OBSERVATIONS ON THE ULTRASTRUCTURE AND HYDROPHOBICITY OF THE WINGS OF THIRTEEN NEOTROPICAL FAMILIES OF DIPTERA (INSECTA) WITH COMMENTS ON THEIR FLIGHT

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ABSTRACT
Insects' wings are complex surfaces that have become a new field to study towards the design of flying devices and hydrophobic surfaces. Several orders and families of insects have been analyzed for their hydrophobic properties and the microstructures related. We studied the wings of 13 dipteran families under scanning electron microscopy and used a goniometer to measure the corresponding static contact angle. Common structures as well as distinct features were found in the samples. None of the wings was superhydrophobic; the contact angles raged from 67.9° to 109.9°. Wings' ultrastructure and cuticle elements are associated with these differences, and play a crucial role during flight.

Keywords: flies, microtrichia, mimicry, neotropic, setae

OBSERVACIONES SOBRE LA ULTRAESTRUCTURA Y LA HIDROFOBICIDAD DE LAS ALAS DE TRECE FAMILIAS NEOTROPICALES DE DIPTERA (INSECTA) CON COMENTARIOS SOBRE SU VUELO

RESUMEN
Las alas de los insectos son superficies complejas y un nuevo campo para el estudio hacia el diseño de dispositivos voladores y superficies hidrofóbicas. Varias órdenes y familias de insectos han sido analizadas por sus propiedades hidrófobas y las microestructuras relacionados. Estudiamos las alas de 13 familias de dípteros bajo microscopía electrónica de barrido y se utilizó un goniómetro para medir el ángulo de contacto estático. En las muestras se encontraron estructuras comunes, así como características distintas. Ninguna de las alas fue superhidrófoba; los ángulos de contacto de las muestras analizadas se encuentran entre 67,9 ° a 109.9 °. Elementos de las alas y la ultraestructura de la cutícula están asociados con estas diferencias, y desempeñan un papel crucial durante el vuelo.

Palabras clave: moscas, microtriquias, mímica, neotrópico, setas

INTRODUCTION

Insects' wings have played one of the most important roles during the evolution of arthropods by giving them the opportunity to colonize and thrive in new scenarios [1]. The specialization of the wings triggered an incredible diversity of structures within and across insects' groups, towards a better performance, efficiency and stability during flight. However, wings also play secondary roles and are related to other functions as reproduction and protection [2].

At the same time, wings' structures and properties have captured the attention of researchers from different fields; in aeronautics for example, the studies on flight mechanisms led to the invention of flying machines with diverse purposes [3] [4] [2]. Similarly, a different approach focused on the nanostructure of some butterflies' wings inspired a new screen technology for electronic devices [5].

More recently and within the field of mimicry (biomimesis), the hydrophobic information derived from the study of natural surfaces awoke the interest in the development of artificial materials with similar properties [6]. Superhydrophobic surfaces show a 150° or higher static contact angle (CA) for water [7], a property that has been observed in both, animal and plant kingdoms with the “lotus effect” as the best example of a hydrophobic and self-cleaning surface. In that line, there has been a growing interest in the study of particular orders and species of insects to measure this property, as well as the micro/nano structures associated to it [7] [8].

Since other groups have recorded higher CA than flies, only one species of Diptera has been analyzed as representative of this order [7].This paper presents data on the ultrastructure elements of several Dipteran wings and their hydrophobic properties, as well as some comments on the role of such structures during flight.

MATERIALS AND METHODS

We studied 13 families of Diptera; Anisopodidae, Asilidae, Bibionidae, Calliphoridae, Conopidae, Dolichopodidae, Muscidae, Sciaridae, Simuliidae, Stratiomyidae, Syrphidae, Tachinidae and Tipulidae, one dried specimen per family. Specimens were collected in Costa Rica between 2000 and 2006 and were supplied by the Laboratory of Entomology and Arachnology, Biology School, University of Costa Rica.

The right and left wing of each specimen were used to study the ultrastructure and the static contact angle (CA) of the dorsal surface, respectively. The right wing was mounted on double-sided adhesive carbon tape on aluminum stubs, then coated with 50nm of Pt-Pd in a Giko IB-3 sputter coater and photographed with a Hitachi S3700N scanning electron microscope at the Microscope Research Center (CIEMIC), University of Costa Rica. The left wing was mounted on double-sided carbon tape, after which a distilled water bead (0.15-0.25 ul) was dropped directly in the middle area of the wing. The CA as well as the height and width of the droplet were measured in a Ramé-hart Standard Goniometer Model 250-F1, at the Electrochemistry and Chemical Energy Research Center (CELEQ), University of Costa Rica.

RESULTS AND DISCUSSION

Photographs of the wings at 500 and 1000 magnifications are displayed in Fig.1; low magnification images are not shown. Analyzed wings presented an evident venation pattern, fold lines and a rough cuticle at high magnifications (> 5000X). All samples showed a uniform distribution of microtrichia on the entire surface (including veins) except for Asiliidae (Fig. 1 C, D) and Syrphidae (Fig. 1 U, V). The former had these structures restricted to the veins and they were completely absent in the latter.

Flies are known as agile insects during flight [9] but some groups are particularly outstanding in their performance. Predator and hover flies for example exhibit quick movements and high accuracy when hunting and landing; interestingly, the specimens of Asiliidae (predators) and Syrphidae (hover flies) had the lowest presence of microtrichia in this study (Fig 1. C, U). It may be possible that the reduction of these elements enhances their flying ability.

As can be seen in Figures 1 and 2, the size, density, arrangement and design of the microtrichia in the middle area of the wings varied among specimens. The longest microtrichia were observed in Tachinidae which also has the widest base associated to them. Bibionidae had the finest microtrochia followed by Simuliidae; these two showed the highest density of microtrichia (Fig. 1).

High densities of microtrichia were found in small specimens as the Sciarid and the Simuliid, which are generally less skilled in terms of flying (so is Bibionidae but their size is relatively larger). Such densities have been observed in other small insects as "thrips" (Thysanoptera), which at the same time show long setae in the margin of the wings related to maneuverability [10]. Instead of active flight, it is said that small insects glide in the air currents and are less affected by small perturbations due to their resistance to the fluid (air) motion, while large specimens should adjust several parameters during flight [4].

In addition to microtrichia, pores were present in the wing of Tachinidae (Fig. 1 W, X) and Calliphoridae (Fig. 1 G, H); surface depressions associated with the bases of microtrichia were only observed in Sciaridae (Fig. 1 P). All samples presented setae and microtrichia in the front rear of the wing, but their density and number were variable.

Elements and structures of the wings are very variable in insects and a common pattern for these structures is unlikely to be defined [2]. Diptera is considered to have an extremely diversified and simplified wing venation [11]; some small groups in particular lack a clear pattern while other elements as setae and microtrichia are typically found in most of the families.

An apparent relation between the insect's size and its microtrichia is observed in our data; nevertheless, this is not correct as can be inferred in the case of Dolichopodidae and Bibionidae whose body sizes were similar and the microtrichia's were not (Fig.1). Furthermore, the partial or complete absence of these elements in some wings (Fig. 1 D, V) suggests another functionality (as pointed out by [2]) rather than a simple size relation. The evolution paths shared by close groups can also influence common patterns; this taxonomic heritage could be the explanation for the pores in Tachinidae and Calliphoridae (Fig. 1 W, G) which constitute the Calyptrata group along with Muscidae and other families. Interestingly, our specimen of Muscidae did not have such structures, but it does not mean they are absent in other species of this family. Previous research has discussed that microtrichiae could serve as a hydrophobic structure that prevents the wings from wetting in various insects. Non wetting property of insect wings is crucial for the survival of insects in wet environments, as insects could be permanently trapped by water or wet surface. Super-hydrophobic or self-clean property of insect wings could lead to next generation of contamination resistant or self-cleaning materials [12].

Studies on the aerodynamics of insect's flight have elucidated the physics, modeling and mathematical theory behind the properties of the wings [4] [13]. Additionally, computational and experimental analyses have contributed to a better understanding of the forces, surface properties, deformation and composition [2], but most studies avoid the complexity of microstructures in the surface [14] [4]. The most recent papers on the Dipteran flight were published in the late 80s and focused on flight's kinematics and dynamics [9] [15]; neither the composition nor the ultrastructure was assessed in those papers.

The values of the contact angles ranged from 67.85° ± 8.41° to 109.90° ± 0.00° (Table 1); one-quarter of the CAs were above 90°. The highest value was recorded with the wing of Conopidae and the lowest value corresponded to that of Muscidae; it was not possible to obtain the value for Calliphoridae. Droplets' micrographs are presented in Fig. 2.

Different structures and surfaces show hydrophobicity but not all of them superhydrophobicity. Since superhydrophobic surfaces have a CA>150° [7] none of the examined wings was under that category, however, those of Conopidae, Stratiomyiidae and Bibionidaeare noteworthy since they were highly hydrophobic surfaces under the experimental conditions (Table 1).

Table 1. Contact angle and corresponding water droplets obtained for 12 Dipteran wings. Electrochemistry and Chemical Energy Research Center (CELEQ), University of Costa Rica.

	Contact Angle (CA)			**Water Droplet (WD)**	
Family	**Left**	**Right**	**Mean**	**Height**	**Width**
Anisopodidae	87,70	85,40	86.55 ± 1.63	0,58	1,19
Asilidae	86,00	82,00	84.00 ± 2.83	0,76	1,62
Bibionidae	92,10	92,95	92.53 ± 0.60	0,75	1,43
Conopidae	109,90	109,90	109.90 ± 0.00	0,80	1,10
Dolichopodidae	70,90	68,80	69.85 ± 1.48	0,31	0,87
Muscidae	61,90	73,80	67.85 ± 8.41	0,56	1,66
Sciaridae	63,70	72,90	68.30 ± 6.51	0,23	0,63
Simuliidae	88,70	88,50	88.60 ± 0.14	0,48	0,96
Stratyomiidae	92,80	93,20	93.00 ± 0.28	0,54	0,99
Syrphidae	76,90	70,70	73.80 ± 4.38	0,57	1,48
Tachinidae	83,10	83,60	83.35 ± 0.35	0,49	1,10
Tipulidae	70,50	69,30	69.90 ± 0.85	0,48	1,40

When evaluating several orders of insects, the reference [2] found different arrangements and elements related to anti-wetting properties, such as layered cuticles in Lepidoptera, setae in Hemiptera, Coleoptera, Diptera and Hymenoptera, and denticles in Orthoptera and Coleoptera. More interestingly, they also found fractal arrangements in Odonata, Neuroptera and Ephemeroptera. Out of those samples, the highest contact angles were 168°, 165° and 162° (x2) and corresponded to Lepidoptera, Homoptera, Lepidoptera and Odonata, respectively.

Regarding the groups with setae as a major element on the surface, the only Dipteran representative (belonging to the family Tabanidae) had an CA of 156°, which is superhydrophobic by definition and higher than the maximum value that we found (Table 1). Studies carried out by [16] measured the highest CA (> 170°) reported so far in insects and it was found in termites (Isoptera), on specific genera that present a combination of micrasters (star-shaped structures) and hairs (microtrichia) on their wings. The same authors provided data on low CA values in termites that were described as hydrophilic species rather than hydrophobic. A similar observation of the differences within the same order was made by [17] with cicads (Hemiptera), on which there were 4 different types of wing surfaces that showed particular CAs.

Variations in the CAs obtained in this study could be attributed to the wing's microstructures and their distribution (Fig. 2). As stated before, size, density, arrangement, depressions and design of the microtrichia themselves as well as the presence of open areas in the surface varied among the samples, same situation as the protrusions in cicads [17] [18] that led to specific qualities of each surface [7]. We agree with [17] that small-scale arrangements play a major role in the hydrophobicity properties of the surfaces; regrettably, the wing of Calliphoridae was in bad condition for the goniometer test and a possible similarity with Tachinidae's CA could not be defined. That value would have allowed an estimation of the impact of the pores.

Besides flight, wings serve secondary functions in insects' behavior and ecology [2]. The elytra in Coleoptera, intended to protect hind wings underneath [2], or mating movements and coloring patterns in several species [19] are only two examples of their functional diversity.

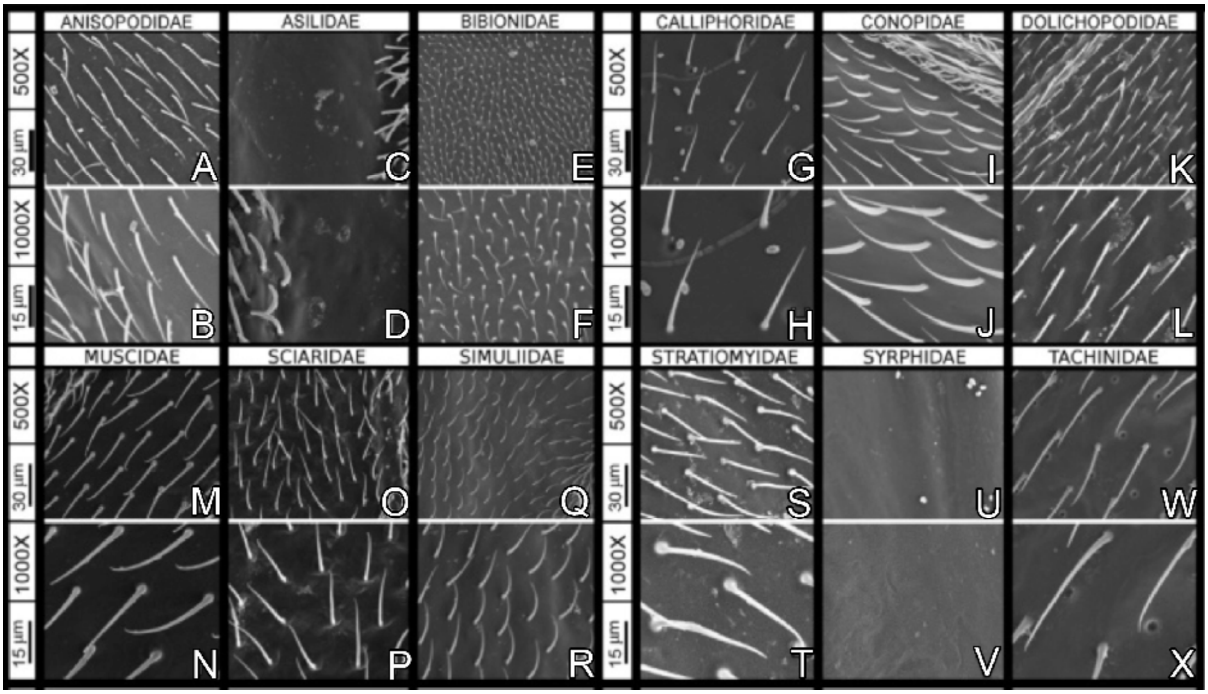

Fig. 1. Micrographs at 500X and 1000X of the dorsal surface of Dipteran wings under scanning electron microscope. Microscope Research Center (CIEMIC), University of Costa Rica.

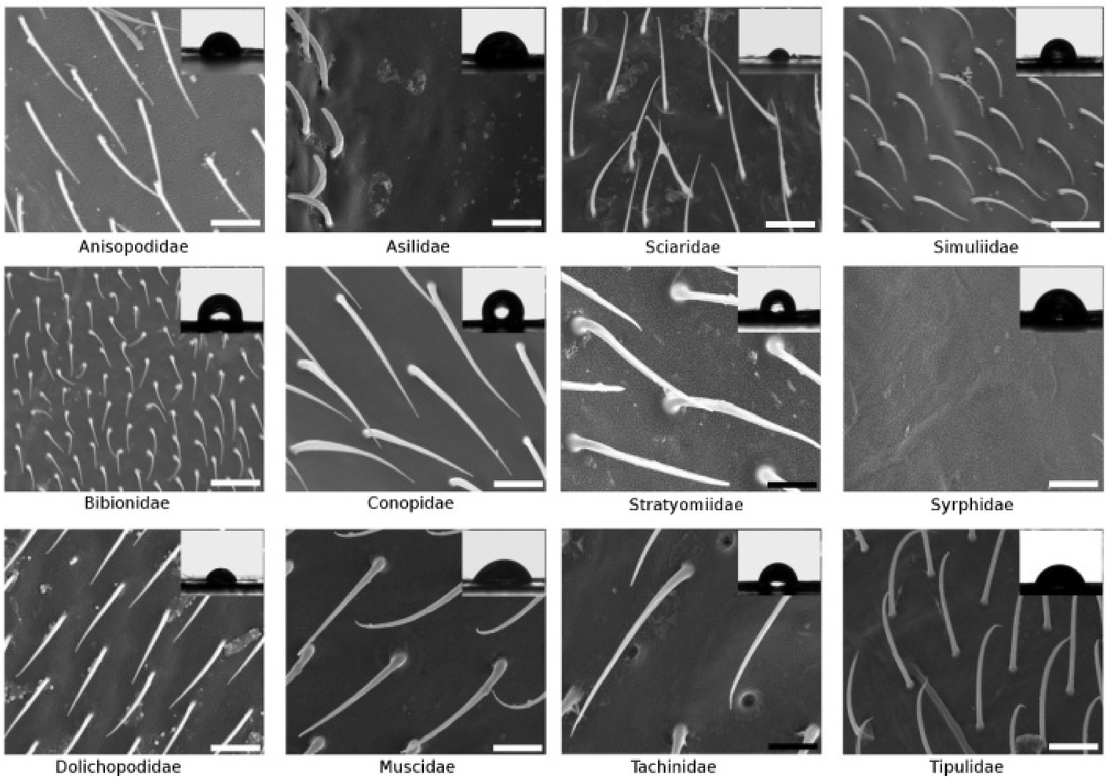

Fig. 2. Micrographs of Dipteran wings under SEM with corresponding pictures (inserted) of a water droplet on dorsal surface. Electrochemistry and Chemical Energy Research Center (CELEQ), University of Costa Rica, scale bar: 10um.

CONCLUSION

The study of the ultrastructural elements of wings in Diptera and their hydrophobic properties as well as the role these structures play in flight, it can be concluded that the small elements and structures in wings could be a key feature during flight since their mechanic properties as well as their density and location can represent an increase or decrease in the resistance to fluids when moving. Besides, the body and shape of the insect interfere with the flight dynamics and consequently, it is important to consider the effect of such features within an integrative approach.

Wings and associated structures require a comprehensive study for a better understanding of their roles in insects' biological history. Regarding hydrophobicity, further studies on new samples of each family need to be undertaken to unravel the effect of the common structures and particular elements on the wings' surfaces.

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REFERENCES

[1] Parchem R., Perry M., Patel N. (2007) "Patterns on the insect wing". *Curr Opin Genet Dev* 17: 300-308.

[2] Combes S. (2010) "*Materials, Structure, and Dynamics of Insect Wings as Bioinspiration for MAVs*". Encyclopedia of Aerospace Engineering, R. Blockley, W. Shyy (eds), John Wiley and Sons, US, 5810p.

[3] Ellington C. (1999) "The novel aerodynamics of insect flight: Applications to micro-air vehicles" *J Exp. Biol* 202: 3439–3448.

[4] Sane S. (2003) "The aerodynamics of insect flight" *J. Exp Biol* 206: 4191-4208.

[5] Coath R. (2007) "*Investigating the Use of Replica Morpho Butterfly Scales for Colour Displays*". University of Southampton, School of Electronics and Computer Science, 9p.

[6] Gao L., MccarthyT. (2006) "A Perfectly Hydrophobic Surface (θ_A/θ_R = 180°/180°)" *J Am Chem Soc* 128: 9052-9053.

[7] Byun D., Hong J., Saputra K. J., Lee Y., Park H., Byun B., Lukes J. (2009) "Wetting Characteristics of Insect Wing Surfaces" *J Bionic Eng* 6: 63-70.

[8] Watson J., Hu H., Cribb B., Watson G. (2011a) "*Anti-Wetting on Insect Cuticle – Structuring to Minimise Adhesion and Weight*". On Biomimetics, L. Pramatarova (ed.), InTech, Europe, pp. 395-418.

[9] Saigusa T. (2006) "*A new intrepretation of the wing venation of the order Diptera and its influence on the theory of the origin of the Diptera (Insecta: Holometabola)*". Homology of wing venation of dipteral, 6th International Congress of Dipterology, Fukuoka, Japan, pp. 4-20.

[10] Ennos R. (1988) "The kinematics and aerodynamics of the free flight of some Diptera" *J Exp Biol* 142: 49-85.

[11] Kuethe A. (1975) "*On the mechanics of flight of small insects*". Swimming and flying in Nature, T.Y. Wu, C. Brokow and C. Brennen. (eds), Springer, US, pp. 803-813.

[12] Qi-Ke W., Yan-Zhi Y., Mei-Qin L., Dong Z. (2014) "Fine Structure of Delia platura (Meigen) (Diptera: Anthomyiidae) Revealed by Scanning Electron Microscopy" Microsc Res Tech 77:619–630.

[13] Wang Z. (2005). "Dissecting Insect Flight". *Annu Rev Fluid Mech* 37:183–210.

[14] Ramamurti R., Sandberg W. (2002) "A three-dimensional computational study of the aerodynamics mechanisms of insect flight" *J Exp Biol* 205: 1507–1518.

[15] Miyan J., Ewing A. (1988) "Further observations on Dipteran flight: details of the mechanism" *J. Exp Biol* 136: 229-241.

[16] Watson G., Cribb B., Watson J. (2011b) "Contrasting Micro/Nano Architecture on Termite Wings: Two Divergent Strategies for Optimising Success of Colonisation Flights" *PLoS ONE* 6 (9): e24368

[17] Sun M., Watson G., Zheng Y., Watson J., Liang A. (2009) "Wetting properties on nanostructured surfaces of cicada wings" *J Exp Biol* 212:3148-3155.

[18] Sun M., Liang A., Watson G., Watson J., Zheng Y. (2012) "Influence of Cuticle Nanostructuring on the Wetting Behaviour/States on Cicada Wings" *PLoS ONE* 7(4): e35056.

[19] Sivinski J., Pereira R. (2005) "Do wings markings in fruit flies (Diptera: Tephritidae) have sexual significance?" *Fla Entomol* 88(3): 321-324.