THE EFFECT OF SUPRA-FRONTO-ORBITAL (SFO) BRISTLE REMOVAL ON MALE MATING SUCCESS IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Here we present the findings of a laboratory study in which male Mediterranean fruit flies (medflies), *Ceratitis capitata*, (Wiedemann) had one or both supra-fronto-orbital (SFO) bristles artificially removed, for comparison with unoperated controls. All the flies were weighed and had their wings measured. The time at which a male began pheromone-calling was correlated with its weight, lighter males beginning calling earlier, but there was no effect of weight on mating success. Mated males had significantly longer wings than unmated males although there was no correlation with wing width. Although males missing both bristles were rejected more by females than those with one or two bristles, the loss of a single bristle had no effect on female response. The presence of bristles was not essential for successful mating. This study does not support the idea of females visually assessing males on the basis of their bristle symmetry.

Key Words: *Ceratitis capitata*, female choice, fluctuating asymmetry, medfly, sexual selection

RESUMEN

A continuación se presentan los descubrimientos de un estudio de laboratorio en el cual machos de la mosca del Mediterráneo, *Ceratitis capitata* (Wiedemann) presentaban una o ambas setas supra-fronto-orbitales (SFO) artificialmente removidas, para comparación con los controles no modificados. Todas las moscas fueron pesadas y sus alas fueron medidas. El tiempo en el cual un macho inició su llamado a través de una feromona se correlacionó con su peso, los machos más livianos iniciaron su llamado más temprano, pero no hubo ningún efecto por parte del peso en el suceso de apareamiento. Los machos apareados presentaban alas significativamente mas largas que los machos no apareados, sin embargo, no se presentó ninguna correlación con el ancho de las alas. Aunque los machos que carecían de ambas setas fueron mas rechazados por las hembras en comparación con aquellos que poseían una o las dos setas, la perdida de una sola de las setas no produjo ningún efecto en la respuesta de las hembras. La presencia de las setas no fue esencial para un apareamiento exitoso. Este estudio no apoya la idea de que las hembras analizan visualmente a los machos en base a la simetría de sus setas.

Mediterranean fruit flies (medflies), *Ceratitis capitata*, are sexually dimorphic, with males possessing features that are either less exaggerated in the female or absent. These include more brightly colored eyes, front legs with more numerous and longer hairs, a brighter white 'chin' or labrum (which in the female is a duller cream color), and a pair of supra-fronto-orbital (SFO) bristles which are elongated and modified to form a spatula shape at the tip (Féron 1962).

The medfly has a lek mating system (Prokopy & Hendrichs 1979, Arita & Kaneshiro 1985) in which males gather beneath neighboring leaves on a fruit tree and release a pheromone to attract females. They then perform a courtship ritual which involves more pheromone calling, wing fanning and wing buzzing, and head rocking (Féron 1962) before the male jumps onto the female and attempts to mate.

The female may reject the male at any stage up to and including the jump, simply by dropping away from the leaf. Male mating success varies greatly, with a small proportion of the males gaining most of the matings (Arita & Kaneshiro 1985, Whittier et al. 1992, 1994). It has been estimated that in the wild over 93% of all courtships end in rejection by the female (Whittier et al. 1994). Given these facts, there is considerable scope for both male competition and female choice to be taking place.

Hunt et al. (1998) carried out laboratory studies to investigate a possible role for the SFO bristles in the mating success of the male. Groups of 50 males and 50 females were allowed two hours to form pairs. Symmetrical males had higher mating success than asymmetrical males. The experiments were repeated in field cages in Guatemala and Crete using wild flies, and again

symmetrical males were found to be more successful (Hunt et al. in prep.).

When wild males emerging from oranges in Crete were housed in wooden (rather than plexiglass) cages they were sometimes observed to have lost one or both of their bristles. It was found that males missing both bristles were less likely to be accepted by females than males with one or two bristles (Hunt et al. in prep.).

Interestingly there was no significant reduction observed in the female acceptance rate of males when only one bristle was missing. It seemed therefore that this extreme form of asymmetry had no effect on a male's mating success, a conclusion which rested on the assumption that males that had lost a single bristle formed a random sample of all fitness types. It remained possible, however, that less fit males may have been more prone to accidental bristle loss. Here we report a repeat of the experiment under laboratory conditions, during which bristles were removed surgically from a random sample of flies.

MATERIALS AND METHODS

A sample of flies collected from coffee beans in Guatemala and sent directly to Manchester as pupae, formed the basis of the laboratory colony used in the present investigation. This colony had been under laboratory conditions for approximately 50 generations and was reared according to the techniques described by Hunt et al. (1998).

Virgin adults were collected in the laboratory within 24 hours of eclosing. For both rearing and mating tests, the flies were maintained at $25 \pm 2°C$ and $68 \pm 4\%$ rh. The sexes were kept separately until the adults were seven days old. The day before an experiment, a group of nine flies was transferred by aspirator and held immobile in a mosquito net bag ($15 \text{ cm} \times 30 \text{ cm}$) and marked individually with a dot of paint on the thorax. While still in the bag the flies were placed under a binocular dissecting microscope and both SFO bristles were removed from three of the flies with a fine pair of curved forceps. Three others had one bristle removed and the remaining three were manipulated in a similar manner, although both bristles were left intact. The flies were then returned to their rearing cage.

For the mate trials we simulated the natural conditions of field cage trials by developing a smaller indoor version (50 cm height × 40 cm diameter) containing a small potted orange tree measuring approximately 45 cm in height. The cage was made of fine black mesh so the flies and their marks were easily visible though it. The experiments were begun 30 minutes after the lights were switched on. No food and water was provided during the test due to its short duration.

On the morning of the seventh day the nine males were released into the cage and allowed fifteen minutes to acclimatize before nine marked females were released. All interactions between the flies were noted including the time at which the males began pheromone calling, the time at which each courtship attempt took place and the outcome. Each experiment lasted for two hours and was replicated 20 times. The small number of flies being observed allowed accurate recordings to be made using paper and pencil. For this purpose event tables were used.

At the end of each experiment, the flies were removed from the cage and immobilized in the freezer for one minute before they were weighed, after which they were preserved in 70% ethanol. Both wings were dissected and fixed onto microscope slides under a cover-slip using glycerol gelatin (Sigma Diagnostics, St. Louis, USA). The length and width of the wings were measured under a binocular microscope using a graticule eyepiece.

Statistical Analysis

Mating success was defined as whether a male mated or not within the two hours of the experiment. The mean acceptance rate of a male by females was calculated by dividing the number of its successful copulations by the number of its courtship attempts (matings plus rejections). This index was designed to take into account the amount of 'effort' a male had to expend before being accepted or not by a female. It therefore excluded those males that made no courtship attempt. T-tests were used to determine if there was a difference in the lengths and widths of wings in the mated and unmated males, and between males that attempted pheromone calling or courtship and those that did not. Tests of association between wing dimensions and acceptance rates were calculated using Spearman's rank correlation. The association between the number of bristles and male mating success, pheromone calling behavior and courtship attempts was calculated using Chi-squared tests. Associations between the number of bristles possessed by a male and its mating success, the time at which it began pheromone calling and the time to acceptance were determined using Kruskal-Wallis tests. The associations between time to pheromone calling and time to acceptance with weight were determined using Spearman's rank correlations. Logistic regression was used to determine if there was a difference in the weight of mated and unmated males. ANOVA was used to test for any association between the number of bristles, male weight and male wing dimensions. All statistical tests were carried out using the statistical package SPSS.

RESULTS

Out of a total of 180 male flies used in the experiments, 31 died during the trial or could not be used, leaving a final total of 149 males, out of which 86 (57.7%) mated. In the 20 replicates, the percentage of males mating ranged from 25 to 100%.

There was no difference in weight between males with 0, 1 or 2 bristles ($F_{2,146}$ = 1.294, P = 0.277). Nor was there any difference in mean wing length ($F_{2,146}$ = 0.048, P = 0.953) or mean wing width ($F_{2,146}$ = 0.234, P = 0.788). The mean wing dimensions and weight in the different bristle categories are listed in Table 1.

Male SFO Bristles

The numbers of males that mated in each bristle category are shown in Table 2. There was no association overall between mating success and the number of bristles (χ^2 = 1.601, df = 2, P = 0.449). Comparisons between 0, 1 and 2 bristles also showed no significant differences (*0,1* χ^2 = 0.344, df = 1, P = 0.558; *0,2* χ^2 = 1.593, df = 1, P = 0.207; *1,2* χ^2 = 0.334, df = 1, P = 0.563).

There was no overall association between acceptance rate and number of bristles (Kruskal-Wallis χ^2 = 5.64, df = 2, P = 0.06), although this value was close to significance. However significant differences were observed between the acceptance rate of males with no bristles and males with either one bristle (Mann-Whitney U = 345.5, P = 0.039) or two bristles (Mann-Whitney U = 446.0, P = 0.045) (see Fig. 1 and Table 1). No significant difference was found in the acceptance rate of flies with one versus two bristles (Mann-Whitney U = 629.0, P = 0.883).

The number of bristles possessed by a male was not associated with whether it pheromone called or not (χ^2 = 1.029, df = 2, P = 0.598) or whether or not it initiated a courtship (χ^2 = 0.757, df = 2, P = 0.685).

There was no significant difference in the time taken to begin pheromone calling between flies with 0, 1 or 2 bristles (Kruskal-Wallis χ^2 = 0.328, df = 2, P = 0.849), nor was there a significant difference in time taken to begin copulation (Kruskal-Wallis χ^2 = 0.077, df = 2, P = 0.962). See Table 1 for mean values in each bristle category.

Male Body Weight and Wing Dimensions

There was a significant positive correlation between wing length and width (r_s = 0.859, N = 149, P < 0.001), wing length and body weight (r_s = 0.651, N = 149, P < 0.001), and wing width and body weight (r_s = 0.657, N = 149, P < 0.001).

Mated males had significantly longer wings than unmated males (χ^2 = 7.251, df = 1, P = 0.007) (Fig. 2), although there was no difference in wing width (χ^2 = 2.415, df = 1, P = 0.120).

No association was found between male mating success and male body weight (χ^2 = 1.185, df = 1, P = 0.276).

Neither mean wing length (R_s = 0.099, N = 149, P = 0.321) nor mean wing width (R_s = 0.001, N = 149, P = 0.995) were associated with acceptance rate.

The weight of a male was not associated with its acceptance rate (R_s = -0.004, N = 102, P = 0.967).

Neither wing length nor wing width were associated with whether or not a male engaged in pheromone calling (length: t = 1.045, df = 147, P = 0.294, width: t = 0.306, df = 147, P = 0.760). There was also no difference in wing width between those males which initiated a courtship and those that did not (t = 1.665, df = 147, P = 0.098), but males which initiated a courtship had significantly longer wings than those which did not (t = 2.663, df = 147, P = 0.009).

The weight of a male was not associated with whether it pheromone called or not (t = 1.472, df = 147, P = 0.143) or whether or not it initiated a courtship (t = 0.262, df = 147, P = 0.793).

There was no significant association between time to begin pheromone calling and either wing length (R_s = 0.02, N = P = 0.835) or wing width (R_s = 0.045, N = P = 0.637). However there was a correlation with weight, the lighter males beginning pheromone calling earlier (R_s = 0.261, N = 111, P = 0.006), although the weight of a male was not correlated with the time it took to begin copulation (R_s = -0.079, N = 149, 86, P = 0.489).

DISCUSSION

Males with no supra-fronto-orbital bristles were less readily accepted by females than males with either one or two intact. This suggests that the SFO bristles play some role in either the ability of the male to perform an adequate courtship, or in the decision of a female to accept a male as

TABLE 1. THE NUMBERS (AND PERCENTAGES) OF MALES IN EACH BRISTLE CATEGORY THAT MATED AND THOSE THAT DID NOT.

	0 bristles	1 bristle	2 bristles
Pheromone called	35 (77.8%)	34 (69.4%)	42 (76.4%)
No pheromone calling	10 (22.2%)	15 (30.6%)	13 (23.6%)
Attempted courtship	30 (66.7%)	32 (65.3%)	40 (72.7%)
No attempt	15 (33.3%)	17 (34.7%)	15 (27.3%)
Mated	23 (51.1%)	28 (57.1%)	35 (63.6%)
Unmated	22 (48.9%)	21 (42.9%)	20 (36.4%)

TABLE 2. MEAN WEIGHT, WING LENGTH AND WIDTH, TIME TO PHEROMONE CALL (PC) AND ACCEPTANCE RATE OF MALES IN EACH BRISTLE CATEGORY.

	0 bristles (±se)	1 bristle (±se)	2 bristles (±se)
Mean weight (mg)	6.956 (0.172)	6.584 (0.154)	6.764 (0.152)
Mean wing length (mm)	4.322 (0.025)	4.327 (0.023)	4.332 (0.020)
Mean wing width (mm)	2.302 (0.011)	2.291 (0.013)	2.296 (0.010)
Mean time to pc (secs)	1105.086 (262.167)	1420.353 (370.162)	1088.214 (276.478)
Mean acceptance rate	0.558 (0.075)	0.763 (0.064)	0.748 (0.059)

a mate. However under the experimental conditions reported here, the influence of the bristles was not strong enough to affect a male's final mating success.

Several theories have been proposed to explain the role of the SFO bristles in the medfly. Arita & Kaneshiro (1985) suggest that the most important determinant of male mating success is its ability to successfully direct a pheromone towards the female during courtship, and the SFO bristles may be playing a role in this (Kaneshiro cited in Hunt et al. 1998). However Briceño et al. (1996) and Mendez et al. (1998) note that during the wing buzzing and head-rocking phase, when bristles are in movement, the male is no longer emitting pheromones.

Briceño et al. (1996) and Mendez et al. (1998) believe it is more likely that bristles function as display devices due to the fact that the stalk and spatulate regions are different colors, the stalk being clear and the spatulate end black. However there seems to be geographical variation in this feature since many male bristles in Hawaii are all black (D.O. McInnis, USDA/ARS, Honolulu, unpublished data).

It has been suggested that fluctuating asymmetry (FA) is an important component of sexually selected characters (Moller 1990), reflecting the quality of a male, and that males with low levels of FA are more successful than their more asymmetrical rivals. Hunt et al. (1998) found that laboratory mated male medflies with bristles that were symmetrical in their length had a higher mating success than males with asymmetrical bristles, a result later confirmed in the wild in tow separate locations, in Guatemala and Crete (Hunt et al. in prep.). However it was impossible to tell from these studies whether symmetrical males were being actively selected by females or whether such males were simply better or more assiduous in their courtship.

Our field studies on the effect of accidental loss of one or both bristles (to be published later) suggested that female choice on the basis of visual symmetry could not be supported. Acceptance rates of males with one or two bristles present were significantly higher than those of males with no bristles. The present laboratory study also indicates that the loss of one bristle has no adverse effect on acceptance rate. Males missing one bristle are absolutely asymmetrical and yet they have the same acceptance rate as males with two bristles intact. The fact that these results could be replicated in the laboratory discounts the possibility that in the wild flies, only the less fit males were losing bristles. The present experiments also show that the actual removal of the bristles in the laboratory does not adversely affect the males
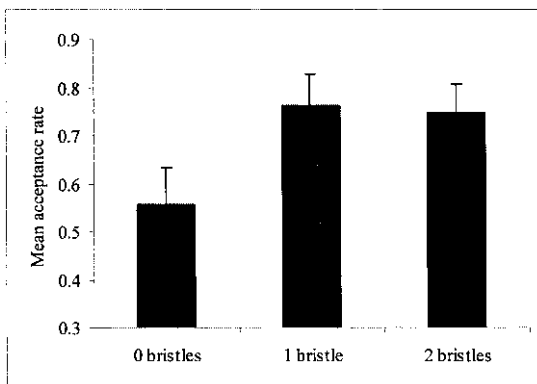

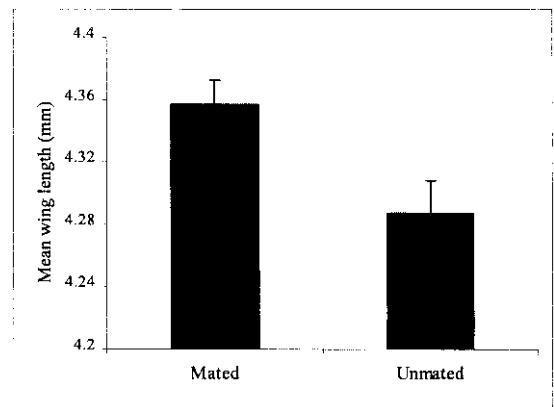Fig. 1. The effect of male bristle number on acceptance rate.

Fig. 2. The mean wing lengths of mated versus unmated males.

mating behavior, and that the relatively poor acceptance rate of males with no bristles was due to their absence rather than the process by which they were removed or any resulting trauma.

The question still remains about how the bristles are actually functioning. Accepting that their effect is visual, a recent suggestion is that they create a 'halo' effect above the male's head as they move from side to side during head-rocking (O. Hasson & P. Taylor, pers. comm.). If this is so, it might explain why males with one bristle are as readily accepted by females as males with two bristles, since one bristle should still be able to create the 'halo' effect above the males head.

At a different level it is possible that the bristles could be functioning as a species recognition signal, as discussed by Hunt et al. (1998). To investigate this theory it would be necessary to study the other species of *Ceratitis* in which the males possess SFO bristles.

In summary our work on SFO bristles presents a complex picture. Although we have demonstrated that symmetrical males have higher mating success, whether in the laboratory (Hunt et al. 1998) or in the field (Hunt et al. in prep.), the present study does not support the conclusion that this occurs as a result of female mate choice on the basis of visual symmetry. This agrees with the suggestion of Mendez et al. (1998) that symmetry in bristle length is unlikely to be acting as an indicator of male quality to the female, because the position of the bristles on the male's head makes it difficult for the female to assess either their size or symmetry with any degree of accuracy. Despite this, we found that the presence of one bristle, although making the male absolutely asymmetrical, is better than having no bristles at all. We can therefore conclude from these experiments that the SFO bristles are indeed important in encouraging acceptance by females, although the reason remains unclear.

We have previously demonstrated in the Guatemalan field cage studies that males with wider wings had a higher mating success (Hunt et al. in prep.). In the current study, we did not find this effect, but instead found that males with longer wings were more successful, and were also more likely to initiate a courtship. These results have since been shown to be repeatable in a second laboratory study (M. K. H., unpublished data). Furthermore, in the Guatemalan field study, male mating success was close to being significantly positively associated with wing length. It is clear that the wings are important in male mating success. The importance of wings in determining mating success is not surprising as they are used in both olfactory and auditory stimulation in the courtship sequence. A particular shape or size of wing may be better than others at performing these functions although wing morphology will obviously be constrained by natural selection on flying ability as well as its function in courtship. The present studies indicate that the optimum wing design for courtship may differ between samples or between laboratory and field cages. The reason for this variation is unclear.

Investigations of male body size made by Arita & Kaneshiro (1988) and Whittier et al. (1992, 1994 & 1995) led to the conclusion that size has no influence on male mating success. Arita & Kaneshiro (1988) found that smaller males from coffee were preferred over larger males from cherry in some mate trials, but they concluded that 'characters other than body size are essential determinants of mating success of the males'. In contrast, both Churchill-Stanland (1986) and Orozco & Lopez (1990) found that large laboratory males had a greater mating success than small males, although Orozco & Lopez (1990) found that size was less important in wild strains. In our work in field cages (Hunt et al. in prep.) we found that male body size may be important to some degree, since smaller (lighter) males begin pheromone calling earlier. In the current study we have demonstrated that this also occurs in laboratory experiments, suggesting that it may be evidence of an alternative mating strategy by the males. Perhaps smaller males need more time to achieve the same degree of mating success. Dunn et al. (1999) found that smaller males of several seaweed fly species were more willing to mount a female than larger males and suggested that this may be because smaller males are more active, or because they develop faster than large males and thus have first access to females. They also suggested that larger males may be longer lived and therefore have a longer time in which to gain access to females. Several studies have shown a positive association between male size and longevity, for example Butlin & Day (1985) studying seaweed flies, and Banks & Thompson (1985) studying damselflies. An association between size and longevity in medflies has not been reported, although Sivinski (1993) found it in domestic *Anastrepha ludens*, a species of Tephritid related to the medfly.

Other investigations into male body size have focused on the nutritional status of the male. Blay & Yuval (1997) found that protein-deprived males are smaller and have lower mating success then the protein-fed males which mate earlier and have a higher probability of mating. Yuval et al. (1998) found that males of all sizes (measured by wing length) participate in leks but that lekking males tend to be heavier and they explain this as a result of these males having greater nutritional reserves. These findings cannot explain the results from our current study in which smaller males pheromone call earlier. Their small size cannot be explained by any nutritional deficiencies. If this were the case we would expect them to begin calling later rather than earlier. However, Blay & Yuval (1997) conclude that male medfly

size has no effect on the overall reproductive success of the female, which suggests that size may not be an important criterion for female choice.

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