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Morphometry and Distribution of Sensilla on the Antennae of *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae)

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Abstract

Antennal sensilla of *Anastrepha fraterculus* (Wied.) were examined using scanning electron microscopy. In the flagellum, there are trichoid, basiconic, clavate type I and II, and styloconic sensilla and microtrichia. Only microtrichiae and chaetica sensilla were observed in the scape and pedicel. The number of sensilla in the flagellum was similar between sexes. At the apex there was a higher density of trichoid and an absence of clavate sensilla, while basiconic sensilla were more abundant in the proximal region.

Introduction

Anastrepha fraterculus (Wied.) is a major pest in South America due to its direct impact on fruit production in a wide variety of hosts (Aluja 1994, Kovaleski *et al* 2000, Clark *et al* 2005). Host localization, oviposition, feeding, and mating in Tephritidae species can be mediated by airborne substances from plants (Robacker *et al* 1990a,b, Fletcher & Prokopy 1991, Landolt *et al* 1992). The antennal sensilla may be associated with chemical perception and behavioral responses (Zacharuk 1980) as observed in Tephritidae species such as *Ceratitis capitata* (Wied.) (Levinson *et al* 1987), *Bactrocera tryoni* (Froggatt) (Giannakakis & Fletcher 1985), and *Anastrepha ludens* (Loew) (Dickens *et al* 1988).

A better understanding of the morphology and types of the chemosensilla located at the antenna, integrated with input from electrophysiological and behavioral studies, may facilitate a comprehensive model of olfactory systems in tephritid fruit flies (Zacharuk 1980, 1985). Therefore, we aimed to identify and compare the sensilla available in the antennae of males and females of *A. fraterculus* as a step towards understanding their role in chemical communication in this species.

Material and Methods

Insects

Anastrepha fraterculus emerged from individuals reared in papaya (*Carica papaya*) (Caricaceae) that were grown in controlled conditions (25 ± 2 ºC, 70 ± 10% RH, and photoperiod of 12h).

Microscopy

The heads of one-day-old flies (males and females) were removed and immersed in 3% glutaraldehyde in a 0.2 M phosphate buffer during 15 days. Samples were then washed three times (30 min/wash) in 0.1 M phosphate, dehydrated in a graded series of acetone (30, 50, 70, 90 and 100%), critical-point dried (Balzers CPD030) and sputtered with gold (SPUTTER COATER – Balzers SCD050) before analysis in a SEM (JEOL - JSM 6060) at 10 kV. The structures were identified and measured using the computer program Image Tool version 3.0 (Wilcox *et al* 2002). The length of the central region of the scape, pedicel, and flagellum, and the width of the largest diameter in each segment were measured. The flagellum was analyzed in dorsal and ventral views and sensilla density recorded in proximal (49 μm^2), median (49.4 μm^2), and distal (35 μm^2) parts of the flagellum. A pair of antennae was attached to stubs, with six replicates of each surface for each sex. Images of 1.657 μm^2 were recorded from six sites, on the dorsal and ventral surfaces of each third of the flagellum, and the number and type of sensilla and microtrichiae was recorded.

The total number of sensilla and microtrichiae was estimated by the sum of the average numbers present on three sample areas (each measuring 1.657 μM^2) at the proximal, middle and distal regions and on the dorsal and ventral surfaces of the flagellum. The values were extrapolated to the total area.

Statistics

Data on the antenna measurements (segments, sensilla and microtrichae) and the estimated number of sensilla were compared between sexes and tested for significance ($P < 0.05$) by ANOVA (Tukey test) (Sokal & Rohlf 1995).

Results and Discussion

The antennae of *A. fraterculus* have three segments: scape, pedicel, and flagellum (Fig1a), similar to other species of Tephritidae (Giannakakis & Fletcher 1985, Dickens *et al* 1988, Hull & Cribb 1997, Arzuffi *et al* 2008). The arista was inserted on the dorso-proximal end of the flagellum, and a sensorial pore was present on the lateral surface as reported for several other species by Dickens *et al* (1988). The length of the antennae and of each segment and arista did not differ between females and males (Table 1).

The sensilla on the antenna of *A. fraterculus* are divided into six types, based on their shape: trichoid, basiconic, clavate (I and II), styloconic, chaetica, and microtrichia (Figs 1 b-h), which all had similar sizes in both males and females (Table 2). No chaetica sensilla were found at the flagellum. Microtrichiae were distributed over the whole antenna. Our observations agree with those of Giannakakis & Fletcher (1985), who also recorded six types of sensilla on the antennae of *D. tryoni*. However, those authors refer to two types of trichoid (type I and II) and only one clavate sensilla, while we found one trichoid and two types of clavate sensilla. Dickens *et al* (1988), in studies of *C. capitata*, *A. ludens*, *Dacus cucurbitae* (Coquillett), and *Dacus dorsalis* (Hendel) also registered the same types found in this study, but used different names: non-porous sensilla (NPS) for chaetica, multiporous grooved sensilla (MPGS) for styloconic, and multipore sensilla (MPS) for trichoid and basiconic.

There were no significant differences ($P > 0.05$) in the number of sensilla and microtrichiae at the dorsal and ventral sides of the flagellum and between sexes. However, these numbers varied among the distal, median, and proximal regions of the flagellum (Table 3). The total number of structures at the flagellum was estimated: trichoid (803.3), basiconic (815), clavate (72.8), clavate II (55.1), styloconic sensilla (304.3), and microtrichia (49.2).

The scape and pedicel in *A. fraterculus* are covered by microtrichiae and chaetica sensilla on the distal and dorsal surfaces of the pedicel, close to the articulated region of the flagellum. The numbers of these sensilla and microtrichiae were not different ($P > 0.05$) between the scape and pedicel of females and males of *A. fraterculus*.

The longest and most conspicuous sensilla were of the trichoid type (Table 2), as observed in *D. tryoni* (Giannakakis & Fletcher 1985, Hull & Cribb 1997), *C. capitata* (Levinson *et al* 1987), *T. curvicauda* (Arzuffi *et al* 2008), and in *C. capitata, A. ludens, D. cucurbitae* and *D. dorsalis* (Dickens *et al* 1988).

The number of trichoid sensilla was highest at the distal region and significantly different among the regions of the flagellum ($F = 147.66$; $P < 0.0001$) (Table 2), as observed for other tephritids (Levinson *et al* 1987, Dickens *et al* 1988). However, these sensilla were uniformly distributed on the flagellum of *D. tryoni* (Giannakakis & Fletcher 1985). The chemoreception function of trichoid sensilla in *C. capitata* is indicated by the presence of pores in their cuticle at the distal region (Levinson *et al* 1987), which are indicated in the perception of the attractant trimedlure (Dickens *et al* 1988) and the male sexual pheromone (Levinson *et al* 1990).

Basiconic sensilla are curved proximally and characterized as digitiform (finger-like) with a rounded point, smooth surface and pores along the wall (Fig 1f) (Giannakakis & Fletcher 1985, Hull & Cribb 1997, Arzuffi *et al* 2008). These sensilla are distributed throughout the flagellum, but their number was significantly higher ($F = 31.13$; $P < 0.0001$) at the proximal region (Table 3), as in other Tephritidae (Dickens *et al* 1988).

Clavate sensilla type I and II of *A. fraterculus* are similar to the basiconic sensilla, but are larger and club-like (Fig 1d and e). Clavate types I and II differed in their distal diameters, being larger in type I ($F = 59.77$; $P < 0.0001$) (Table 2). Giannakakis & Fletcher (1985) recorded these sensilla at the proximal region of the flagellum in *D. tryoni* and verified differences in length and width. Clavate sensilla (I and II) were more abundant ($F = 26.32$; $P = 0.0001$ and $F = 11.95$, $P = 0.002$) at the proximal region of the flagellum in *A. fraterculus,* and were not found at the distal region (Table 3), as in *T. curvicauda* (Arzuffi *et al* 2008).

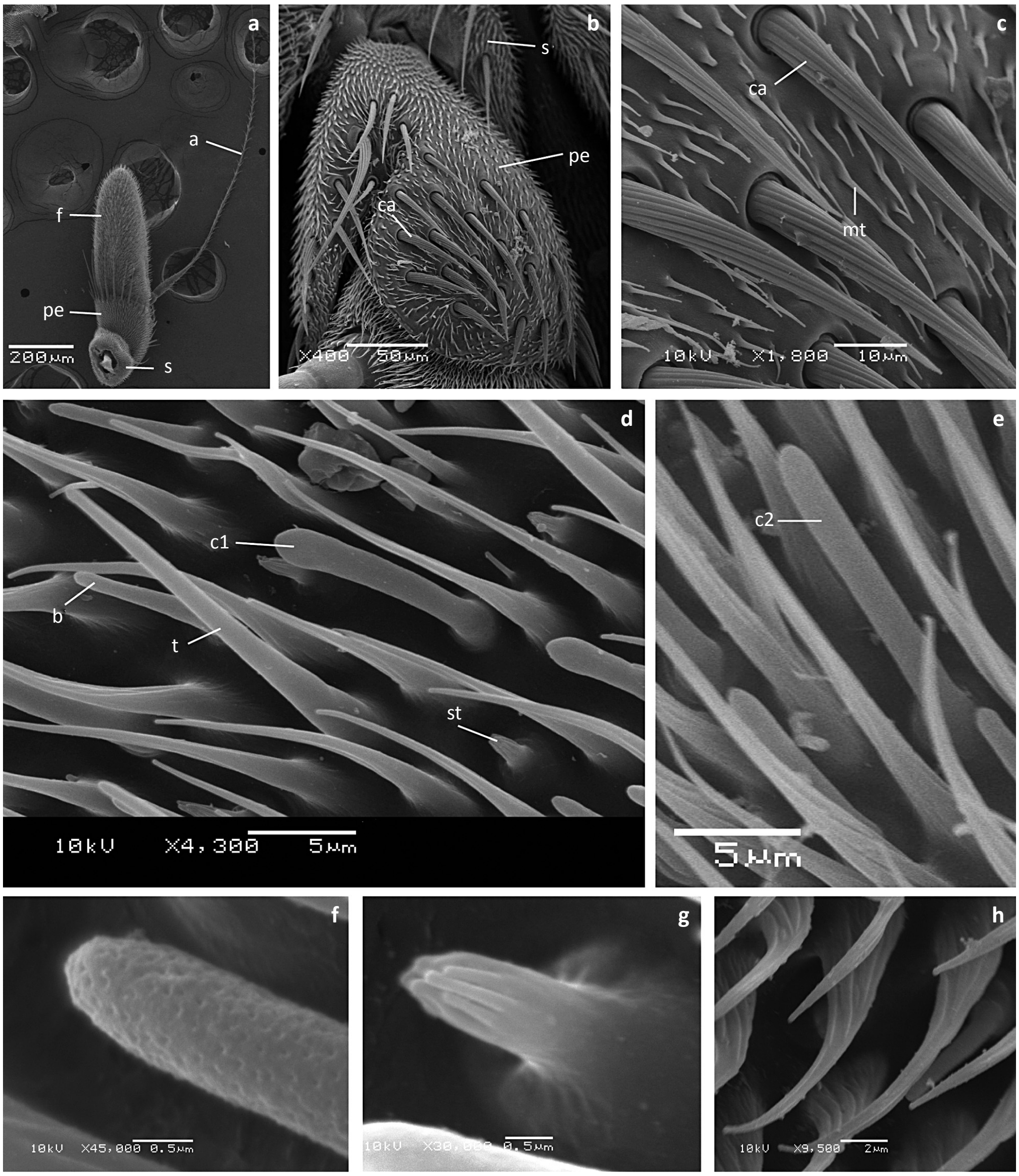

Fig 1 SEM images of the antennae of *Anastrepha fraterculus*. a) general view: a, arista; s, scape; pe, pedicel and f, flagellum; b) s, scape and pe, pedicel with ca, chaetica sensilla; c) ca, chaetica sensilla and mt, microtrichia; d-e) Sensilla on the flagellum: t, trichoid; b, basiconic; st, styloconic; c1, clavate type I; c2, clavate type II and mt, microtrichiae; f) Pores on the surface of the basiconic sensilla; g) Styloconic sensilla with finger like processes; h) Microtrichiae with grooves along the length.

Styloconic sensilla are present on the whole surface of the flagellum, but more abundant at the median region ($F = 6.83$; $P = 0.0036$) (Table 3). They were characterized by the presence of finger-like processes above the proximal third, with distinctive longitudinal grooves (Fig1g). The styloconic sensilla of *B. tryoni* were shown to respond to different odors and changes in temperature (Hull & Cribb 1997), and are common to other Tephritidae species (Giannakakis & Fletcher 1985, Levinson *et al* 1987, Dickens *et al* 1988, Hull & Cribb 1997, Arzuffi *et al* 2008).

Table 1 Mean (± SE) of the length and width (µm), at the larger diameter, in the antennae segments of females and males of *Anastrepha fraterculus*.

	Length		Width	
	Females	Males	Females	Males
Scape	92.2 ± 6.36	95.7 ± 3.55	162.2 ± 6.83	158.5 ± 4.78
Pedicel	140.2 ± 6.52	135.9 ± 6.04	192.4 ± 8.44	184.5 ± 4.56
Flagellum	436.0 ± 3.78	409.3 ± 7.37	188.9 ± 6.83	190.8 ± 3.88
Arista	1072.6 ± 18.48	1079.0 ± 29.33	-	-

Table 2 Sensilla length and width (μm) (apical and basal) of males and females of *Anastrepha fraterculus* on the flagellum (mean ± SE; n =15).

Sensilla	Length		Width		
	♀	♂	Region	♀	♂
Trichoid	21.9 ± 0.67	23.6 ± 0.4	Basal	1.9 ± 0.04	2.0 ± 0.05
			Apical	0.7 ± 0.03	0.7 ± 0.01
Basiconic	8.7 ± 0.03	9.3 ± 0.24	Basal	1.2 ± 0.03	1.4 ± 0.04
			Apical	0.8 ± 0.03	0.9 ± 0.03
Clavate I	12.8 ± 0.25	13.9 ± 0.29	Basal	1.4 ± 0.02	1.4 ± 0.02
			Apical	1.8 ± 0.04	1.6 ± 0.03
Clavate II	13.7 ± 0.41	12.6 ± 0.46	Basal	1.6 ± 0.08	1.5 ± 0.03
			Apical	1.4 ± 0.06	1.2 ± 0.07
Styloconic	3.0 ± 0.06	3.6 ± 0.15	Basal	1.0 ± 0.02	1.1 ± 0.04
			Apical	0.7 ± 0.02	0.6 ± 0.02

Table 3 Mean number (± SE) of the antennal sensilla and microtrichia, in a defined area (9,942 μm^2) on the distal, median, and proximal regions on the flagellum of *Anastrepha fraterculus* (n = 6).

Sensilla/ microtrichia	Region		
	Distal	Median	Proximal
Trichoid	97.9 ± 3.61 a	69.9 ± 3.20 b	22.8 ± 2.46 c
Basiconic	48.7 ± 1.97 b	56.4 ± 1.99 b	73.8 ± 2.85 a
Clavate I	0.0 ± 0.00 c	3.5 ± 0.74 b	11.3 ± 1.31 a
Clavate II	0.0 ± 0.00 b	2.2 ± 0.86 b	9.0 ± 1.78 a
Styloconic	18.5 ± 1.67 b	27.4 ± 1.77 a	20.9 ± 1.85 b
Microtrichia	332.6 ± 8.37 b	367.1 ± 9.74 ab	390.5 ± 12.54 a

Means followed by the same letter on the lines are not statistically significant different (P > 0.05).

The chaetica sensilla were observed only on the distal region of the scape and pedicel. They are 113.1 ± 7.96 μm long and are distally pointed and longitudinally ridged, inserted into sockets (Fig 1c).

Dickens *et al* (1988) and Hull & Cribb (1997) characterizated trichoid, basiconic, clavate and styloconic sensilla as chemoreceptors, and the chaetica type (a non-porous sensilla) as a mechanoreceptor (Dickens *et al* 1988, Arzuffi *et al* 2008).

Microtrichiae, the most abundant hairs, were distributed on the whole antenna and accounted for approximately 70% of the total number of structures observed on the flagellum of *A. fraterculus* (Table 3). Their length was 17.3 ± 0.43 μm and their density was significantly higher (F = 7.90; P = 0.0019) at the proximal region of the flagellum (Table 3). They correspond to the structures referred to as microtrichia (Levinson et al 1987, Dickens *et al* 1988, Hull & Cribb 1997, Miranda 2000, Arzuffi *et al* 2008) or as non-innerved, curved and longitudinally ridged setae (Giannakakis & Fletcher 1985) (Fig 1h).

The morphological aspects of the olfactory sensilla in the antennae of males and females of *A. fraterculus* are thought to provide the basis for future electrophysiological bioassays aimed to define the functional significance of the sensory cells associated with the different types of sensilla described in this study.

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