17 Automatic Measurement of Honeybee Wings

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CONTENTS

INTRODUCTION

Honeybee wings are one of the most frequently measured insect body parts. These measurements are most often used to distinguish between honeybee subspecies (Ruttner, 1988a). This discrimination is required in honeybee breeding in order to preserve the morphological characteristics of selection lines (Ruttner, 1988b), with breeding programs often being related to conserving honeybee biodiversity (Rortais et al., 2004). Discrimination between subspecies is essential for monitoring and controlling Africanized honeybees on the American continents (Strauss and Houck, 1994). There is also a growing number of studies using honeybee wing measurements, not only in biogeography but also genetics (Brückner, 1976), development (Smith et al., 1997) and ecology (Higginson and Barnard, 2004).

The process of measuring of honeybee wings is tedious and time consuming. In subspecies discrimination studies, the measurement of a single individual is not enough to obtain 100 per cent accuracy (Daly and Balling, 1978). Usually, more than 10 workers from one colony are used. In biogeography studies, it is recommended that measurement of 10 workers from five to six colonies per apiary be taken (Radloff et al., 2003). Also, the number of wing measurements per individual is large, usually ranging from 15 (Ruttner, 1988a) to 38 (Smith et al., 1997).

WING MOUNTING

Before a wing can be measured it needs to be mounted. Mounting allows the wing to be flattened and positioned on a plane perpendicular to the optic axis of the measuring instrument or image acquisition system. The mounted wing can also be easily labelled and stored.

Specimens are usually mounted on microscopic slides or glass photographic slide frames. Wings mounted on microscopic slides are fixed in place by Canada balsam (Adsavakulchai et al., 1999; Weeks et al., 1999b), Euparal (Batra, 1988; Smith et al., 1997) or transparent tape (Yu et al., 1992; Kokko et al., 1996). Using mounting substances is tedious and their optical properties deteriorate with time. Mounting with the use of transparent plastic tape is quick; however, the optical quality of images obtained from such preparations is markedly reduced.

The optimal solution seems to be mounting the wings in photographic glass frames. These do not reduce optical properties because the wing is kept in place by the glass and no mounting medium is required. Moreover, mounting is quick and easy and remounting is possible if the wing needs to be cleaned of dust or dirt. Wings mounted this way are ready for scanning, projecting with a slide projector or photographing under a microscope. If numerous samples of worker front wings are taken from a single colony, they can be mounted in one slide frame. On the other hand, if right and left wings are required (e.g. in fluctuating asymmetry studies), all the wings of a single individual can be mounted in one slide frame. The position of the wings in the frame can provide information as to whether it is the left or right wing. A slight problem is that the smaller hind wings, which are thinner than the front wings, tend to slide down because the glass plates are pushed away by the front wings. This problem can be solved by using a small amount of water-soluble glue. This should be applied at the wing base, which provides little information for the automatic identification of species.

IMAGE ACQUISITION

In most contemporary studies, a wing image is acquired before measurements are made. In earlier studies, PAL or NTSC video cameras were mainly used to obtain the images. These required a frame grabber that converted an analog video signal into a digital image. Resolutions obtained by PAL or NTSC video cameras can be relatively low (often less than 0.5 Mpixel). This can be improved by the use of digital still cameras, which produce images of up to 12 Mpixels. The cameras can be connected to various microscopes or lenses to obtain an image of the appropriate magnification and quality. They are versatile and can take wing images even in the case of live insects and museum specimens, when wings cannot be detached from the insect's body (Houle et al., 2003). Another advantage of these cameras is fast image acquisition, which can be important in the case of live insects anaesthetized for only a short time.

When the wing can be detached from an insect's body and mounted in a photographic slide frame, scanners might be preferred. These are easily accessible and available in a wide range of prices – from inexpensive flatbed scanners with a slide adapter to more expensive high-resolution photographic slide scanners. There are attachments to some of the scanners allowing the automatic scanning of multiple frames, making image acquisition faster and requiring less human intervention. The resolution of a photographic slide scanner can be up to 4000 dpi (equivalent of about 22 Mpixels when a whole slide frame is scanned), which is more than most digital cameras produce. When scanners are used, scaling is not required because the resolution of the image (in dpi) is known. Images obtained by scanning have a uniform background, which makes wing detection and analysis easier (Weeks and Gaston, 1997). Producing a uniform background with a microscope is much more difficult and requires a precisely aligned light source.

WING MEASUREMENTS

The first large-scale measurements of honeybee wings were undertaken by Alpatov (1929). At that time microscopes equipped with ocular micrometers were used. DuPraw (1964) used a microscope-slide projector to obtain a wing image on a piece of paper, which he pricked with a pin to mark the vein junctions. He then measured angles and distances in the traditional way with a ruler and protractor.

Daly et al. (1982) combined the projector with a digitizer tablet. In doing this they were able to indicate the landmarks that were automatically recorded by the computer. This procedure increased the speed of measurements, their precision and their repeatability, and eliminated errors related to data input or calculations.

A more sophisticated system was described by Batra (1988). In this system wing images were obtained through the use of a microscope equipped with a camera. This system required substantial user intervention, but allowed the automatic detection of 16 vein junctions. It was based on specific and expensive hardware, which reduced its accessibility.

In most recent studies wing images acquired by a camera or scanner are transferred to the computer and analysed using data acquisition software. Either specialized honeybee wing measurement software (e.g. Beemorph; see Talbot, 2002) or general-purpose data acquisition software (e.g. tpsDig; see Rohlf, 2005) can be used. Usually, a computer mouse is used to indicate the vein junctions on a computer screen and the data acquisition software provides the coordinates, which can be used to calculate distances and angles.

IMAGE ANALYSIS

Despite several attempts to automate honeybee wing measurements, the methods currently in use are, to a large extent, manual. This is surprising because image analysis allows the automatic detection of vein junctions on which the measurements are based (Batra, 1988; Steinhage et al., 1997). One of the computer programs specialized in the image analysis of insect wings is *DrawWing* (Tofilski, 2004). It is particularly suitable for automatically measuring wings because it is able to recognize and position wings in an image. *DrawWing* software is able to recognize all vein junctions of the honeybee forewing, which allows for fully automatic measurements. The image analysis of honeybee wings is performed in two main steps: the first step is the detection of the wing outline and the second step is the detection of the wing venation.

Detection of the Wing Outline

In a fully automated measurement system, wings need to be detected in an image without user intervention. The process of wing detection is based on the image histogram, which represents the number of pixels at each greyscale value within the image. This works very well if the wings are darker and presented against a relatively uniform light background (Figure 17.1), which is always the case if the image has been obtained by a scanner.

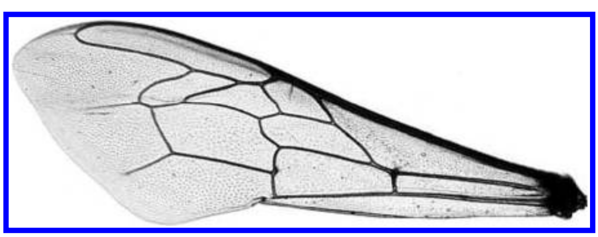

FIGURE 17.1 The front wing of a worker honeybee.

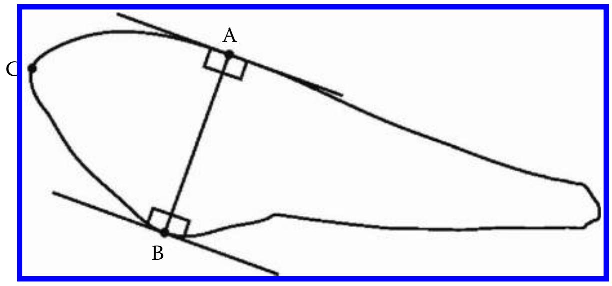

FIGURE 17.2 The wing outline with three characteristic points marked: A: anterior point, B: posterior point and C: apex point. Tangents to the outline at the anterior and posterior points are perpendicular to the line crossing the points. The anterior and posterior points demarcate the wing width, which can be used for wing positioning and scaling. There are two extrema of the outline on opposite sides of the wing width. The apex point is the extremum at which the outline curvature is smaller.

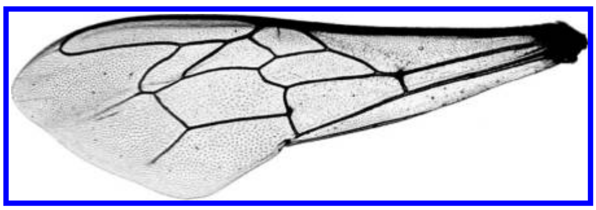

FIGURE 17.3 The standard wing image produced by *DrawWing* from the image depicted in Figure 17.2.

If the background is uniform, the image histogram is markedly bimodal. The first mode corresponds to wing pixels and the second mode corresponds to the background. The colour corresponding to the minimum between the maxima is used as a threshold for conversion of the greyscale image to black and white. At this stage wings (and some other dark artefacts) are represented as black objects. Outlines of all the objects are detected by contour tracing (Rohlf, 1990) and their size and shape are analysed to decide whether or not they are potential wings. Only the potential wings are subjected to further processing.

In order to remove artefacts (e.g. hairs or dust particles) from the wing edge, the outline is smoothed. The smoothing is achieved by contour dilation equal to earlier erosion. The smoothing algorithm removes only the thin protrusions of the outline and minimally affects the wing shape.

On the honeybee wing outline there are three characteristic points: anterior, posterior and apex (Figure 17.2). The anterior and posterior points demarcate the wing width. The tangents to the wing outline at those two points are parallel to each other and perpendicular to the line crossing the points. The anterior and posterior points are automatically detected by examining outline points in pairs until they meet the aforementioned criteria. There are two extrema of the outline on opposite sides of the wing width. The apex point is the extremum at which the outline curvature is smaller. Finally, the wing is rotated in software and cropped from the original image. The resulting image is called a 'standard wing image' (Figure 17.3).

In order to avoid negative coordinates, the origin of the Cartesian coordinate system is defined by the bottom and left extreme of the wing outline. Traditionally, right wings are depicted in the drawings and images (Mason, 1986). However, if the right wing is presented in the Cartesian coordinates system, zero on the x-axis has to be assigned to the wing base. It is better to avoid this because the wing base is not very well defined. When the wing is detached from the body, it can break off in different places. Moreover, the wing base can be invisible when wing images are acquired from live specimens. Therefore, in a standard wing image, left wings are depicted. Then the zero on the x-axis is defined by the wing apex, which is always visible. Right wings are flipped

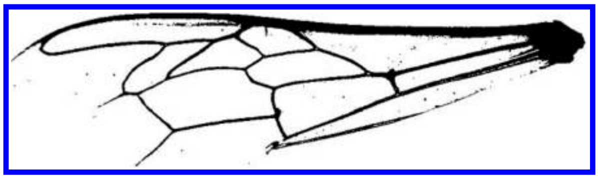

FIGURE 17.4 The honeybee front wing after extraction of venation outline.

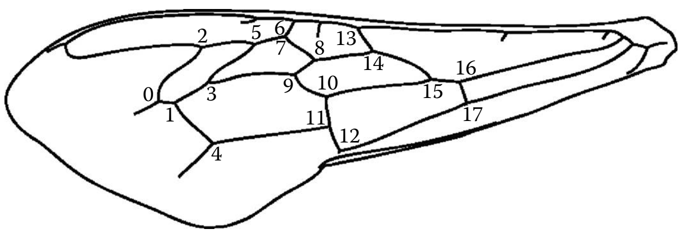

FIGURE 17.5 The wing diagram depicting only the wing outline and the venation skeleton.

horizontally before they are presented in the standard wing image. Multiple standard wing images are produced if the input image contains more than one wing.

Detection of Junctions

The veins are darker than the membranous parts of the wing, but the intensity of their colour varies. This makes it difficult to choose the optimal threshold for extracting the venation outline. In this case, the method used to detect the wing outline is not always satisfactory and a more complex method needs to be used. A gradient for the image (Shen and Castan, 1992) can be calculated and the gradient's maxima found. The maxima at which the gradient is relatively high correspond to the veins' edges. The median of the maxima can then be used as the threshold. Pixels darker than the threshold are considered 'veins' and their outline (Figure 17.4) is found by contour tracing (Rohlf, 1990). The vein outline is reduced to its skeleton using a thinning algorithm (Rohlf, 1990). The skeleton pixels with three of four neighbours are vein junctions. The junctions are compared with the expected junctions from a typical honeybee wing. Junctions that fit those expected are reported to the user. In order to avoid misidentification, both coordinates of the junctions and tangents at which veins approach the junction are used in the comparison. Apart from the list of vein junctions, a wing diagram is produced (Figure 17.5). It depicts the wing in the same position as the standard wing image but only the wing outline and skeleton of venation are visible.

RELIABILITY AND PRECISION OF THE AUTOMATIC MEASUREMENTS

As a rule, differences between wings of individuals belonging to the same species are small. For example, venation of two honeybee subspecies – *Apis mellifera carnica* and *A. m. ligustica* – differs mainly by lengths of a cubital vein, which are 0.56 and 0.59 mm, respectively (Nazzi, 1992). This difference is small in comparison with precision of the venation measurements: 0.023 mm for the ocular micrometer and 0.007 mm for the tablet (Daly et al., 1982). The measurements of the wing length and width proved to be even less precise (Dedej and Nazzi, 1994). Therefore, the precision is one of the most important characteristics of any automated wing-measurement method. Other important factors are reliability and the speed of measurement.

Material and Methods

In order to test reliability of the *DrawWing* software, measurements from 300 honeybee workers were used. Their wings were torn off and mounted in glass photographic frames (Rowi 260). In every frame all wings of one worker were mounted (two front wings and two hind wings). Images of the wings were obtained using a Nikon Coolscan 5000 ED scanner equipped with SF-210 slide feeder. The original resolution of the images was 4000 dpi. However, this was converted to 2400 dpi before analysis. The images were analysed by *DrawWing* software and the number of correctly recognized front wings was counted. Correctly recognized wings were checked if all vein junctions were detected.

In order to compare precision and speed of the automatic and manual wing measurement, 40 front wings of honeybee workers were used. Three images were produced for every wing and the positions of 18 landmarks determined. All landmarks were vein junctions. These landmarks were either manually pointed with a computer mouse using tpsDig2 software (Rohlf, 2005), or determined automatically by *DrawWing*. Manual measurements were made by three different inexperienced persons. Procrustes superimposition was used to align the junctions found on three images of the same wing. The precision was measured as the mean distance between the three junctions and their centroid (Arnqvist and Martensson, 1998). The results are reported as mean ± standard deviation.

Results

Out of 600 front wings in the 300 images, 552 wings (92.0%) were correctly detected and saved as standard wing images. In the standard wing images, out of 9936 expected vein junctions, 9894 (99.6%) were detected. The automatic measurements proved to be significantly more precise than the manual measurements (Mann–Whitney test: $Z = -27.38$, $N_1 = 720$, $N_2 = 2160$, $p < 0.001$; Table 17.1). The mean precision of the automatic and manual measurements was 5.20 ± 4.44 and 9.37 ± 5.22 µm, respectively. In the 2400-dpi images, those values correspond to 0.49 ± 0.42 and 0.88 ± 0.49 pixels, respectively. There were significant differences between precision of measurements performed by three different manual data collectors (Kruskal–Wallis test: $H = 283$, df = 2, $N = 2160$, $p < 0.001$; Table 17.1). The automatic measurements were also much faster than the manual measurements (Mann–Whitney test: $Z = -9.47$, $N_1 = 40$, $N_2 = 120$, $p < 0.001$). The time required to analyse one wing automatically and manually was 6.4 ± 0.83 and 68.8 ± 18.40 s, respectively.

Discussion

The detection rate of the front wings of honeybee workers was satisfactory, but it can be improved further. The main reason for the detection failures was damages of the wing edge, which led to incorrect detection of wing width. This problem can be solved to some degree by changing the algorithm of the wing width search; however, if the wing edge is damaged at the anterior or posterior point (Figure 17.2), correct detection of the wing width is impossible. In a few cases, there were also problems with artefacts near the wing base arising from tearing the wings off the thorax. This problem could be avoided by cutting the wings off with scissors. The detection of vein junctions was much better than the detection of wings – close to 100 per cent. The problems with the vein junctions' detections were mainly caused by unusual shapes of the venation.

These results show clearly that the automatic measurements are more precise and much faster than the manual measurements. This test used only one scanner, but it is probable that differences between scanners will be much smaller than the differences between persons. This is important when results from different studies are compared. The precision of automatic measurements is close to the limits set by resolution of the images. In order to increase the precision further, images of higher resolution need to be used, but this requires changes to the software, which is optimized for 2400-dpi images.

TABLE 17.1
Precision[a] of Automatic and Manual Measurements of Honeybee Wing Venation

Junction no.	Automatic	Manual			
		A	B	C	Mean
0	4.31 ± 3.68	6.94 ± 3.54	8.26 ± 4.59	10.57 ± 4.57	8.59 ± 4.23
1	4.70 ± 4.14	6.30 ± 3.57	9.54 ± 4.66	10.49 ± 5.79	8.78 ± 4.68
2	5.69 ± 3.30	7.42 ± 3.80	9.06 ± 5.25	10.83 ± 5.87	9.10 ± 4.98
3	3.70 ± 2.69	6.90 ± 3.71	8.51 ± 4.53	10.56 ± 5.54	8.66 ± 4.59
4	5.94 ± 3.78	7.67 ± 3.59	9.85 ± 5.08	12.19 ± 5.68	9.91 ± 4.78
5	4.08 ± 2.59	6.75 ± 3.88	8.87 ± 5.05	10.50 ± 5.54	8.71 ± 4.83
6	5.77 ± 3.58	9.19 ± 5.71	15.90 ± 9.17	12.32 ± 6.37	12.47 ± 7.08
7	3.83 ± 2.34	6.92 ± 4.16	8.83 ± 4.94	9.89 ± 5.56	8.55 ± 4.89
8	4.07 ± 3.04	6.42 ± 4.00	7.64 ± 3.57	10.55 ± 5.18	8.20 ± 4.25
9	4.01 ± 2.34	7.04 ± 6.46	8.36 ± 7.37	12.10 ± 6.54	9.17 ± 6.79
10	4.67 ± 3.26	7.14 ± 3.84	8.44 ± 4.46	11.10 ± 5.94	8.89 ± 4.75
11	5.11 ± 4.30	7.85 ± 4.03	8.46 ± 4.91	10.80 ± 5.65	9.03 ± 4.87
12	5.94 ± 4.33	6.30 ± 3.26	8.38 ± 5.08	10.60 ± 5.22	8.43 ± 4.52
13	11.21 ± 13.28	12.26 ± 7.08	19.54 ± 11.08	14.76 ± 8.19	15.52 ± 8.78
14	5.33 ± 5.66	8.64 ± 5.84	8.28 ± 4.41	10.60 ± 5.68	9.17 ± 5.31
15	5.11 ± 7.38	6.94 ± 3.83	7.83 ± 4.23	10.12 ± 6.17	8.30 ± 4.74
16	5.81 ± 7.64	6.85 ± 4.24	9.02 ± 5.01	9.76 ± 5.18	8.54 ± 4.81
17	4.28 ± 2.60	7.57 ± 4.08	8.92 ± 5.38	9.40 ± 5.57	8.63 ± 5.01
Mean	5.20 ± 4.44	7.50 ± 4.37	9.65 ± 5.49	10.95 ± 5.79	9.37 ± 5.22

Note: The manual measurements were performed by three persons (A, B and C). The veins' junctions are numbered according to Figure 17.5.

[a] In microns; mean ± SD.

WING-BASED SPECIES DISCRIMINATION

Wings seem to be the best morphological structure for the automatic identification of insects. They differ much more between species than within species (Matias et al., 2001; Baylac et al., 2003). Even within species the variation is large enough to distinguish subspecies (Daly and Balling, 1978; Ruttner, 1988a). There is no problem with the positioning of wings in three dimensions because they are flat and can be considered as two dimensional (Weeks and Gaston, 1997). High-quality wing images can be obtained using relatively inexpensive scanners, which allows the process to be semi-automated.

When wings of different species are compared there is a need for scaling and rotating them to a standard position (Lane, 1981; Albrecht and Kaila, 1997). The wing outline can be used for the positioning because, unlike venation, its shape is similar in all species of insects. The outline is always rounded at the apex and narrower at the wing base, which can be used for wing detection and positioning. The width of such outline can be chosen as a baseline and the two-point registration (Bookstein, 1991) can be used to produce a standard wing image and wing diagram by a combination of scaling, translating and rotating. Ideally, the baseline should be positioned along the longest wing diameter and across the wing centroid (Zelditch et al., 2004). However, this is difficult to achieve because one side (the wing base) of the longest diameter is not well defined and can be invisible.

Both the standard wing image and the wing diagram can be important elements of an automatic species discrimination system. In comparison with unprocessed images, these are a better form of storage and medium of exchange for information about wings. They contain only relevant information, which reduces both storage space and retrieval time. The standard wing image can be used by pixel-based species identification systems that, instead of extracting characteristic points from the image, use the intensity of all pixels (Weeks et al., 1997; Roth et al., 1999a; MacLeod et al., in press). In those systems, the position of a wing in the image is essential for the correct discrimination of species. The manual positioning of wings has proven to be time consuming and imprecise (Weeks et al., 1999a, 1999b).

FUTURE PROSPECTS

The system described here has been tested on honeybee wings; however, it should work with other species of insects as well. The detection of a wing outline is based on properties common to the wings of all insects; therefore, *DrawWing* should generate the standard wing image, even for butterfly wings. If wings are membranous with clearly visible veins, a wing diagram can also be produced. Extraction of the standard wing image was also tested on three species of wasps and the results were satisfactory (unpublished data). The earlier version of this software was already able to produce wing diagram of various species (Tofilski, 2004). Only the detection of junctions in the wing of a particular species requires a list of expected junction coordinates for this species.

It has been shown that insects' wings can be used to discriminate between species (Weeks and Gaston, 1997). Methods based on wing outline (Rohlf and Archie, 1984), vein junctions (Schröder et al., 2002) and all image pixels (Weeks et al., 1997; Roth et al., 1999a) have proven successful. Various statistical methods of species discrimination have been described as well (Strauss and Houck, 1994; Roth et al., 1999b). However, there are relatively few tools for efficient data acquisition and wing visualization. *DrawWing* proved to be successful in this area. It will be developed further to become a general-purpose tool for the processing of insect wing images.

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REFERENCES

Adsavakulchai, S., Baimai, V., Prachyabrued, W., Grote, P.J. and Lertlum, S. (1999) Morphometric study for identification of the *Bactrocera dorsalis* complex (Diptera: Tephritidae) using wing image analysis. *Biotropica,* 13: 37–48.

Albrecht, A. and Kaila, L. (1997) Variation of wing venation in Elachistidae (Lepidoptera: Gelechioidea): Methodology and implications to systematics. *Systematic Entomology,* 22: 185–198.

Alpatov, W.W. (1929) Biometrical studies on variation and races of the honey bee (*Apis mellifera* L.). *Quarterly Review of Biology,* 4: 1–58.

Arnqvist, G. and Martensson, T. (1998) Measurement error in geometric morphometrics: empirical strategies to asses and reduce its impact on measures of shape. *Acta Zoologica Academiae Scientiarum Hungaricae,* 44: 73–96.

Batra, S.W.T. (1988) Automatic image analysis for rapid identification of Africanized honey bees. In *Africanized Honey Bees and Bee Mites* (ed G.R. Needham), Ellis Horwood, Chichester, UK, pp. 260–263.

Baylac, M., Villemant, C. and Simbolotti, G. (2003) Combining geometric morphometrics with pattern recognition for the investigation of species complexes. *Biological Journal of the Linnean Society,* 80: 89–98.

Bookstein, F.L. (1991) *Morphometric Tools for Landmark Data: Geometry and Biology.* Cambridge University Press, Cambridge, 435 pp.

Brückner, D. (1976) The influence of genetic variability on wing symmetry in honeybees (*Apis mellifera*). *Evolution,* 30: 100–108.

Daly, H.V. and Balling, S.S. (1978) Identification of Africanized honeybees in the Western Hemisphere by discriminant analysis. *Journal of the Kansas Entomological Society,* 51: 857–869.

Daly, H.V., Hoelmer, K., Norman, P. and Allen, T. (1982) Computer-assisted measurement and identification of honey bees (Hymenoptera: Apidae). *Annals of the Entomological Society of America,* 75: 591–594.

Dedej, S. and Nazzi, F. (1994) Two distances of forewing venation as estimates of wing size. *Journal of Apicultural Research,* 33: 59–61.

DuPraw, E.J. (1964) Non-Linnean taxonomy. *Nature,* 202: 849–852.

Higginson, A.D. and Barnard, C.J. (2004) Accumulating wing damage affects foraging decisions in honeybees (*Apis mellifera* L.). *Ecological Entomology,* 29: 52–59.

Houle, D., Mezey, J., Galpern, P. and Carter, A. (2003) Automated measurement of *Drosophila* wings. *BMC Evolutionary Biology,* 3: 1–13.

Kokko, E.G., Floate, K.D., Colwell, D.D. and Lee, B. (1996) Measurement of fluctuating asymmetry in insect wings using image analysis. *Annals of the Entomological Society of America,* 89: 398–404.

Lane, R.P. (1981) A quantitative analysis of wing pattern in the *Culicoides pulicaris* species group (Diptera: Ceratopogonidae). *Zoological Journal of the Linnean Society,* 72: 21–41.

MacLeod, N., O'Neill, M.A. and Walsh, S.A. (in press) A comparison between morphometric and artificial neural-net approaches to the automated species-recognition problem in systematics. In *Biodiversity Database: From Cottage Industry to Industrial Network* (eds G. Curry and C. Humphries), Taylor & Francis, London.

Mason, W.R.M. (1986) Standard drawing conventions and definitions for venational and other features of wings of hymenoptera. *Proceedings of the Entomological Society of Washington,* 88: 1–7.

Matias, A., de la Riva, J.X., Torrez, M. and Dujardin, J.P. (2001) *Rhodnius robustus* in Bolivia identified by its wings. *Memorias do Instituto Oswaldo Cruz,* 96: 947–950.

Nazzi, F. (1992) Morphometric analysis of honey bees from an area of racial hybridization in northeastern Italy. *Apidologie,* 23: 89–96.

Radloff, S.E., Hepburn, R. and Bangay, L.J. (2003) Quantitative analysis of intracolonial and intercolonial morphometric variance in honeybees, *Apis mellifera* and *Apis cerana*. *Apidologie,* 34: 339–351.

Rohlf, F.J. (1990) An overview of image processing and analysis techniques for morphometrics. In *Proceedings of the Michigan Morphometrics Workshop* (eds F.J. Rohlf and F.L. Bookstein), The University of Michigan Museum of Zoology, Ann Arbor, pp. 37–60.

Rohlf, F.J. (2005) tpsDig, digitize landmarks and outlines, version 2.04. Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F.J. and Archie, J.W. (1984) A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Systematic Zoology,* 33: 302–317.

Rortais, A., Arnold, G., Baylac, M. and Garnery, L. (2004) Analysis of the genetic diversity of honeybees in France and establishment of bees conservatories. In *First European Conference of Apidology,* Udine, Italy, p. 48.

Roth, V., Pogoda, A., Steinhage, V. and Schröder, S. (1999a) Integrating feature-based and pixel-based classification for the automated identification of solitary bees. In *Jahrestagung der Deutschen Gesellschaft für Mustererkennung,* 21, pp. 120–129.

Roth, V., Steinhage, V., Schröder, S. and Cremers, A. B. (1999b) Pattern recognition combining de-noising and linear discriminant analysis within a real world application. *8th International Conference on Computer Analysis of Images and Patterns,* Ljubljana, pp. 251–266.

Ruttner, F. (1988a) *Biogeography and Taxonomy of Honeybees,* Springer, Berlin, 284 pp.

Ruttner, F. (1988b) *Breeding Techniques and Selection for Breeding of the Honeybee,* BIBBA, London.

Schröder, S., Wittmann, D., Drescher, W., Roth, V., Steinhage, V. and Cremers, A.B. (2002) The new key to bees: automated identification by image analysis of wings. In *Pollinating Bees – The Conservation Link between Agriculture and Nature* (eds P. Kevan and V.L. Imperatiz-Fonseca), Ministry of Environment, Brasilia, pp. 209–216.

Shen, J. and Castan, S. (1992) An optimal linear operator for step edge detection. *CVGIP: Graphical Model and Image Processing,* 54: 112–133.

Smith, D.R., Crespi, B.J. and Bookstein, F.L. (1997) Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. *Journal of Evolutionary Biology,* 10: 551–574.

Steinhage, V., Kastenholz, B., Schröder, S. and Drescher, W. (1997) A hierarchical approach to classify solitary bees based on image analysis. In *DAGM-Symposium* (eds E. Paulus and F.M. Wahl), Springer, Berlin, pp. 419–426.

Strauss, R.E. and Houck, M.A. (1994) Identification of Africanized honeybees via non-linear multilayer perceptrons. *Proceedings of the IEEE International Conference on Neural Networks,* 5: 3261–3264.

Talbot, R. (2002) Beemorph, version 1.0.7. http://www.hockerley.plus.com/.

Tofilski, A. (2004) *DrawWing*, a program for numerical description of insect wings. *Journal of Insect Science,* 4: 1–5.

Weeks, P.J.D. and Gaston, K.J. (1997) Image analysis, neural networks, and the taxonomic impediment to biodiversity studies. *Biodiversity and Conservation,* 6: 263–274.

Weeks, P.J.D., Gauld, I.D., Gaston, K.J. and O'Neill, M.A. (1997) Automating the identification of insects: a new solution to an old problem. *Bulletin of Entomological Research,* 87: 203–211.

Weeks, P.J.D., O'Neill, M.A., Gaston, K.J. and Gauld, I.D. (1999a) Automating insect identification: exploring the limitation of a prototype system. *Journal of Applied Entomology,* 123: 1–8.

Weeks, P.J.D., O'Neill, M.A., Gaston, K.J. and Gauld, I.D. (1999b) Species-identification of wasps using principal component associative memories. *Image and Vision Computing,* 17: 861–866.

Yu, D.S., Kokko, E.G., Barron, J.R., Schaalje, G.B. and Gowen, B.E. (1992) Identification of ichneumonid wasps using image analysis of wings. *Systematic Entomology,* 17: 389–395.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. and Fink, W.L. (2004) *Geometric Morphometrics for Biologists: A Primer,* Elsevier/Academic Press, London, 443 pp.