

Male position and calling effort together influence male attractiveness in leks of the medfly, *Ceratitis capitata* (Diptera: Tephritidae)

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Despite the close association between lek mating systems and the study of female mate choice, male mating success in leks is often associated with other aspects of sexual selection as well as female choice of male display traits. Males of the medfly *Ceratitis capitata* form leks on the undersides of leaves of their host plants. By experimentally creating artificial leks, we show that male success at attracting females depends not only on male calling effort (pheromone production dispersed by wing movement), but also on the position of the male within a lek. Males in the highest position in the artificial lek (closest to the light) attracted more females, and received more visits from those females. In our experiment, we deliberately minimized the visual cues that females approaching a male could use and, under these conditions, found no associations between male attractiveness and male size, weight or fluctuating asymmetry, either of the wings or sex setae (a pair of bilateral supra-fronto-orbital bristles). The latter result contrasts with earlier studies showing a significant negative association between sex setae fluctuating asymmetry and mating success. Accordingly, we conclude that symmetry of the male sex setae has no role in nonvisual communication (e.g. through directing pheromone plumes). Mating patterns associated with this insect are therefore every bit as complex as those in vertebrate leks. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **95**, 479–487.

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INTRODUCTION

Understanding the patterns of nonrandom mating in leks has played a key part in attempts to disentangle the processes of sexual selection (Andersson, 1994; Högländ & Alatalo, 1995). Subsequent to the characterization of the so-called ‘paradox of the lek’ (Borgia, 1979), lekking species have been extensively studied to test the benefits that accrue to females mating with particular males in the lek (Högländ & Alatalo, 1995). These benefits are often considered to be indirect, in

the form of beneficial traits inherited by their offspring, which make them fitter or more attractive (Kirkpatrick & Ryan, 1991; Maynard Smith, 1991; Andersson, 1994; Johnstone, 1995; Jennions & Petrie, 2000), although it has become appreciated that females visiting leks may also obtain direct benefits (e.g. through increased fertility; Reynolds & Gross, 1990; Jones, Quinnell & Balmford, 1998). The evolution of the actual lek mating system itself has also attracted great interest, with the development of numerous models to explain why males form aggregations to attract and mate with females (Högländ & Alatalo, 1995). Despite this general interest, it is birds and mammals that have played the major role in the development and testing of lek

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theory, with lekking insect species playing far less a role (Shelly & Whittier, 1997). This is despite the success of novel tests of theory that insects systems have allowed (Jones *et al.*, 1998; Jones & Quinell, 2002; Drone, 2003).

Female choice and the evolution of leks come together when female behaviour influences lek formation, such that the dynamics of the lek interacts with the mating success of the males within that lek. For example, patterns of female choice for attractive males may lead other males to aggregate around these attractive males (as envisaged by so-called 'hotshot' models of lek formation; Beehler & Foster, 1988; Kokko & Lindström, 1996). However, even within leks based around female habitat preferences (e.g. 'hotspot' lekking models: Bradbury, Gibson & Tsai, 1986; Höglund & Alatalo, 1995), positions within the lek may influence male mating success, with females preferring males in certain positions within the lek. These position effects may either be independent of the males present, or influenced by variation in male attractiveness, such that mate preferences may be context-dependent (Bateson & Healy, 2005). Male success in a lek may therefore be a function of both the absolute and relative quality of a male's courtship display, as well as his position in a lek.

In the present study, we consider how these different aspects of lekking influence male attractiveness in the Mediterranean fruit fly (medfly) *Ceratitidis capitata* (Wiedemann). Male medflies form leks, numbering between three and six males, on the undersides of sun-lit leaves of host trees (Prokopy & Hendrichs, 1979; Arita & Kaneshiro, 1989; Whittier, Kaneshiro & Prescott, 1992; Shelly, Whittier & Kaneshiro, 1994; Eberhard, 2000; Field, Kaspi & Yuval, 2002), where they release and disperse a pheromone to attract females. Components of the male sex pheromone also attract other males, a phenomenon sometimes associated with aggressive male-male interactions as males compete for territory location, attempting to drive out individuals holding preferred territories (Whittier *et al.*, 1994; Eberhard, 2000).

Having gained an individual leaf territory, the male medfly raises its abdomen and begins 'pheromone-calling' from an extruded gland in the rectal epithelium, the pheromone being then dispersed by wing movement (Burk & Calkins, 1982; Arita & Kaneshiro, 1986; Whittier *et al.*, 1992). Within this male hormone, 69 components have been detected, six of which show significant activity when tested individually or as a blend, with three being identified as the main components (Jang *et al.*, 1989; Baker *et al.*, 1990; Heath *et al.*, 2000). Heath *et al.* (2000) also reported that females proved highly responsive to a particular dose of pheromone. The arrival of a female stimulates the male courtship display face to face

(Féron, 1962; Arita & Kaneshiro, 1985, 1989; Harris, Wood & Bailey, 1986; Briceño, Ramos & Eberhard, 1996). There is female choice for male courtship ability and, if she is not receptive or prefers not to mate, she is able to reject the courtship at any given stage by turning away from the male or, if the male has already jumped onto her back, by dropping from the leaf (Arita & Kaneshiro, 1989).

One target of female choice is the conspicuous pair of male 'sex setae', which are elongated bristles on the supra-fronto-orbital area of the head, with a light-coloured stalk bearing a striated, diamond-shaped terminal expansion. Hunt *et al.* (1998) demonstrated a significant negative correlation between fluctuating asymmetry (FA) in the length of the sex setae (hereafter referred to as bristles) and mating success (i.e. the more symmetrical, the more attractive; see also Hunt *et al.* (2004). A direct visual stimulus appears likely although it has also been suggested that the bristles, together with the wing movements, may influence how male pheromones are directed to females (Mendez, Briceño & Eberhard, 1999). Therefore, as well as influencing close range female choice, bristles may influence pheromone calling more broadly. The male's wing movements also produce sound, but its significance as an acoustical component of female attraction and courtship is not clear (Sivinski, Calkins & Webb, 1989; Eberhard, 2000).

Despite work on these close-up cues, it is not known whether females also choose specific mating places within the lek on the basis of their location, as envisaged by hotspot models of lek formation. We report a study conducted on artificial leks in laboratory cages under conditions in which calling males were kept at a distance from approaching females, behind black netting. Under these conditions, the four aims of the study were to investigate whether: (1) the position of a male in a lek affects success in attracting females; (2) calling males vary in their intrinsic attractiveness to females, irrespective of location; (3) the duration of pheromone calling influenced male attractiveness; (4) the attractiveness of a calling male is correlated with a number of morphological characters, including sex setae FA, when visual cues are obscured from the approaching females.

MATERIAL AND METHODS

THE FLIES

Flies used in the present study were from the Petapa strain reared at the 'Moscamed' mass rearing factory near the town of Petapa in Guatemala. A subculture of this strain was established at the University of Manchester and had been maintained for 3 years at the commencement of this study, on a carrot-based

diet using the rearing procedures of Busch-Petersen & Wood (1986). The flies were kept at $25 \pm 2^\circ\text{C}$ and 68 ± 5 RH, under a 12 : 12 h light/dark cycle, for both rearing and experiments.

EXPERIMENTAL DESIGN

To mimic lek size in the field, we experimentally created leks with three males, and let eight females visit the lek. The experiment was replicated in twelve independent trials, using flies sexed and separated within 24 h of eclosion from independent batches of separately reared larvae. For each replicated trial, eight female virgin flies and ten male virgin flies were kept in separate holding containers ($345 \times 245 \times 210$ mm) and provided with food (3 : 1 mixture of yeast and sugar) and water. After 4 days, we individually colour-coded each female with a spot of enamel paint on the dorsal mesonotum of the thorax. From each group of ten males, three pheromone-calling individuals were selected for each trial, and they were paint marked at the age of four days in the same way. The flies of both sexes were allowed 24 h for recovery from the marking before the trial began.

For each trial, we transferred the eight females to an experimental cage (Fig. 1), prepared in the following manner: a potted, 45-cm tall, *Citrus* (orange) sapling (approximately 70 leaves) was placed inside a cylindrical black net-cage (height 54 cm, diameter 40 cm). We illuminated the cage from above using four 6-W fluorescent tubes, as well as by the natural laboratory fluorescent lighting. Three transparent plastic tubes were fitted onto the cage wall on different positions relative to the *Citrus* sapling to act as artificial male territories (Fig. 1). Each tube consists of two cylindrical chambers (length 12 cm, diameter 4 cm), joined by a connecting piece fitted with a sliding partition between the two chambers (Fig. 2). The tubes were fitted in such a way that the black net of the cage wall came between the interior (female) chamber and the connecting piece attaching to the exterior (male) chamber (Fig. 1). The colour of the net was chosen to inhibit visual cues between the two sexes. With the sliding partition open, the only barrier between the two chambers was provided by the black net. To remove traces of male pheromones from previous experimental trials, all parts of the apparatus were cleaned thoroughly with ethanol (70%), rinsed with water, and left soaking in water at 25°C for 24 h before drying.

We next transferred each of three males into one of the exterior chambers, capped with a netted cover to allow for aeration. The flies were then given 20 min for acclimatization with the sliding partition closed (it was determined in preliminary experiments that an



Figure 1. The experimental cage, showing the positioning of the three artificial male territories. Each territory is a tube fitted in such a way that the net of the cage wall comes in between the interior (female) chamber and the connecting piece attached to the exterior (male) chamber.

interval of 5–10 min was usually sufficient for the males to resume pheromone-calling). The partitions were then opened, and the cage observed for 45 min. We recorded the number and duration of female visits to the three interior compartments of the male tubes. A brief stop at or near the mouth of a chamber was not scored as a visit, nor was lingering on the outer surfaces of the chamber. Males were checked every 2 min to determine whether they were still visibly calling. Calling males were oriented apparently randomly within their chambers (i.e. not particularly towards the cage interior). After 45 min had elapsed, any ongoing female visits to male tubes were allowed to continue until they left, after which both sexes were subsequently re-transferred to their respective holding containers. All trials were started within the first hour of the photoperiod because the Petapa strain was observed to be most active in the early morning.

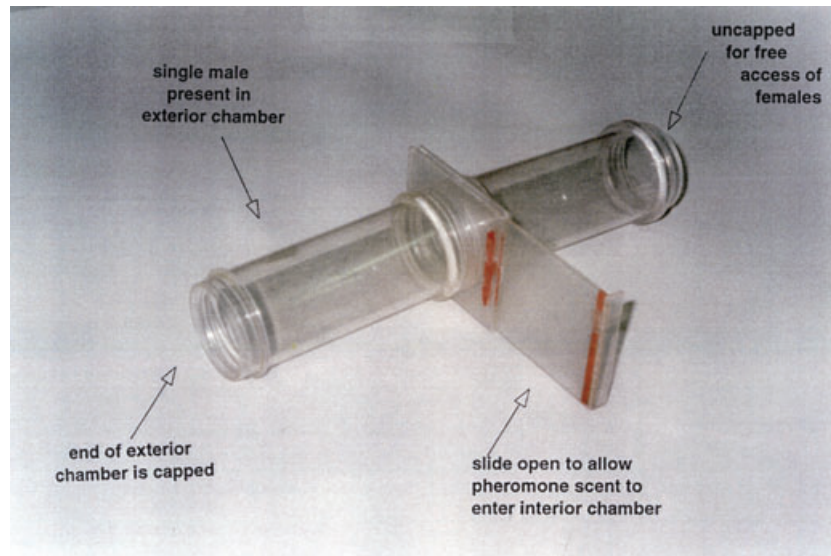


Figure 2. The artificial male territory used in the experiment, consisting of two chambers attached to a connecting piece fitted with a sliding partition.

Within each trial, we repeated this protocol twice more for the same set of male and female flies for the next 2 days, with the males rotating positions. Each trial thus consisted of three parts (called observation periods), each of which was conducted on one of three consecutive days. Twelve replicates of the above tripartite trial were performed, providing information on 36 males, each tested three times.

At the end of the third day of each trial, the male flies were removed from the cage and immobilized by holding them at -15°C for 2 min. Male weight was determined to the nearest 0.1 mg using an electronic balance (Mettler AC100), whereupon they were preserved in ethanol (70%). Both wings were later dissected and fixed onto microscope slides under a coverslip using glycerol gelatin. The length and the width of the wings were measured under a stereo dissecting microscope at a 20-fold magnification using a calibrated eyepiece graticule. The procedure for measuring bristle length was adopted from a field technique devised by Hunt *et al.* (2004), which allows the bristles to remain *in situ*. They showed the method to be effective, as the measurement error was smaller than between-sides variation (see below). The fly was placed in a transparent plastic bag, which was held under a stereo dissecting microscope; the fly was then gently pressed into a position where the bristles adhered to the bag and the bristle length was measured at 45-fold magnification. To quantify measurement error, we measured both bristles and wings twice (on separate days). To make the study directly comparable with that of Hunt *et al.* (1998), FA was measured as the absolute difference between mean right and mean left (FA1 in Palmer, 1994).

STATISTICAL ANALYSIS

We performed a series of tests on our measures of bristle length, wing length and wing width to determine whether the patterns of symmetry in these traits departed from that of FA (see Palmer, 1994). First, for each trait, the mean length of the two measurement replicates of the right side was subtracted from that of the left side to obtain signed asymmetries. We performed a Kolmogorov–Smirnov goodness-of-fit test on these signed asymmetries to determine if the data were normally distributed. For all three traits, the signed asymmetries were not significantly different from a normal distribution ($\chi^2 = 0.86\text{--}1.04$, d.f. = 2, all $P > 0.05$). None of the asymmetry distributions were significantly skewed ($t < 1.30$, all $P > 0.05$), and there was no significant kurtosis for bristle length or wing length asymmetries ($t < 1.90$, all $P > 0.05$). Wing width asymmetry was significantly leptokurtic however ($g_2 = 1.985$, SE = 0.768, $t = 2.585$, $P < 0.05$). For each trait, the mean unsigned asymmetry was not significantly different from zero ($t_{35} < 0.52$, $P > 0.05$).

The assessment and reporting of measurement error are essential in studies of asymmetry, as measurement error generates the same asymmetry pattern as FA (Palmer, 1994; Watson & Thornhill, 1994). To determine whether the pattern of symmetry was significant after removal of measurement error, we performed a two-way analysis of variance in accordance with Palmer (1994). For all three traits, the variance among individuals was significantly greater than measurement error ($F_{35,72} = 2.54\text{--}3.43$, $P < 0.05$). The repeated measure-

ments on all traits were highly significantly correlated ($r_{35} = 0.53\text{--}0.88$, $P < 0.001$).

The signed asymmetries of both bristle length and wing length revealed a pattern of between-sides variation consistent with FA; no departures from FA with respect to directional asymmetry or anti-symmetry were detected. Signed asymmetries of wing width were significantly leptokurtic and were therefore omitted from further analysis.

Male success was scored within trios as a ranking from most to least successful, in terms of both number of visits and duration of time visited. We tested whether male success was associated with: (1) bristle length asymmetry, (2) wing length asymmetry, (3) wet weight, (4) wing length, and (5) body condition. Wing length was used as a correlate of body size, and body condition was estimated from the residuals of the regression of body weight on wing length ($R^2 = 0.21$, $P = 0.005$, $N = 36$). Given the non-normal distribution of much of the data, we used nonparametric Kruskal–Wallis tests. Associations between male success, with respect to number of visiting females and number of visits, and other variables were investigated by carrying out Kruskal–Wallis tests. All statistical tests were conducted using SPSS (SPSS Inc.).

RESULTS

Up to 20 (out of 24) females would visit a single male across the three observation periods, and together they would make up to 39 visits of greatly varying duration (visits lasted 1–44 min; mean \pm SD = 5.46 ± 5.62). Males spent between 20 % and 100 % (mean \pm SD: $93.79 \pm 15\%$) of the available observation period (45 min) actively calling.

Male success at attracting females varied with position within the lek. When males were in the top tube they received significantly more visits than when they were in the middle or bottom position (number of visiting females: $W = 23.97$, d.f. = 2, $P < 0.001$; number of visits: $W = 46.62$, d.f. = 2, $P < 0.001$; Fig. 3A, B). However, the fewer females visiting the males in the bottom position tended to spend longer with those males ($W = 9.18$, d.f. = 2, $P < 0.05$; Fig. 3C). Male position had no effect on pheromone calling by males ($W = 0.75$, d.f. = 2, $P > 0.05$). There was no significant variation in either male calling behaviour or female visitation between observation periods (time spent calling: $W = 0.24$, d.f. = 2, $P > 0.05$; number of females visiting: $W = 0.26$, d.f. = 2, $P > 0.05$; total number of visits: $W = 1.82$, d.f. = 2, $P > 0.05$; duration of visits: $W = 1.84$, d.f. = 2, $P > 0.05$). There was also no significant variation between replicates in calling: $W = 6.67$, d.f. = 11, $P > 0.05$; number of females visiting: $W = 17.20$, d.f. = 11, $P > 0.05$; total number of visits: $W = 6.07$, d.f. = 11, $P > 0.05$). Only with respect

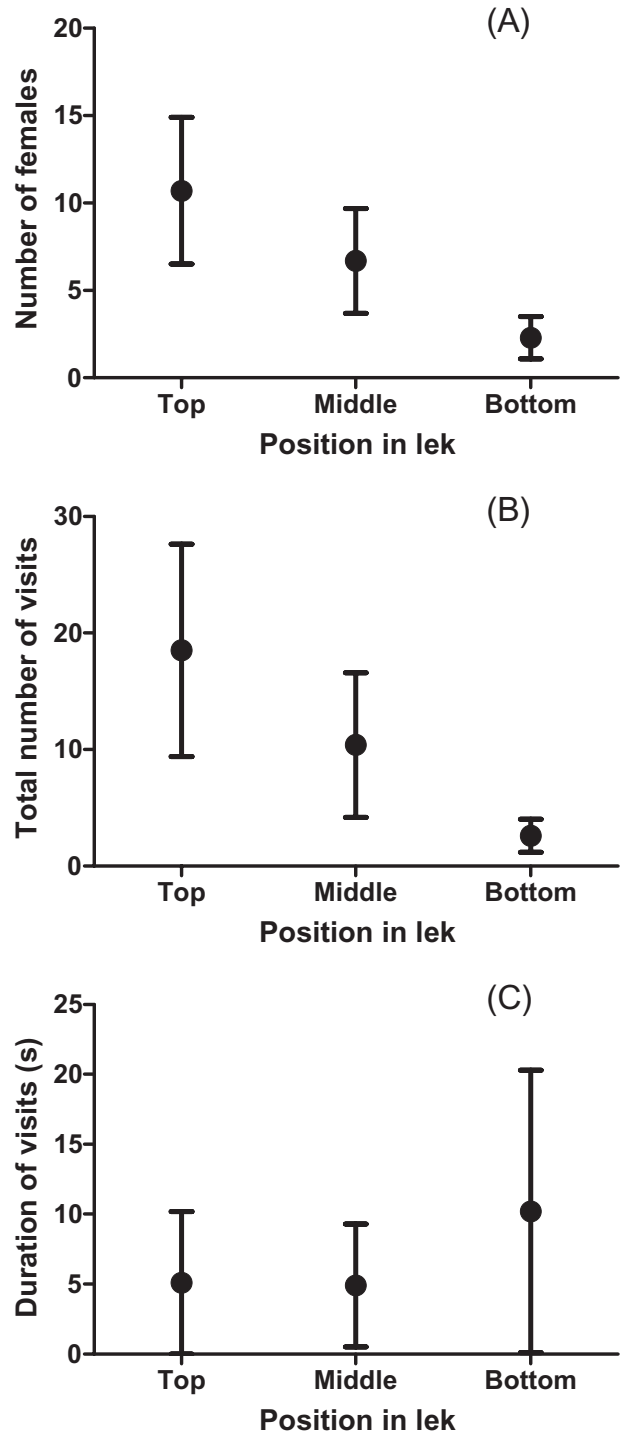


Figure 3. Male success in attracting females from the different territory positions in an artificial lek in terms of (A) number of females, (B) number of visits summed across females, and (C) duration of female visits. Error bars indicate standard deviations.

to duration of visits was variation between replicates significant ($W = 22.7$, d.f. = 11, $P = 0.02$). Adult behaviour is expected to vary to some extent because of differences in larval nutrition among cultures but, under the conditions of the experiment, this affected only the duration of female visits. Across the three parts of a trial (with each male in each position), male success in attracting females showed a significant positive association with calling effort, establishing that males vary in their intrinsic attractiveness, irrespective of their location. Males that performed more calling behaviour had both more females visiting them ($r_s = 0.196$, $N = 36$, $P < 0.05$), and also more visits in total ($r_s = 0.191$, $N = 36$, $P < 0.05$). The duration of visits was not significantly correlated with time spent calling ($r_s = 0.121$, $N = 108$, $P > 0.05$).

Male attractiveness, in terms of number of visiting females, or total number of visits, was not associated with any of the male morphological characters considered. Successful males were not larger (number of females: $W = 3.61$, $P > 0.05$; number of visits: $W = 4.61$, $P > 0.05$), heavier (number of females: $W = 3.60$, $P > 0.05$; number of visits: $W = 1.36$, $P > 0.05$), in better condition (number of females: $W = 4.45$, $P > 0.05$; number of visits: $W = 0.32$, $P > 0.05$), more symmetrical in bristle length (number of females: $W = 0.40$, $P > 0.05$; number of visits: $W = 0.60$, $P > 0.05$) or more symmetrical in wing length ($W = 0.96$, $P > 0.05$; number of visits: $W = 0.48$, $P > 0.05$).

DISCUSSION

Male *C. capitata* attractiveness within an artificial lek was positively associated with male calling effort (confirming the results of Whittier *et al.*, 1994) and also with calling position in relation to a light source. Males calling from the top position, nearest to the lights, scored the highest numbers of visits, whereas those in the bottom position scored the lowest. Our experiment was designed to restrict the influence of close-up visual cues known to influence male mating success following mate attraction, focussing instead on longer-distance male–female interactions and lek structure. Contrary to the discussion in Field *et al.* (2002), our data are consistent with the idea that leks form around areas attractive to females (e.g. hotspot models of lek formation; Höglund & Alatalo, 1995), but that individual male attributes are also important. Male calling did not vary with position, suggesting that males do not alter their display behaviour relative to the positional ‘quality’ of their territory. Clearly, understanding male mating success in medflies requires consideration of the whole process for males: from the formation of leks and gaining the best

possible territory within the lek, through to female attraction, and then successful close-up courtship and insemination.

The territory-dependent female visiting rate observed in the experiment appears consistent with the observation by Arita & Kaneshiro (1985) that a large proportion of females entering a lek seem to move directly into preferred territories (i.e. territories preferred in terms of their position). In the field, it may be assumed that the best males compete for the best positions to engage both in this activity and in the courtship that follows. In the present study, in which the influence of position and calling activity of males are experimentally separated, and male–male competition is restricted solely to calling activity, some females entered a higher territory even when occupied by an inferior male, while other females were diverted by pheromone influence (and possibly sound) towards a superior male in an unfavourable position. The interaction between success in any male–male competition for lek position and male calling, however, remains to be formally quantified.

It is unsurprising that the best position in the lek in this experiment is at the top of the laboratory cage, closest to the lights. In nature, light is clearly a major stimulus, both for its warming effect as well as for illuminating the upper surface of the leaves. Each male occupies the shaded underside of a single sunlit leaf, as observed in field-caged host trees and also under free-living conditions (Prokopy & Hendrichs, 1979; Shelly *et al.*, 1994; Eberhard, 2000). The first part of the morning is a favoured time for courtship before it gets too hot, and wind can also affect the males’ positioning (Arita & Kaneshiro, 1985). In field cages (3 × 3 m; height 2.5 m) under still conditions, flies are to be found resting near the roof of the cage in the early morning from where they disperse downwards to form leks in the enclosed coffee bush as the warmth of the sun increases (R. J. Wood, pers. observ.). In the present study, the position of the lights above the cage was aimed at mimicking the natural stimulus except that clearly the illumination was kept constant. Also to be considered is the presence within our experimental cage of a miniature orange tree. Light intensity in the middle and bottom tubes will be lower due partly to the shading of the leaves of the tree.

The duration of female visits varied significantly between tube positions, and was longest in bottom-position tubes compared with the other two positions. A longer duration may have resulted from a lesser degree of confrontation among competing females. Female activity was much less concentrated on bottom-position tubes; female visitors may thus have been less disturbed by the presence of competitors. The role of female–female interactions and interfer-

ence in structuring leks and determining male mating success has not received as much attention as the more straightforward male–male competition and female choice (Saether, Fiske & Kålås, 2001; Bro-Jorgensen, 2002).

Across each trial, with the males in the three different positions on successive days, male success in attracting females (assessed in terms both of number of females visiting and total number of visits) was significantly associated with the time it spent calling ('calling effort'). This may be referred to as their 'intrinsic attractiveness' (i.e. their relative attractiveness irrespective of position). Male attractiveness was not significantly correlated with fluctuating asymmetry in the sex setae or wing length. Neither was it associated with weight, wing length (as an indicator of body size), or body condition, which suggests that the quantity or quality of pheromone (or possibly sound) produced is not affected by these variables.

The absence of an association between a male's attractiveness and any particular physical attribute is unsurprising given the experimental conditions, designed to confine the male at a distance from the female behind black netting. It does, however, raise the general issue about physical characteristics, and fluctuating asymmetry in particular, concerning whether it is the characteristic itself that is attractive or a genetically associated 'fitness' quality. In the case of sex setae FA, several studies have supported the idea of an indirect effect (Mendez *et al.*, 1999; Hunt *et al.*, 2002, 2004) without identifying what this might be. The present study provides no evidence that it has to do with 'calling effort'.

As to the function of the sex setae, this remains a matter of debate. Visual display is not the only function proposed for these bristles. Two other hypotheses are listed by Mendez *et al.* (1999): (1) a tactile influence on the female during courtship and (2) a means of helping to direct male pheromones towards the female. Mendez *et al.* (1999) favour the visual explanation, and note that visual information provided by the bristles might relate to species or sex recognition, as well as some aspect of male 'quality'. In experiments involving the surgical removal of one or both bristles, the presence of even a single bristle proved attractive to females (in terms of acceptance rate; Hunt *et al.*, 2002). Although mating by males without bristles did take place, it was significantly delayed. Mendez *et al.* (1999) showed that the removal of both bristles affects sexual competitiveness without affecting courtship behaviour.

As well as highlighting the role of position effects, the dynamics of medfly leks are important for the ongoing attempts at biocontrol of this species (Field *et al.*, 2002). *Ceratitis capitata* is a serious economic pest of many fruit crops across the globe. Its out-

standing success in terms of dispersal is attributed chiefly to a highly polyphagous nature (White & Elson-Harris, 1992). The mating behaviour of the medfly has therefore attracted interest in connection with attempts to control medfly populations by the mass release of sterile males [sterile insect technique (SIT); Hendrichs *et al.*, 2002]. Successful SIT programmes are predicated on sterile males being successful at obtaining mates when in competition with wild-type, fertile males (for a review of progress in *C. capitata*, see Robinson, Cayol & Hendrichs, 2002). Although matings do occur outside the lek, studies have indicated that lek matings are much more common (Eberhard, 2000). Sterile male success in mating will therefore generally depend on attracting females into a lek. It has been established that mass reared male medflies do participate in lek formation in the field (Cayol *et al.*, 1999; Robinson *et al.*, 2002). It remains to be seen to what extent male success in leks has a genetic component and whether male success can be artificially selected. Published genetic studies on male mating success have not involved lek formation but have instead concentrated on courtship (Boller, Calkins & Chambers, 1984; Harris *et al.*, 1986; Harris, Wood & Bailey, 1988). The results of the present study suggest that male success in competition for the preferred positions within a lek needs to be considered when attempting to generate sexually competitive sterile male medflies.

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