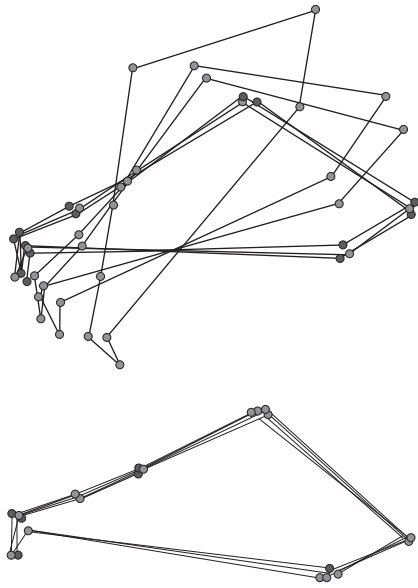


Fig. 25.6. Two more steps of the Procrustes superimposition, the scaling (top) and the rotation (bottom) steps. *Top:* All configurations, after translation (see Fig. 25.5), have been scaled for size (the very small configurations seen Fig. 25.5 are now of equal size). *Bottom:* Objects are then rotated to allow the best superimposition possible across all landmarks. Consensus is not shown. Different statistical techniques exist for finding the best fit, the one most commonly used is based on the minimum Procrustes distance. Residual differences are size-free variation. MOG software, version 0.67 (Dujardin). See color plates.

between one configuration and another. After obtaining Procrustes residuals, an additional step allows the production of another set of variables called *partial warps*: They define the positional changes at each landmark in relation

to a consensus or reference ordered by geometric scale. Partial warps are true *shape variables*, and have the properties of isometry-free variation. This geometric description of variation may still, however, contain allometric effects (the contribution of size variation to shape changes), and its construction relative to a reference means that shape variables depend on that reference. That is, the same individual may have different partial warps scores depending on the reference used, that is, according to the other individuals making part of the analysis. For a complete description of shape one must separately compute uniform (see below) terms (two for 2D data) [82].

25.3.3.1 Uniform and nonuniform components of shape

The shape variables may be decomposed into two components called the *uniform* (or *affine*) and *nonuniform* (or *nonaffine*) components. These two components both describe a different kind of shape change relative to a reference object, one (affine, or uniform) is a global change that is the same everywhere, the other one (nonaffine or nonuniform) is made of locally distorting changes [9]. Figure 25.7 shows separate affine and nonaffine transformations with increasing size (affine transformations leave unchanged the parallelism of the grid, but see Ref. [8]). As these components are just the mathematical decomposition of shape variation, it is generally not advisable to analyze them separately [10].

25.3.3.2 Relative warps

Relative warps are the principal components of the partial warps scores plus the uniform terms (they are produced by a simple principal component analysis). In comparing groups and/or visualizing group differences and patterns of variation, relative warps (or part of them) may be used as input for a discriminant analysis.

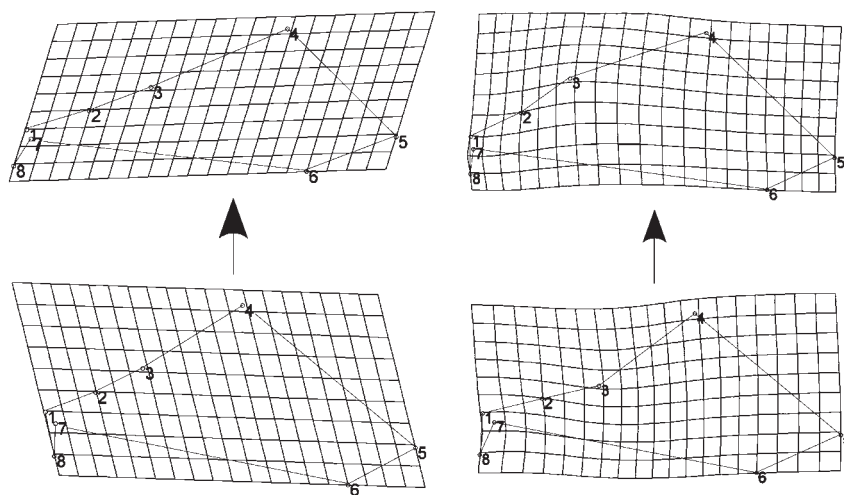


Fig. 25.7. Polygons connecting eight landmarks collected on the wing of *R. prolixus*, and inserted into a grid showing deformations from consensus. The figure illustrates the changes of shape due to growth heterogeneity among sympatric individuals (Pampanito, Venezuela), that is, it allows the visualization of allometry. Plots are thin-plate splines showing uniform (left) and nonuniform (right) changes from the consensus for the smallest (bottom) and the largest (top) wings. To help visualization, changes were amplified 10 times. tpsRelw software (Rohlf).

25.3.3.3 Thin-plate spline The thin-plate spline is a spatial interpolation function that can produce D’Arcy Thompson-like plots showing the geometry of shape changes between objects [9]. The TPSPLINE program [76], as well as the TPSRELW [77] or TPSREGR [78] programs, perform the necessary computations and plot the resulting differences as a transformation grid (see Fig. 25.7). Morpheus et al. [86] and Edgewarp [11] can compute both 2- and 3D thin-plate spline representations.

25.3.4 Which Shape?

When comparing samples of conspecific individuals, one usually wants to factor out exclusively allometric size to remove the effects of differential growth (or aging, in vertebrates). Indeed, among conspecific individuals living in sympatry, allometric changes are most probably attributable to growth heterogeneity. In case of significant residual variation after removing allometric changes, causes other than simple differential growth may be suspected. This conceptually interesting approach to explore intraspecific variation is limited by its own assumption of a common allometric axis, and more commonly applied in traditional morphometrics where this axis is expected to be linear. A common axis of growth, at least for the characters used, is not always verified (the NTSYS® software provides a relevant statistical procedure), and of course, less easy to find when populations are geographically distant, adapted to different environments, or actually when they belong to different species. In these situations where causes other than physiology are expected to play a role (genetic adaptation, microevolutionary trends, interspecific differences, etc.), scaling for isometric variation is a common practice in both traditional and geometric approaches. It is then recommended to verify (and quantify) the residual allometry by regressing shape onto centroid size.

25.4 MORPHOMETRICS AND MEDICAL ENTOMOLOGY

Medical entomology focuses on medically important insects. Most recent morphometric studies related to medical entomology have been dedicated to Triatominae and Phlebotominae.

Triatominae (Hemiptera: Reduviidae) constitute a small subfamily containing all the vectors of Chagas disease occurring in Latin America (see the corresponding chapter in this encyclopedia). They are large bugs (from one to four centimeters) with long generation times (a few months to more than 2 years, according to the species), and obligatory hematophages at all stages of their cycle, larvae or adults, males or females. Phlebotominae (Diptera: Psychodidae) are very small flies (less than 3 mm) transmitting *Leishmania* spp., bacteria and viruses (see Fig. 25.8). As in mosquitoes, only females are hematophagous, they have short generation time (1 month), and cover cold and tropical areas worldwide. As both of these insects have medical importance, their correct identification is a crucial component of epidemiological study.



Fig. 25.8. Sandflies are very small, hematophagous Diptera (1–3 mm); their identification needs dissection, mounting and microscope amplification. Here we see the hyaline wing of *Lutzomyia* sp. (top), with parts of the thorax, abdomen, and genitalia (bottom) (from “Ciberatlas de los Flebotominae de Bolivia”, Le Pont et al., see <http://www.mpl.ird.fr/morphometrics>).

Initially, morphometrics applied to medical entomology had the objective of aiding systematic research, so that it has been traditionally associated with description of species, often based on very few specimens [63,96] and described by a few ratios. This is unfortunate, because morphometrics is a population approach rather than a description of individuals. To be consistent, morphometric studies need more appropriate sampling techniques than used in taxonomy, and more adapted statistics than simple ratios. The use of simple ratios, although useful in some cases [45], is not a recommended practice [21], especially when used as a statistical technique to reduce the influence of size on shape [92]. As an illustration, see the obvious correlation of a head ratio with head size itself (Fig. 25.9), which is frequently used in the systematics of Triatominae. In medical entomology, ratios have been used as a taxonomic tool for decades; the first attempt to use multivariate techniques in removing size effects on a medically important insect is only a few years old [50].

The main interest of using morphometrics as a tool for species distinction in medical entomology is that it does not require the high level of entomological expertise to make an accurate species diagnosis in a specific group of insects. Health personal or nonspecialized laboratories could use similar concepts and techniques to classify insects in very different groups. This idea has been recently discussed for mites [7].

25.4.1 Systematics

25.4.1.1 Phlebotominae The taxonomy of Phlebotominae is still a very debated issue: This group of insects is huge (more than 1000 species [96]) and poorly understood. Cryptic species are not uncommon [15,34,59]. To help in distinguishing morphologically close species, multivariate

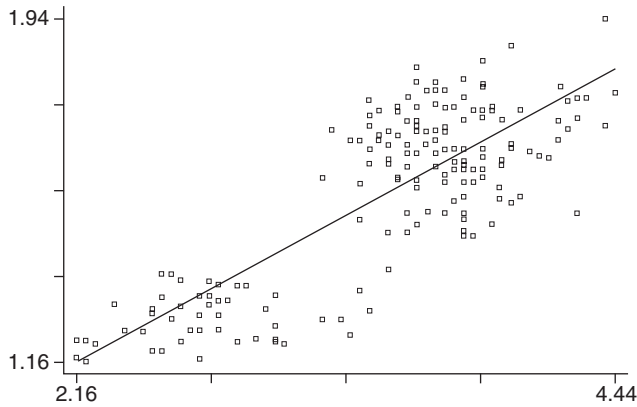


Fig. 25.9. The vertical axis shows the values of the AO/PO ratio in the genus *Rhodnius*: It is the ante-ocular distance (AO) of the head relative to the post-ocular one (PO). The horizontal axis presents the total length of the head in millimeters. This head ratio AO/PO is frequently used in the systematics of Triatominae, it is supposed to remove the influence of (head) size but, as shown here, it behaves as another estimator of size.

techniques on raw data have been suggested [58], and applied a few years later [1,18]. The partitioning of size-included and size-free variation has been successfully used to explore or detect hidden speciation [31,35,36,43], or as an additional taxonomic criteria in species description [60]. Very recently geometric morphometry has also been used, showing unexpected power in detecting cryptic species [56].

25.4.1.2 Triatominae There are less classification problems in the small group of Triatominae (137 species [41]). However, morphometrics can be particularly helpful in Triatominae systematics because these insects, unlike Phlebotominae, often lack discrete, qualitative characters allowing their discrimination (except color patterns) [63]. Multivariate techniques of size and shape partitioning were applied to one of the most confusing taxonomic problems in Triatominae: *R. robustus* and *R. prolixus* [49,70,72]. Between laboratory lines of these two taxa, that is, insects sharing the same environment during one generation after their field capture, allometry-free variation and geometric techniques disclosed consistent differences [94], allowing species distinction even on a single specimen [68].

25.4.2 Geographic Variation

25.4.2.1 Phlebotominae In sandflies, geographic variation of size is frequent and could interfere with species diagnostics [57]. The removal of size by means of multivariate techniques is intended to explore the stability of size-free metric properties. In a study covering 10 species of the genus *Lutzomyia*, it was shown that within large eco-geographical regions of South America, size-free variation was generally stable within species; however, this intraspecific stability was compromised when comparing samples from

different ecological regions [32]. Geometric techniques seemed to provide more stable characters [33].

25.4.2.2 Triatominae Geographic studies partitioning size and shape (size-free variation) have been performed on a few species, and showed the influence of geography on metric properties [30,90]. A geographic study is expected to focus on conspecific populations, but the inclusion of another species may provide interesting taxonomic information. Two possibilities are (i) the within-species, geographic variation is lesser than interspecific variation, which is an expected feature or (ii) the geographic heterogeneity is wider and includes some other species. In this latter case, the study questions either the included species or the geographic populations themselves. For instance, in *T. dimidiata*, size-free divergence between cave populations of *T. dimidiata* and other populations of *T. dimidiata* were commensurate with interspecific metric distances either to *T. mexicana*, *T. pallidipennis* or *T. ryckmani* [13]. This too-wide geographic variation called for a taxonomic revision of *T. dimidiata*. Another example used landmark coordinates of the wings, showing that the controversial species *T. melanosoma* did not depart from the geographic variation of *T. infestans* [47]. This result, already disclosed by traditional morphometrics [40], did not support *T. melanosoma* as a different species, and was in agreement with genetic studies [71].

25.4.3 Comparisons of Morphometric with Genetic Variation

25.4.3.1 Phlebotominae Among closely related species, two studies have shown fair agreement between MLEE (multilocus enzyme electrophoresis) and morphometric variation [38,44]. Concordant results were also found for very distant, Old World and New World taxa (see Fig. 25.10) [37].

25.4.3.2 Triatominae The size-free variation of head and wings in the *Rhodnius* genus produced a classification in global agreement with phylogenetic reconstruction [29].

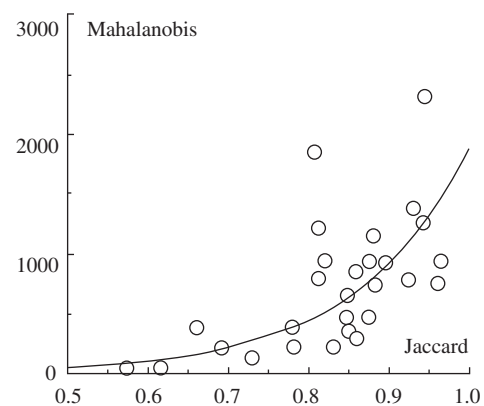


Fig. 25.10. Relationship between distances as computed from metric (vertical axis) and genetic (horizontal axis) characters, used to classify distant taxa of Old World and New World Phlebotominae (from Ref. [37]).

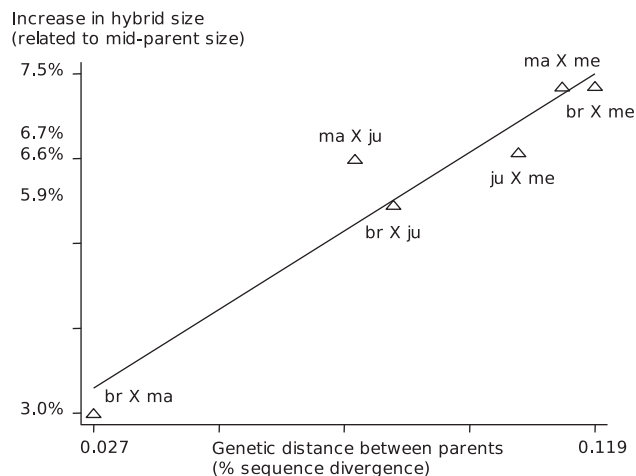


Fig. 25.11. Relationship between size in hybrids and genetic distances between parents; on vertical axis, the increasing of size relative to mid-parent size; on horizontal axis, the genetic distance between parents as inferred from mitochondrial DNA sequence; ma, macromelasoma; me, melanica; br, brasiliensis; ju, juazeirensis (from Costa et al., unpublished data).

A good fit between morphometric and evolutionary relationships was also found among close species in a small group of the genus *Triatoma* [73]. Such agreement between morphometrics and genetics has been verified in various instances in Triatominae [26,71,90,94]. Altogether, it seems that, at least for closely related species, there is a frequent accord between morphometric and genetic classifications [41]. Such agreement becomes higher within species, when comparing subspecies or geographic populations. Between seven laboratory colonies of *T. protracta* or between each of the five subspecies of *T. protracta*, each hybrid had an intermediate centroid size between parents (Dujardin et al., unpublished data). However, centroid size was increased in hybrids obtained from two other subspecies, *T. b. brasiliensis* and *T. b. juazeirensis*. It was larger than the mid-parent size, suggesting a heterosis, itself pointing to a probably consistent genetic divergence of parents (Costa et al., unpublished data). Extending this study to experimental hybrids among the four members of the Brasiliensis complex, it was possible to show a linear relationship between the genetic divergence of the parents [20] and the increase of size of their offspring (Fig. 25.11).

25.4.4 Topics Specific to Triatominae

In Triatominae, metric variation was also used to explore other topics, like their transition from sylvatic to laboratory or domestic environments, their reinfestation behavior, and their migrating history.

25.4.4.1 Adaptation to new ecotope The most epidemiologically meaningful niche adaptation of Triatominae is the transition from the natural, sylvatic to the domestic, or artificial environments [41,67] where the insect actually transmits the parasite to humans. It has been shown that the

body is larger for specimens collected in natural conditions, versus their counterpart reared in laboratory [42,97]. Although the laboratory cannot be equated to domestic conditions of life, this decrease of size in artificial ecotopes seems to parallel a similar trend from sylvatic to domestic conditions [48,97]. Sylvatic specimens of *T. dimidiata* [97], *T. infestans* [25], *T. brasiliensis* [12], *Panstrongylus geniculatus* [51], and *P. megistus* [6] are larger than their domestic counterparts. This sometimes includes sexual size dimorphism. On average, females are larger than males [63], but old laboratory colonies of *R. neglectus* showed strongly reduced sexual head size dimorphism, and so did domestic populations of *T. infestans* relative to their sylvatic counterparts [42]. Reduced sexual size dimorphism was not found however for head or wing dimension of a five-generation laboratory colony of *P. geniculatus* [51].

From natural to artificial ecotopes, after three to five laboratory generations, for head and wings dimensions in *T. infestans* and *R. stali*, there was not only a decrease of size but also a larger variance (Matias et al., unpublished data). In accord with this observation, fluctuating asymmetry was also shown to increase from sylvatic to domestic environments [40].

So far, all these changes were putatively related to environmental changes (the transition from one habitat to another), but these studies need to be refined by experimental work controlling some confounding variables like, for instance, blood source, insect density, or feeding patterns.

25.4.4.2 Migration history A cline of decreasing size also seems to follow the progression of past geographic migrations (generally passive migrations) of domestic vectors. It is apparent in *T. infestans* and in *R. prolixus* from their supposed original countries to the present periphery of their distribution: size decreases [39] and developmental instability increases [23]. It is indirectly supported in some cases where an insect is found in a limited area, outside its current territory (generally in domestic conditions only): These specimens are generally smaller than current representatives [2]. In such isolated or peripheral populations, metric changes and local adaptations [40] may even lead to undue new species description, see for instance *T. melanosoma* [17,47,62,71] or *P. herreri* (Gumiel et al., unpublished data) [65].

25.4.4.3 Reinfestation studies A few studies proposed morphometrics as helpful criteria for making decisions in entomological surveillance. Provided that samples were available from the population before insecticide application, relative metric similarities could suggest the origin of reinfestation specimens [27]. As a residual population is supposed to be the same generation as or the next generation to the individuals subjected to insecticide spraying, such an application is based on the supposition that an insect is more similar to its parents than to other insects. It is also based on the idea that insects reared in the same microenvironmental conditions (a few houses, a village) would share a significantly larger amount of metric similarity. Such idea would be less applicable to insects having



Fig. 25.12. Triatominae, or “kissing bugs,” are generally large insects (1–4 cm). A North American species has been shown Figure 25.2. Here is an adult specimen of *T. infestans* (Hemiptera, Reduviidae), the main vector of Chagas disease in South America. As Hemiptera, it has two pairs of wings. The anterior pair is the best anatomical structure for morphometric studies: It is almost two-dimensional, so that the measurement error can be reduced, and it shows well-defined venations, so that homologous parts are easily recognized. In addition, these are bilateral structures, allowing studies about the symmetry of the insect (from the “Ciberatlas de los Triatominae,” Dujardin and Matias, see <http://www.mpl.ird.fr/morphometrics>).

short generation times (like Phlebotominae); its applicability actually depends on the stability of metric properties in one place from one generation to another, a feature that has to be explored in each case rather than theoretically predicted. This has been done for *T. protracta* by examining two successive generations of seven laboratory colonies: The geometry of the wing, male or female, was useful to assign any single specimen to its parents or close relatives in more than 90% of cases [24]. Thus, the geometry of the wing could be an interesting candidate to assess the origin of reinfesting specimens, either the descendants of previously killed bugs or immigrants from an external focus.

Traditional morphometrics (head measurements) already gave satisfactory results, as verified by genetic markers [26].

25.5 AUTHORS CONTRIBUTION TO MORPHOMETRICS SOFTWARE

With an intuitive understanding of multivariate and geometric analyses, it is often possible to perform valid morphometric studies thanks to specialized software, some of them freely available at <http://life.bio.sunysb.edu/morph/> and

<http://www.mpl.ird.fr/morphometrics>. We just mention here our contribution to this production.

25.5.1 Software for Multivariate Analyses

25.5.1.1 BAC BAC (for “Bootstraps, Analyses of principal Components”), beta version, multiplatform (Windows, freeBSD and Linux), presently commented in Spanish, performs different kinds of principal component analyses with limited graphical output. Assuming data are log-transformed measurements, BAC allows the use of bootstrapping to estimate the variation of allometric coefficients (eigenvector stability). After successive PCA using the same characters, BAC computes the angle between first principal components. Applied to *partial warps data*, BAC computes the corresponding relative warps.

25.5.1.2 PAD PAD (for Spanish words “Permutaciones, Analisis Discriminante”), multiplatform, beta version, performs a multivariate discriminant analysis (DA) with limited graphical display. For log-transformed data, if requested, PAD removes the isometric change of size, performs a PCA on isometry-free variables to produce isometry-free components, a representative set of which is then used for discriminant analysis. A similar approach is offered for *landmark-based data*. PAD allows random permutation of individuals among groups to test the significance of each pairwise Mahalanobis distance. PAD allows a reclassification of individuals, with and without cross validation. Supplementary data may be introduced.

25.5.2 Software for Landmark-Based Data Analyses

25.5.2.1 COO COO (for “Collection of Coordinates”), multiplatform (Windows, Linux) and beta version, is designed to collect coordinates of anatomical landmarks.

25.5.2.2 MOG MOG (for the Spanish words “Morfometria Geometrica”), multiplatform (Windows, Linux) and beta version, allows users to visualize the different steps of a Procrustes superimposition, generating Procrustes residuals, partial warps (shape variables) and centroid size for each individual. The output format is ready to use for multivariate analyses programs described above (BAC, PAD). Some of the illustrations used here were made with MOG.

25.5.3 Comprehensive Software

25.5.3.1 Morpheus et al. Morpheus et al. (Morpheus) is a program for morphometric research [86]. It supports a variety of data types, such as n -dimensional points, curves, images, user variables, and dynamic measurements. The program provides extensive import/export functions, including the estimation of landmark locations from truss distances, and advanced graphical capabilities for the visualization of morphometric data and results.

25.6 CONCLUSION

Morphometrics is a dynamic branch of biology. Technical and conceptual advances have considerably increased its resolution power. In medical entomology, it is no more a mere taxonomic tool, but it has also become a powerful way to explore intraspecific variation. Its relevance to quantify phenotypic changes makes it a valuable complement for biological studies on laboratory or natural populations, with possible applications to epidemiology. As long as phenotypic evolution is a relevant factor of speciation [85], morphometrics is one of the best quantitative approaches to evolutionary biology.

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