

16 Modern Morphometrics of Medically Important Insects

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16.1 Introduction

The phenotype is the product of the interaction between genes and environment. Phenotypic variation is then an expected outcome of more than one factor. It can be scored by measurable changes in anatomy, morphology, physiology, life history, behavior, etc. (West-Eberhard, 1989; Gadagkar and Chandrashekara, 2005). This chapter focuses on the phenotype as a set of metric properties and their variations. Morphometric changes are generally recorded as variation in size and shape, although these two metric traits are not independent ones. Their interdependence (allometry) is worth considering in intraspecific studies, but is never complete. Therefore, the two metric properties are often considered separately for their genetic determinism, their heritability, their sensitivity to the environment, and their capacity to provide indirect information about the genetic differentiation of natural populations.

16.1.1 *Modern and Traditional Morphometrics*

Morphometric techniques aim at measuring size, shape, and the relation between size and shape (allometry). Before the so-called “revolution” (Rohlf and Marcus, 1993), shape was an abstraction, a residue after scaling for size, and it was not possible to visualize the “residue.” The replacement of initial variables describing a distance between two anatomical points by the coordinates of these points, and the subsequent visualizing techniques, represented a giant step in the direct study of forms. The shift from traditional morphometrics to more complex geometric functions was facilitated by the development of image processing tools. Not only landmark methods but also “outline methods” (Rohlf and Marcus, 1993) and other techniques exploring textures and surface patterning (Lestrel, 2000) are used today. This chapter deals mainly with landmark-based geometric morphometry.

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16.2 Landmark-Based Geometric Morphometry

The coordinates of anatomical landmarks contain not only size, such as distances between landmarks, but also shape, such as their relative position.

In common practice, size and shape are derived from a configuration of landmarks collected on a non-articulated part, often a single organ (but see Adams, 1999). A few anatomical landmarks available on a wing (or any measurable part of the body) do not completely describe the wing, nor do they describe the complete body. However, provided there is anatomical correspondence among individual landmarks, only a partial capture of shape is needed to allow valid comparisons among populations and species.

The choice of suitable landmarks relies on their operational homology. In the morphometrics practice, homology is “correspondence of parts” with no specification about whether the parts correspond with respect to structure, development, or phylogeny (Smith, 1990). If individuals belong to a single species, homologous landmarks are probably similar due to common descent because all members of the species come from a common ancestor. If they belong to different species, there is no guarantee that homologous landmarks are similar due to common descent, except if they are known to be descending from a common ancestor (Lele and Richtsmeyer, 2001). This homology is one of the criteria making landmark-based morphometrics a suitable tool for systematics (see Section 16.6.1). Bookstein (1991) described various categories of landmarks with decreasing levels of precision.

16.2.1 Size

To avoid the problem of multidimensionality, traditional systematists often selected one single dimension to represent body size. For an insect, the length of the wing along its largest axis is frequently used as an estimator of body size (Nasci, 1990; Siegel et al., 1992; Lehmann et al., 2006). Such a relationship is often assumed rather than demonstrated (Siegel et al., 1992; Morales et al., 2010).

Size Variable: The Centroid Size

The centroid size (CS) is the square root of the sum of the squared distances from the centroid to each landmark (see Gower, 1971 in Rohlf, 1990). It thus can detect change in various directions. In the case of small, circular variation at each landmark, this estimator of isometric change of size is not correlated to shape variation (Bookstein, 1991). It is expressed in pixels, or units relative to the resolution of the viewing device (most often a computer display). As a scalar it is less sensible to small digitization errors and can be shared among systematists provided the pixels have been converted into absolute length units (inches, centimeters, millimeters, etc.). Thus, to allow for exchangeability of CS, an image on which landmarks may be collected should contain a scale for size (for instance the picture of a reticule) allowing the conversion of pixels to absolute units.

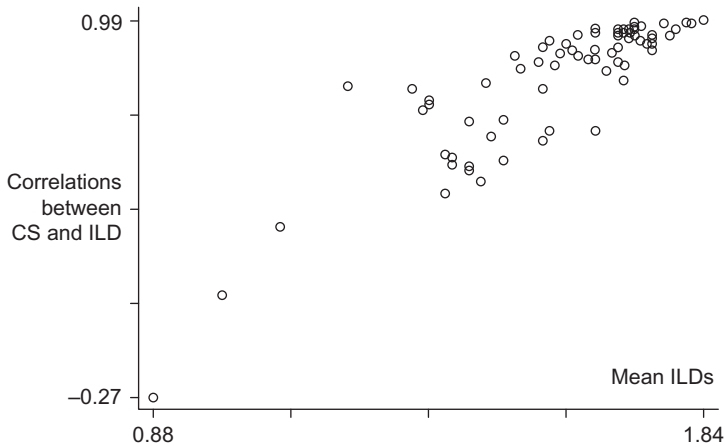


Figure 16.1 Each circle represents on the vertical axis the correlation found between an ILD and the CS of the wing computed from the total set of landmarks. The value of each ILD on the horizontal axis is an average (mean ILD) obtained from the total number of wings (78) examined in this sample. The wings belong to *Ae. aegypti*.

The relationship of CS values and the traditional wing length in the mosquito *Aedes aegypti* showed good correlation (Morales et al., 2010). Actually, the correlation of CS values with traditional interlandmark distances (ILD) is itself correlated to the relative dimensions of ILD: the largest the ILD, the highest its correlation with CS (see Figure 16.1).

16.2.2 Shape

Not only in medical entomology but also in many fields where morphometrics is applied, shape has been traditionally described as the ratio of one dimension to another. Although intuitively the ratio may appear as capable of scaling for size, it often does not (Burnaby, 1966; Albrecht et al., 1993; Klingenberg, 1996; Dujardin and Slice, 2007). Moreover, the ratios introduce some well-known statistical drawbacks (Albrecht et al., 1993). Angles do not improve the situation since they are another kind of ratio (Burnaby, 1966).

Shape Variables: The Procrustes Residuals, the Partial Warps, the Relative Warps

In geometric morphometrics, the shape of a configuration of landmarks is represented by their relative positions as contained in their coordinates. However, these coordinates also contain artifactual variation due to position, size, and orientation. Thus, shape must be described by new variables having removed these artifacts. This is

obtained through the Procrustes¹ superimposition on a consensus configuration. If using the least squares fit as an optimality criterion, the statistical procedure of superposition is called Generalized Procrustes Analysis (GPA). It is currently the most common procedure, but other techniques also exist (Zelditch et al., 2004). The residual coordinates after a GPA provide a shape description relative to the consensus configuration of landmarks, they thus depend on the composition of the group under study. If other specimens (i.e., coordinates) are added to the analysis, shape variables must be recomputed accordingly (Rohlf and Marcus, 1993; Adams et al., 2004).

Furthermore, the residual coordinates lie in a weird mathematical shape space. When working on a two-dimensional space, the residual coordinates have lost 4 degrees of freedom (Rohlf, 1996). They lie in the Kendall space or not depending on the kind of Procrustes distance used, full or partial one (Slice, 2001), but they lie in a curved, non-Euclidean space unsuitable for standard statistical tests (except resampling methods). Since Procrustes residuals² lie in a non-Euclidean space, they must be further modified by a rigid rotation so that they can be studied using classical statistical tools (Rohlf and Bookstein, 2003).

Using for rigid rotation the eigenvectors of the variance–covariance matrix of the Procrustes residuals, the resulting projection is described by the principal components scores (“Procrustes components”) and can be used for standard statistical analyzes and comparison tests. Using the eigenvectors of the bending-energy matrix (Bookstein, 1991), the resulting shape variables are called “partial warps” scores (PW). The PW, or their principal components, namely the “relative warps³” (RW), may be used in classical statistical analyzes and in visualization of shape changes through the deformation grids (i.e., D’Arcy Thompson-like plots showing the geometry of shape changes between objects; Bookstein, 1991).

The obvious interest of using principal components (either Procrustes components or RW) is that the number of input variables can be reduced: the few first RW generally represent a significant fraction of shape. The subset of first principal components to use is rather subjective, but it can follow some rules (Baylac and Frieß, 2005).

16.2.3 Allometry

Since each shape can be explained by the change in linear dimensions, it is obvious that size and shape are not independent attributes. The relationship between size and shape is called allometry. Geometric shape variables (see previous paragraph) are not allometry-free variables: they remove the isometric component of size change.

¹ Procrustes, 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 onto the bed (Grose Educational Media, 1997–1998, <http://www.groseculturalmedia.ca/greekm/mythproc.html>).

² Procrustes residuals are the differences between the residual coordinates of each object and the residual coordinates of the consensus configuration.

³ A complete glossary of the many technical terms related to GM can be found at <http://life.bio.sunysb.edu/morph/>.

Assuming a common model of growth is not rejected, one can use the growth model to predict allometry-free shape variation among groups after fixing size to one value (MANCOVA). The tentative removal of the allometric effect on shape can be justified for intraspecific studies (Klingenberg, 1996; Caro-Riaño et al., 2009; Morales et al., 2010). It is less justified for interspecific comparisons, where allometric variation is likely to be part of the evolutionary differences relevant to systematics.

16.2.4 Measurement Error

As explained above, the extraction of shape information from raw coordinates is computed relative to the consensus configuration derived from a specific group of samples; this thwarts mixing the final variables with other such variables computed from other individuals. Only raw coordinates could be shared, to the condition there was no error introducing artifactual differences between two sets of homologous landmarks. The measurement error exists at various steps of morphometric analysis (Arnqvist and Mårtensson, 1998). The mounting technique of specimens or organs, the photographing conditions, and the user's skill in collecting landmark coordinates may produce artifactual variation. Generally, similar techniques are used to process similar organisms, and digital techniques of modern photography provide adequate resolution for correct recognition of landmarks under different conditions. Whatever the quality and reproducibility of landmark digitization, the recommended way to perform morphometric comparisons is to allow one single user to produce the data.

Even when performed by a single user, digitization should be repeated at least once, allowing one to measure the precision and to reduce the error by averaging the two digitizations. The precision is estimated by the "repeatability" (R) index as described by Arnqvist and Mårtensson (1998), which is a Model II one-way ANOVA on repeated measures, where "R" is provided by the ratio of the between individual variance and the total variance.

16.3 Nonenvironmental Sources of Metric Change

16.3.1 Shape as a Polygenic Character

Shape appears as a classical polygenic character (Klingenberg and Leamy, 2001). Evidence for strong genetic determinism of shape was suggested by significant association with chromosome polymorphism (Bitner-Mathé and Klaczko, 1999; Orengo and Prevosti, 2002; Hatadani and Klaczko, 2008), and confirmed by quantitative genetic studies (Breuker et al., 2006; Patterson and Klingenberg, 2007). When studies on quantitative trait loci (QTL) were applied to the shape and size of mouse mandible, many QTL were identified for shape (Klingenberg et al., 2004), many more than for size (Klingenberg et al., 2001; Workman et al., 2002). Few studies are found in insects, also fitting the idea of genetic determinism (Iriarte et al., 2003) and polygenic inheritance (Shrimpton and Robertson, 1988; Long et al., 1995).

16.3.2 Genetic Drift

Since shape seems the output of a cascade of genes, it is expected that in natural conditions genetic drift will be a common factor of shape variation. Field observation has frequently observed significant shape differences between geographic areas (De la Riva et al., 2001; Dujardin et al., 2003; Gumiel et al., 2003; Dujardin and Le Pont, 2004a; Camara et al., 2006.; Aytekin et al., 2007; Henry et al., 2010). Laboratory experiments reproducing conditions favoring genetic drift between lines sharing the same environment were performed in *Ae. aegypti*. Using a set of three isofemale lines of *Ae. aegypti* monitored during 10 generations, a significant shift of shape appeared in one line, with nonsignificant changes in corresponding size (Jirakanjanakit et al., 2008). In this experiment, the change apparently produced by genetic drift did not affect the same landmarks as those affected by larval food or density variation (Jirakanjanakit et al., 2007).

16.3.3 Heritability

Heritability is depending on the genetic variability related to the trait under study, it is then depending on the population under study. Its measurement is not indispensable to the interpretation of natural metric variation, but it can provide valuable information about the adaptiveness of metric traits. In insects, morphological traits commonly have the highest heritability values compared to other trait categories such as life history, probably because the former are less concerned with fitness.

Geometric techniques allow separate estimations of size and shape heritabilities. Size in insects may show consistent heritability values (Daly, 1992; Lehmann et al., 2006), so that they can be experimentally selected to constitute subpopulations genetically distinct for size (Anderson, 1973; Partridge et al., 1994). Various studies examining cross-environment heritability of wing shape in Diptera produced high and stable heritability, reaching 60% or more (Roff and Mousseau, 1987; Bitner-Mathé and Klaczko, 1999; Gilchrist and Partridge, 2001; Hoffman and Shirriffs, 2002). The consistent values of shape heritability suggest that a large fraction of morphometric divergence seen between natural populations of insects (Camara et al., 2006.; Henry et al., 2010; Morales et al., 2010) may be due to additive effects of genes.

In *Ae. aegypti*, shape appears to be more heritable than size. When comparing size and shape cross-environment heritability on the same populations in *Ae. aegypti*, much higher values for shape (Figure 16.2) than for size were found, providing indirect evidence for different genetic sources of variation (Morales et al., unpublished data).

16.3.4 Hybridism

CS was increased in hybrids obtained from two close species, initially considered as two subspecies (Costa and Felix, 2007), *T. brasiliensis* and *T. juazeirensis*. It was larger than the mid-parent size, and larger than the largest parent's size, suggesting heterosis pointing to a consistent genetic divergence of the parents.

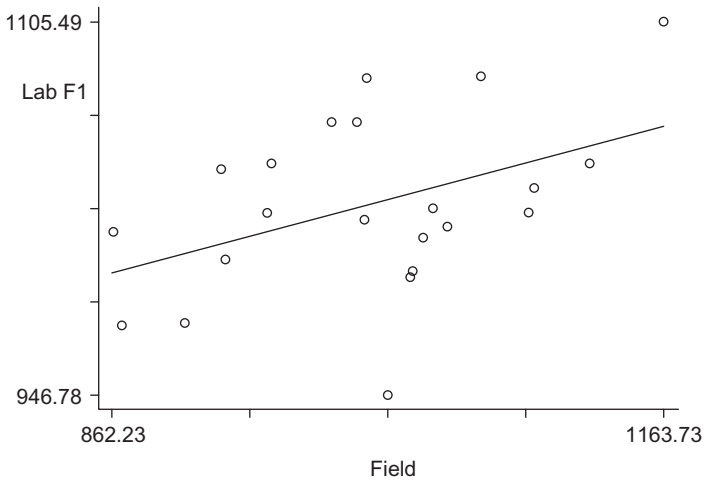


Figure 16.2 *Ae. aegypti*: regression of the first relative warps (RW1) of laboratory daughters on the RW1 of corresponding field-collected mothers in a cross-environment study of the heritability of the wing shape at 18 landmarks (Morales et al., unpublished data). Lab F1, female specimens obtained after crossing field-collected specimens.

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 (Costa et al., 2001; Costa and Felix, 2007) and the increase in size of their offspring. Contrary to size, shape of the hybrids remained intermediate between parents (Costa et al., 2008).

Between cryptic species of *Diachasmimorpha longicaudata*, a hymenopteran parasitoid of fruit flies, hybrids showed intermediate shape on the morphological space obtained from the two first RW. The size of the hybrids was larger than that of mid-parents, although not significantly larger (Kitthawee and Dujardin, 2009).

These two studies indicate different genetic mechanisms affecting size and shape, suggesting size as a character prone to show heterosis in case of genetically differentiated parents. This behavior of size could help exploring the degree of genetic differentiation between populations, especially when they are suspected to undergo speciation. Thus, when shape and size remain at intermediate values between parents, the latter are probably not genetically distinct taxa. Between seven laboratory colonies of *T. protracta* (Dujardin et al., 2007), or between each of the five subspecies of *T. protracta*, each hybrid had an intermediate CS between parents. Accordingly, geometric shape was also intermediate between parents (Dujardin, unpublished data). These observations were in agreement with allopatric conspecific *T. protracta* populations instead of with distinct taxa. Indeed, as observed in this group of insects, like in *T. platensis*–*T. infestans* hybrids or in *T. brasiliensis* (Costa et al., 2008), genetically differentiated parents, or distinct but phylogenetically close species, would have produced an exaltation of some body dimensions and other phenotypic traits in their progeny.

16.4 Environmental Sources of Metric Changes

Environment often affects metric properties. However, it is important to specify which metric property is affected, size, and/or shape, and how it is affected. No simple rule can be formulated. Moreover, since there is no detectable polarity in metric changes, the present aspect of the phenotype does not contain any signal of its own history. As stated by Losos (2000), "... we cannot go back in time and expressly determine why a trait evolved ... the best we can do is enumerate tests suggested by a particular adaptive hypothesis."

There are different hypotheses about the way the environment affects shape. They describe situations which can be partially reproduced in laboratory experiments and/or manipulative field experiments. We will briefly consider only the following ones: phenotypic plasticity (PP), character displacement (CD), genetic assimilation, and accommodation.

16.4.1 *Phenotypic Plasticity*

The genotype does not give rise to the phenotype, but to a range of phenotypes. The "reaction norm" is the whole repertoire of possible phenotypes that may occur for a given genotype in all environments (Dobzhansky, 1971; Schlichting and Pigliucci, 1998). The reaction norm can easily be explored in laboratory experiments (Hillesheim and Stearns, 1991; David et al., 1994; Debat et al., 2003; Jirakanjanakit et al., 2007; Caro-Riaño et al., 2009). By definition, PP is the occurrence of phenotypic variation of a single genotype interacting with different environments (Schlichting and Pigliucci, 1998).

A new phenotype expressed in a new environment may be adaptive. To this condition, PP can aid speciation by making available a different phenotype upon which natural selection can act. In such scenario, speciation would start with PP, not reproductive isolation (Görür, 2005). In Triatominae, such a scenario is apparent (Dujardin et al., 1999b) and many examples exist of morphologically and ecologically recognized species which can still interbreed (Dujardin et al., 2009). Understanding the causes and consequences of phenotypic variation is important for understanding the mechanisms of evolution. However, the genetic mechanisms underlying the evolutionary importance of PP (Waddington, 1953; Thompson, 1971; West-Eberhard, 1989; Schlichting and Pigliucci, 1998) have to date received few experimental confirmations (Rutherford and Lindquist, 1998; Suzuki and Nijhout, 2006).

Contrary to its evolutionary importance, the ecological importance of PP is easy to understand: populations or species having wider adaptive plastic responses can enlarge their ecological niches. For instance, among the more than 140 species of Triatominae, the vectors of Chagas disease, a few species have been able to colonize human structures. Within some of these species, the comparison of "domestic" and sylvatic subpopulations highlighted significant size differences, sylvatic insects

being generally larger. Were these species more plastic than the others so that they could reduce their size as apparently required by survival in artificial ecotopes? Was the size a secondary event selected by the domestic environment? Using emerging adults of an inbred line of *R. pallescens*, Caro-Riaño et al. (2009) evaluated size and shape variation of the heads and the wings under simulated conditions of sylvatic (low population density, low feeding frequency) and domestic (higher density, higher feeding frequency) habitats. Results demonstrated that selection was not needed to account for observed changes between sylvatic and domestic ecotopes. The significant size reduction was shown to be a plastic response to combined population density and feeding frequency.

In addition to explain diversity and adaptation, PP also impacts our understanding of taxonomy, because it suggests that species characteristics are not immutable, but are influenced by the environment and can be highly variable (Ananthkrishnan, 2005).

16.4.2 Character Displacement

The initial definition of the “character displacement” concept (Wilson and Brown, 1955; Brown and Wilson, 1956) did not predicate the real complexity of its demonstration: “the situation in which, when two species overlap geographically, the differences between them are accentuated in the zone of sympatry.” Specifically, in sympatry selection was supposed to minimize attempts at hybridization (by mistaken identity) as well as competition between the two species.

The difficulties of obtaining unambiguous evidence from natural observations have been discussed by Grant (1972), and, more recently, by Losos (2000). Typically, CD was suspected when more difference was observed between species developing in sympatry than in allopatry; “soon after the theory was promulgated, ecologists and evolutionary biologists were seeing evidence for character displacement everywhere” (Losos, 2000). Various other conditions must be satisfied to assess CD, among which are the level of differences in sympatry (greater than expected by chance), the evolutionary history of sympatry (original or derived situation), the genetic nature of phenotypic differences and, importantly, the connection between characters and competition for resources (Grant and Grant, 2006). CD was demonstrated for behavioral and ecological characters more often than for morphological characters (Grant, 1972; Losos, 2000). Morphologically, the displaced character is expected to be part of the feeding apparatus (Adams and Rohlf, 2000). If the mouthparts have a species recognition function, then displacement may have consequences on speciation as well.

Thus, if PP could be analyzed raising the same genotypes of one species in different environments, CD would then be studied in one single environment, raising a genotype of one species alone and in combination to a genotype of another species. Nevertheless, if one considers for a given species the surrounding ones as making part of the environment, then CD could be considered as a particular case of PP induced by species competition. Medically important insects did not receive much attention.

16.4.3 Genetic Assimilation

Unexpectedly, PP can produce heritable changes. The mechanisms of such phenomenon, which is reminiscent of Lamarckian “inheritance of acquired characteristics,” do not depart from orthodox genetics. They have been named “genetic assimilation” (Waddington, 1953) or “autonomization” according to Schmalhausen (Levit et al., 2006), and more recently “genetic accommodation” (West-Eberhard, 1989).

Waddington defined genetic assimilation as “a process by which a phenotypic character, which initially is produced only in response to some environmental influence, becomes, through a process of selection, taken over by the genotype, so that it is found even in the absence of the environmental influence which had at first been necessary” (Waddington, 1953).

Laboratory experiments, old ones (Waddington, 1953, 1956; Anderson, 1973) and more recent ones (Gibson and Hogness, 1996; Rutherford and Lindquist, 1998; Sollars et al., 2003), unambiguously demonstrated genetic assimilation. Indirect evidence was provided from natural populations of the medically important triatomine bug *Rhodnius pallescens*. Five lines reared at the same temperature, some of them reared over more than 40 generations, have been shown to harbor distinct sizes in accordance with the temperature of their region of origin in Colombia (Jaramillo, Ph.D. thesis). The absence of size convergence at the same laboratory temperature, and the correlation with temperature of initial field conditions, suggested a genetic determinism for size variation (Dujardin et al., 2009). This latter example illustrates that Bergmann size clines in natural populations may have more complex causes (Davidowitz et al., 2004) than a merely developmental process (David et al., 1994; Vanvoorhies, 1996).

16.4.4 Genetic Accommodation

Genetic accommodation (West-Eberhard, 1989, 2003) is a concept very close to genetic assimilation, but wider for two reasons: the nature of the trigger and the outcome of the process (Görür, 2005; Braendle and Flatt, 2006).

First, the hypothesis of genetic accommodation assumes that the trigger at the onset of the phenotypic change is either genetic or environmental, whereas the concept of genetic assimilation typically assumes only an environmental trigger. Second, the expected outcome of genetic assimilation is a new, heritable phenotype insensitive to environmental change (see hereunder the concept of “canalization”), while genetic accommodation can produce both insensitive and sensitive new, heritable phenotypes.

Thus, genetic accommodation is a generalization of genetic assimilation. In this general hypothesis, it is argued that environmentally triggered novelties may have greater evolutionary potential than mutationally induced ones, mainly because of two features (Görür, 2005): (i) they concern populations rather than individuals (while mutations are individual events) and (ii) they represent optimal, or close to optimal, adaptations (while mutations are often counter-selected). With time

(the concept of “recurrence”), these two features increase the likelihood of genetic assimilation (selection of genotypes accidentally producing the same phenotype as the adapted one), ending up in local genetic changes.

Genetic accommodation has been experimentally demonstrated by Suzuki and Nijhout (2006) studying a color polyphenism in *Manduca sexta* (Braendle and Flatt, 2006).

Hidden Genetic Variability

The mechanisms by which an environmentally induced phenotype may become heritable are entirely compatible with concepts of classical neo-Darwinian evolutionary biology. Indeed, the environmental trigger (since this is the disputable one) just uncovers previously cryptic genetic variation (Gibson and Dworkin, 2004).

Thus, there are genetic mutations that can remain masked until the environment (or another mutation) reveals them (Bergman and Siegal, 2003). In case of an environmental trigger, the external stimulus has to be recurrent and consistent in time so that selection can lead to genetic accommodation.

The trigger, either an environmental or mutational one, acts through its effects on “capacitors,” which are proteins able to buffer genotypic variation under normal conditions, thereby promoting the accumulation of hidden polymorphism. Published examples of capacitors for morphological evolution are the heat shock protein Hsp90 (Rutherford and Lindquist, 1998; Debat et al., 2006) or the genes regulating hormonal titers (Pennisi, 2006; Suzuki and Nijhout, 2006).

Hidden genetic variation can also be revealed through epigenetic mechanisms (Sollars et al., 2003), which are heritable changes in gene function that occur without a change in the sequence of nuclear DNA (Jablonka et al., 1992; Jablonka and Lamb, 2002). Epigenetic mechanisms such as DNA methylation, histone acetylation (producing changes to the chromatin packaging of DNA), and RNA interference (regulation of gene-expression control by non-coding RNA), and their effects in gene activation and silencing are increasingly understood to play a role in phenotype transmission and development (Bird, 2007).

16.5 The Regulation of Phenotype

To the many sources of phenotypic changes the organism opposes homeostatic processes. Two components of this homeostasis are canalization and developmental stability. The two components seem to be independent processes (Debat et al., 2000; Réale and Roff, 2003), and are easy to distinguish: canalization is the stability of development in different environments, while developmental stability refers to stability in the same environment. Canalization is thus a buffering process against external and/or mutational perturbation from one environment to another, while developmental stability allows the organism to withstand random accidents during development in the same environment (Graham et al., 1993).

16.5.1 Canalization

The term “canalization” also is due to Waddington, corresponding to the “stabilizing selection” of Schmalhausen (Levit et al., 2006). Here are the terms used by Schmalhausen himself: “Every adaptive modification is an expression of a norm of reaction, which went the long way of historical development under changing conditions. It is connected with the establishment of ‘canals’ through which a certain modification develops (Waddington talks about the ‘canalization’ of development). An external factor operates only to switch the development into one of the existing canals” (Schmalhausen in Levit et al., 2006).

Thus, as for PP, canalization is not a property of a species or of a population, but of a genotype (Dworkin, 2005). However, different traits of a single organism can be examined for their relative canalization by studying their natural variation in different lines, populations, or species. For instance, contrary to size changes, shape changes of the wings induced by striking altitudinal variation as found between the Andes and the Amazon basin could not interfere with species differences in sandflies (Dujardin et al., 2003). A similar study comparing the wing shape of transcontinental populations of two close mosquito species, *Ae. aegypti* and *Ae. albopictus*, showed that species differentiation based on wing shape, but not on its size, was not altered by transcontinental migration during the last decades. Thus, in spite of the many possible situations supposed to affect shape, like environmental changes, possible environmental stress, likely founder effect, possible genetic drift, and species competition, both species were still distinguishable at the same landmark locations (Henry et al., 2010). This relative constancy of shape patterns within each species contrasted with the lability of size. For the same species (*Ae. aegypti*), size was significantly affected by a simple change in the food concentration or in the larval density (Jirakanjanakit et al., 2007). Another example comparing size and shape responses is found in highly inbred lines of *R. pallescens* (Triatominae): the plastic response scored for the CS of the wing to the laboratory conditions of “domesticity” was not observed for the shape, except as an allometric change (Caro-Riaño et al., 2009).

Incidentally, the apparently higher canalization of shape makes this trait a suitable character for populations and species distinction (Dujardin and Le Pont, 2004b).

16.5.2 Developmental Stability

Although development is an individual attribute, its stability is estimated at the population level and can be compared with other populations. The use of morphometrics as an indicator of environmental stress is generally performed by estimating the frequency of abnormal phenotypes (phenodeviants) or the amount of fluctuating asymmetry (FA) (Palmer and Strobeck, 1986). Bilateral symmetry is not supposed to change during development; it is a developmental invariant. Other measures of developmental stability could be used that also are developmental invariants, like fractal dimensions, although they were described for plants and vertebrates only (Graham et al., 1993).

Stress may have many different interpretations, and is probably not the only explanation for increased FA. Stress can be the infection by a virus or parasite, or a difficult conquest of a new habitat. In the few domestic populations of *T. sordida*, a potential vector of Chagas disease in Bolivia and Argentina, significant FA was found, whereas no FA at all could be disclosed in their sylvatic counterparts (Dujardin et al., 1999b).

Because of its relatively strong canalization, geometric shape of the insect wings is not prone to show significant changes under the normal range of developmental conditions encountered by organisms (Birdsall et al., 2000). Nevertheless, the use of shape variation in response to environmental stress has been advocated for insects, although these changes were considered as very subtle ones (Hoffmann et al., 2005). An advantage it could have on asymmetry analyzes would be possible signature changes in landmarks characteristic of a specific environmental stress. A disadvantage is that, contrary to symmetry, which is expected to be perfect, and contrary to the frequency of phenodeviants, which is expected to be zero, there is no “expected shape” and thus no way to use shape changes to measure the degree of stress.

16.6 Applications in Medical Entomology

16.6.1 Species Identification and Detection

The most important objection to the morphological concept of species is the existence of sibling (or isomorphic) species (Mayr, 2000). Sibling (or also cryptic) species are morphologically identical or nearly identical entities recognized as different species according to other, modern concept(s) of species. However, this objection to the typological concept (i.e., to “morphospecies”) is weakened by the possibilities of modern quantitative shape comparisons (Baylac et al., 2003; Becerra and Valdecasas, 2004; Dujardin, 2008). Shape comparisons detect minimal morphological variations, which often are undetectable by traditional morphological studies and even by classical morphometric approaches. Cryptic species of insects showed distinct shapes in kissing bugs (Matias et al., 2001; Villegas et al., 2002; Dujardin et al., 2009), sandflies (De la Riva et al., 2001), scythrindids (Roggero and Passerin d’Entrèves, 2005), parasitoid hymenoptera (Baylac et al., 2003; Villemant et al., 2007; Kitthawee and Dujardin, 2009), syrphids (Francuski et al., 2009), fruit flies (Kitthawee and Dujardin, 2010), and screwworm flies (Lyra et al., 2009). Although morphometric discrimination does not necessarily mean species determination, it has also been used to question species boundaries (Aytekin et al., 2007), or to synonymize controversial taxa (Gumiel et al., 2003).

Geometric morphometry is becoming a fast and low-cost alternative to identify cryptic species that often need the molecular machinery to be distinguished. However, the diagnostic metric features cannot be shared. Because geometric shape is defined relative to the consensus of the specimens under study, shape variables derived from one set of coordinates cannot be compared with shape variables

derived from another set. Coordinates themselves could be used for such comparisons, but the measurement error may represent a significant obstacle, especially when the objective is to distinguish very similar species.

The "User Effect"

Among the sources of measurement error (see Section 16.2.4), user intervention is often the most important. The error is generally due to small but systematic differences in pointing to the exact localization of some landmarks. These subtle discrepancies are amplified by the power of multivariate analysis like the discriminant analysis. Their impact can be reduced averaging repeated collections of the data (Arnqvist and Mårtensson, 1998). However, such correction might not be satisfactory when comparing very close specimens or groups, and measurement error may become a significant obstacle for different users (Jordaens et al., 2002; Rasmussen et al., 2001). As a consequence, user A should not enter his own measurements in a database of coordinates collected by user B, and vice versa.

The Need for a Bank of Reference Images

To circumvent the lack of exchangeability of the morphometric variables, an alternative geometric descriptive system should be developed that separates data gathering and analyzes. It goes through the creation of a bank of reference images from which one can extract raw data and compare it to external, unknown specimens. The chances of successful identification would then depend on the relevance of reference images, on their level of shape divergence and on the classification techniques. Such an initiative is ongoing at <http://www.mpl.ird.fr/morphometrics/clic/index.html> under the name CLIC (Collection of Landmarks for Identification and Characterization). The need for such a database is underestimated, because the power of morphometrics to identify taxa is itself probably underestimated.

16.6.2 Characterization Tool at the Individual Level

In humans, some metric traits allow highly reliable individual identification (fingerprint, iris pattern, etc.). We can expect similar situation in animals. In medical entomology, it might be useful to assign a single individual to its origins. Two applications can be considered, one in systematics, the other one in population structure.

Species are generally well distinguished thanks to qualitative morphological characters, but close species might be difficult to confidently identify based on one single individual. Using geometric shape comparisons, one single individual can generally be accurately classified using a database of images of the candidate species (Matias et al., 2001). As an example, we show here unpublished data about mosquito identification. Each single individual has been allocated to its closest group (according to Mahalanobis distance) without using that individual to help determine a group center ("validated reclassification"). The wing venation patterns

appear to be roughly the same among the genera of Culicidae, they allowed however an almost perfect reclassification of them all (Table 16.1). Within some genera like *Aedes* or *Anopheles*, the species discrimination was also very satisfactory; it was less convincing in the genus *Culex* (Table 16.2). The possibility to perform satisfactory identifications without being an expert in taxonomy is very attractive, but

Table 16.1 Morphometric Identification of Culicidae Based on 13 Landmarks of the Wing

Genera	Ur, Ma	An	Mi	Cu	Ae, Ar, Co
Scores	100%	97%	96%	95%	100%
N	508 (8)	446 (6)	348 (5)	317 (4)	127 (3)

The first column indicates that 100% of the genus *Uranotaenia* (Ur) and 100% of the genus *Mansonia* (Ma) could be recognized when mixed with the six other genera: *Anopheles* (An), *Mimomyia* (Mi), *Culex* (Cu), *Aedes* (Ae), *Armigeres* (Ar), and *Coquilliettidia* (Co). The second column indicates that 97% of the genus *Anopheles* could be recognized when mixed with the genera *Mimomyia*, *Culex*, *Aedes*, and *Armigeres*. The third column indicates that 96% of the genus *Mimomyia* could be recognized when mixed with the genera *Culex*, *Aedes*, and *Armigeres*. The fourth column indicates 95% of the *Culex* could be distinguished from the genera *Aedes*, *Armigeres*, and *Coquilliettidia*. The last column indicates that these three genera were perfectly discriminated by their wing geometry. N, total number of individuals in each analysis; number of genera in the analysis is given in parenthesis. Mosquito collection by A. Henry and P. Thongsripong (University of Hawaii). Morphological identification of the genera by Dr. R. Rattanarithikul (AFRIMS, Thailand). Digitization of wings by J.-F. Lasnes (University of Montpellier).

Table 16.2 Correct Species Attribution Scores Based on the Geometry of the Wings

Species	Scores (%)	n/N
<i>Aedes</i>		
(<i>Stegomyia</i>) <i>aegypti</i>	100	12/12
(<i>Neomelaniconion</i>) <i>lineatopennis</i>	66	10/15
(<i>Aedimorphus</i>) <i>mediolineatus</i>	100	12/12
(<i>Aedimorphus</i>) <i>vexans</i>	83	20/24
<i>Anopheles</i>		
(<i>Anopheles</i>) <i>barbirostris</i>	100	14/14
(<i>Cellia</i>) <i>tessellatus</i>	88	8/9
(<i>Cellia</i>) <i>vagus</i>	91	34/37
<i>Culex</i>		
(<i>Culex</i>) <i>vishnui</i>	55	29/52
(<i>Culex</i>) <i>gelidus</i>	61	11/18
(<i>Culex</i>) <i>quinquefasciatus</i>	91	11/12
(<i>Oculeomyia</i>) <i>bitaeniorhynchus</i>	78	18/23
(<i>Oculeomyia</i>) <i>sinensis</i>	62	18/29
(<i>Culiciomyia</i>) <i>nigropunctatus</i>	91	11/12

Thirteen species belonging to three genera, *Anopheles* (An.), *Culex* (Cx.), and *Aedes* (Ae.), were analyzed for species identification, namely: *Ae. aegypti*, *Ae. lineatopennis*, *Ae. mediolineatus*, *Ae. vexans*, *An. barbirostris*, *An. tessellatus*, *An. vagus*, *Cx. bitaeniorhynchus*, *Cx. gelidus*, *Cx. nigropunctatus*, *Cx. quinquefasciatus*, *Cx. sinensis*, and *Cx. vishnui*. Scores, correct attributions in percentages by species after validated reclassification; n, number of individuals correctly assigned to the species; N, total number of individuals in the species. Mosquito collection by A. Henry and P. Thongsripong (University of Hawaii). Species morphological identification by Dr. R. Rattanarithikul (AFRIMS, Thailand). Digitization of wings by J.-F. Lasnes (University of Montpellier).

more studies are needed to evaluate the full interest of this identification approach in many groups of medically important insects.

More difficult is the identification when comparing few conspecific individuals. Reinfestant specimens after vector control measure may be few, and classical morphology could be unable to suggest their origin (see Section 16.6.4). Provided a database exists of specimens collected before control measures, shape can be used for quantitative comparisons of local and external individuals (Dujardin et al., 2007).

16.6.3 Biodiversity

The transmission of vector-borne diseases has obvious links with the environment. Studies exploring these links suggested that the reduction in global biodiversity is likely to contribute to vector-borne disease transmission through the “dilution effect”⁴ (Chivian and Bernstein, 2004; Keesing et al., 2006). It is therefore highly desirable to quantify the environment. In this kind of study, geometric morphometrics has two advantages: the ability to help identify taxa and its own addition to knowledge about biodiversity.

Biodiversity is expressed as the combination of both species richness (SR), the number of species in a specific environment, and species evenness, the proportion of each of them. Different indexes have been suggested to take into account both richness and evenness, from which the most commonly used are the Shannon–Wiener’s (Shannon and Weaver, 1949) and the Simpson’s (Simpson, 1949) indexes.

In addition to these estimates of biodiversity, complementary information has been searched for in the morphological disparity of organisms. The morphological disparity has been expressed in two ways, one considering the range of shape variation (the difference between extreme forms), the other one the amount of shape variation (the variance of shape). Modern morphometrics is giving these estimations a powerful quantitative tool for accurate measurements and comparisons (Roy and Balch, 2001; Neige, 2003).

The relationship between morphological disparity and biodiversity differs according to the way biodiversity is measured (i.e., taking into account or not the evenness).

Metric Disparity and SR

Does metric disparity increase with the number of species? One could expect greater richness to be the cause of higher morphometric variation, but no such relationship could be confirmed. Trends in SR generally did not match trends in metric disparity (MD). However, one could argue that if selection targets forms rather than species, some relationship is predictable. For a given clade’s history,

⁴ In the “dilution effect” hypothesis, locales with few species capable of sustaining vectors will have higher disease risk because vectors feed more frequently on the species that serve as hosts of the pathogen. In contrast, “dilution” occurs in areas with high biodiversity because more species (not all of which harbor parasites) are available to sustain vectors.

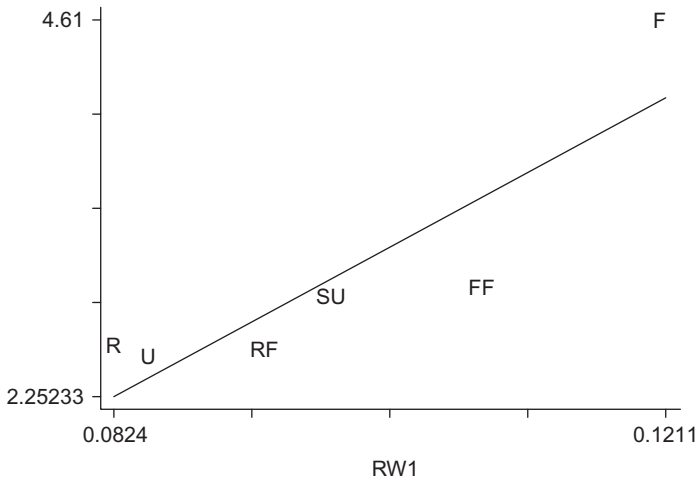


Figure 16.3 Relationship between the Shannon index of biodiversity (vertical axis) and the metric disparity (horizontal axis) computed as the range of the first RW in a region of Thailand (Henry et al., unpublished data). There were 584 mosquitoes defined by their wings at 13 landmarks in different environments: F, forest; FF, fragmented forest; RF, rice field; R, rural; SU, suburban; U, urban. Statistical significance: $P = 0.02$ (down to $P = 0.06$ if forest is removed from the data).

Foote (1993) predicts a high or low ratio MD/SR as depending mainly on the kind of selection during evolution: a selection precluding either intermediate or extreme forms, respectively.

When high, the ratio MD/SR is probably the result of combining a relatively low number of species with a relatively high morphometric variation. What could be the origin of such pattern? In addition to the possible effect of selection promoting extreme forms (Foote, 1993), the answer is probably to be found into what generates morphological heterogeneity: PP, species competition (Ricklefs and Miles, 1994), and of course phylogenetic diversity (Richman and Price, 1992; Shepard, 1998). Based on some idealized scenarios, the ratio MD/SR can help discussion about the geographic origin of some group of species in relatively isolated regions (Neige, 2003).

Taking into Account the Evenness

In addition to being poorly related to metric disparity, trends in SR do not necessarily match trends disclosed by other biodiversity metrics (Roy and Balch, 2001), either Shannon's index (H) or Simpson's index (D). These estimates take into account the proportion of each species (evenness), not only their number. In some occasions, they can show some relationship with morphological disparity. An ongoing study in Thailand about mosquito diversity according to different environments

allowed the capture of most of the tribes and many genera of Culicidae (Henry et al., unpublished data). In this study, neither the richness nor the biodiversity indexes could show any significant correlation with the metric disparity estimates, except the Shannon–Wiener’s index and the range of metric variation as estimated by the first RW (see Figure 16.3).

Heterozygosity

Interestingly, some correlation is observed more frequently when relating the metric disparity of conspecific populations and their genetic diversity (heterozygosity)⁵. The relationship is often, but not always, a negative one. The negative relationship has generally been interpreted as evidence for a higher developmental homeostasis in heterozygotes, but simple inbreeding has been also suggested (David, 1999).

16.6.4 Reinfestation

As long as geometric shape is able to identify the parental generation and to distinguish it satisfactorily from other subpopulations (Falconer, 1981; Dujardin et al., 2007), it might be able to provide relevant information in studies of reinfestation after treatment (Dujardin et al., 1997, 1999a).

Provided that samples were available from the population before insecticide application, relative similarities could suggest the origin of reinfesting specimens: they would be either the descendants of previously killed bugs, or immigrants from an external focus. Shape as extracted from traditional morphometrics (head measurements) of the Chagas disease vector *Triatoma infestans* provided information that could identify the source of reinfesting specimens (Dujardin et al., 1997), and such information has been shown to be in agreement with genetic markers (Dujardin et al., 1999a). The geometry of the wing of the North American *T. protracta* was tested on laboratory populations and was shown to be an interesting candidate to assess the origin of a given individual (Dujardin et al., 2007).

Since a residual population is assumed to be the same generation as or the next generation to the individuals subjected to insecticide spraying, the reinfestation analysis is based on the assumption that an insect is more similar to its parents than to other insects. But the reasons for successful results are not only the high heritability of shape (see Section 16.3.3). In the reinfestation studies, the objective is not to measure gene flow or levels of migration, but rather to distinguish local “inhabitants” from “immigrants.” Thus, the environmental effect on metric traits (the environmental covariance) is a welcome effect. Insects reared in the same microenvironmental conditions (a few houses, a village) would share a significantly larger amount of metric similarity, making a residual population easier to recognize.

⁵ Actually, the Simpson’s index of biodiversity and the heterozygosity or genetic diversity index are obtained from the same probabilistic approach: both indexes consider the probability to sample at random two identical species or genotypes. This probability is actually estimating the homogeneity of the individuals (homozygosity), and to get the opposite, it is subtracted from 1, the total frequency.

Of course, the level of population structuring is an important condition for morphometric characters to be applicable in reinfestation studies. They would be less applicable to highly dispersive insects breaking the population structure at each new generation.

16.6.5 Population Structure

A recurrent need in medical entomology is to quantify the current exchanges of individuals among subpopulations. This quantification would inform on “population” structure, to be distinguished from “genetic” structure, which is defined by the level of gene flow among subpopulations. Although mark-release-recapture studies might be a valid option to evaluate the frequency of active migrants among subpopulations (Tapis and Hausermann, 1975; Harrington et al., 2005), it cannot account for passive migration of nonflying stages of the insect, so that this frequency is currently evaluated by indirect methods; the measurement of gene flow is the technique of choice (Slatkin, 1981, 1985).

Gene Flow and the Flow of Migrants

Gene flow measurement provides indirect information on the level of migration among subpopulations. However, this information is of unequal value depending on its output, either “lack of gene flow” or “complete gene flow.” Lack of gene flow is valid information since in that circumstance (genetic divergence) migrants are highly unlikely. Less valid information is the case of complete gene flow, since no one can affirm that such (lack of) genetic structure is a reflection of the current level of migration. How contemporaneous or recent it depends on the effective size of the populations under study and the evolutionary rate of the genetic marker (McKay and Latta, 2002). Additional problems with genetic markers are that they are relatively costly and they need appropriate infrastructures. As an unfortunate consequence, genetic markers often remain inside research laboratories and have not yet found their way into routine medical entomology.

Environmental Variance of Size Versus Shape

Modern morphometrics is tempting as a candidate population marker because it is a fast, low-cost, easily spread tool; it is informative about current or very recent population events (Falconer, 1981); and it contains information on genetic variation. However, as long as morphometric traits have much higher environmental variance than genetic markers, they are not appropriate for gene flow estimation.

How then to interpret geographic variation of metric properties? After what we learned from natural and experimental studies on PP, an entirely environmental origin of phenotypic variation is not likely among natural populations. Metric variation can be decomposed into size and shape variation, and even if the two properties are not completely independent, their environmental variance can be examined separately. The importance of diversifying selection inflating size or

shape variation among natural populations can be quantified by comparing on the same material (i) the F_{st} index as derived from neutral molecular markers and (ii) the Q_{st} index as computed from metric characters. Q_{st} separates quantitative genetic variation in a manner analogous to F_{st} for single gene markers (Spitze, 1993): if the quantitative characters and the molecular characters are neutral, Q_{st} and F_{st} should converge to the same value (Hiernaux, 1977; Rogers and Harpending, 1983; Whitlock, 1999). Data comparing molecular F_{st} and quantitative Q_{st} are few. They tend to show the following trends: (i) Q_{st} is generally higher, or much higher, than F_{st} , and (ii) the value of Q_{st} depends on character fitness (McKay and Latta, 2002). Within species, traits experiencing the strongest local selection pressures (diverging, or diversifying selection) are expected to be the most divergent from molecular F_{st} (McKay and Latta, 2002).

The small set of comparisons reported by Dujardin (2008) in medically important insects between Q_{st} and F_{st} confirmed the importance of selection modifying the geometric variation among subpopulations. These comparisons allowed two more observations: (i) in agreement with the idea of shape having less environmental variance than size, they confirmed the lower sensitivity of shape (relative to size) in response to diversifying selection, and (ii) in agreement with the infrequent report of a Q_{st} lower than F_{st} , which would suggest homogenizing selection acting on the quantitative trait, no such situation was observed in medically important insects.

Biogeographical Islands

Local elimination of an insect vector of disease is generally held to be feasible only for geographically constrained situations such as islands. The task of population genetics studies is, in a sense, to find and define those biogeographical “islands” (Patterson and Schofield, 2005) of the vector distribution on the mainland. Can modern morphometrics help defining these target areas?

To discuss this application, it is important again to insist on which metric property is considered, either geometric shape or size. Here, modern morphometrics means “geometric shape.” Moreover, since the populations compared are conspecific ones, and especially if size variation is important, allometry-free shape should be preferred to just shape. As seen in the previous sections, geometric shape is made of homologous characters, it is more heritable than size and more canalized than size against environmental disturbances. The following two propositions are related to the geometric shape of the wings, but probably also would apply for the shape of other organs:

1. Because of genetic drift and the polygenic nature of shape, isolation in natural conditions will tend to quickly generate shape changes.
2. When frequent exchanges occur between populations (i.e., when genetic drift is not possible), shape is hardly different even if habitats are different.

The first proposition helps to decide a likely isolation between populations using knowledge about their respective environments. Between truly isolated populations,

differences in shape should develop because of two main reasons: (i) genetic drift is likely to be a major force affecting shape, and (ii) homogenizing selection seems infrequent (or unable to counteract the effects of genetic drift). For the shape of the wing in isofemale lines of *Ae. aegypti*, laboratory observations suggested that genetic drift could occur after a few generations (Jirakanjanakit et al., 2008). However, because of the possible importance of diversifying selection, a decision is not possible when shape differences coexist with habitat heterogeneity.

The second proposition refers to lack of shape changes. The interpretation of shape homogeneity in nature does not require information about the environments of the populations under study. As long as homogenizing selection on geometric shape can be discarded, similarity is suggestive of exchanges between compared populations. This proposition is supported by various studies. Even between populations of houses and of palm trees, the shape of the wing of *R. prolixus* was similar (Felicciangeli et al., 2007). Such similarity strongly suggested exchange of individuals, which was confirmed later by genetic markers (Fitzpatrick et al., 2008). Lack of isolation was also described by both genetic and metric markers for tsetse flies along the Mouhoun River in Burkina Faso (Bouyer et al., 2007). Recently, tsetse flies were collected in the city of Abidjan (Ivory Coast) from a primary forest relict, from the zoological garden, and from the nearby university campus of Abobo Adjame. The three sites are a few miles away from each other. The question was: could the flies from the forest reinvade the university campus or the zoological garden after vector control there? The wings of the flies, either males or females, in spite of size differences in one sex, could not show allometry-free shape differences. The likely connection among three sites was confirmed by microsatellite markers applied on the same specimens (Kaba Dramane, Ph.D. thesis).

The Need for a Heuristic

Considering the cost represented by the molecular machinery in developing countries, these examples suggest that a faster and less expensive morphometric approach could be helpful, even as an orientation technique only. Thus, geometric shape variation could be our guide to quickly identify at low-cost areas where isolation is possible and where it is unlikely. Two directives helping interpretation could be the following:

- If shape does not show differences between populations, the most likely explanation is that populations are not isolated ones.
- If shape shows strong differences, one must consider also the habitats which are compared: isolation is a valid interpretation only in case of similar environments.

These guidelines are based on the hypothesis that genetic drift is the main force in nature producing fast differences in shape among conspecific populations. They refer to contemporaneous time, not to an undefined evolutionary past. They are easy to falsify, so that they invite more natural observations related to population structure and shape variation.

16.7 Conclusion

Because coordinates of anatomical landmarks contain information about both size and shape, modern morphometrics could convert the abstract quantification of shape into a direct visual representation. In the same process the geometric approach provided a global estimate of size independent from shape in the absence of allometry. These two metric properties were examined for their insight in evolutionary biology studies, as well as in relation to medical entomology. Their usefulness in quantifying PP was shown to help in the understanding of the evolutionary and ecological importance of the phenotype. In relation to medical entomology, the following needs were considered: species identification, biodiversity estimation, reinfestation analyzes, and “biogeographical islands” detection. It is suggested that geometric shape, as opposed to size, appears as the property of choice to meet these needs. Concrete propositions are made, one is a bank of reference images, the other one is a heuristic to population structure interpretation.

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