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*Brief Communication*

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## **Do the Eyestalks of Female Diopsid Flies Have a Function in Intrasexual Aggressive Encounters?**

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### **INTRODUCTION**

It is widely recognized that male animals frequently exhibit extravagant traits such as long tails, bright coloration, or aural displays. Explaining the presence of these traits in terms of their selective benefit has been an important focus of behavioral ecology over the last few decades, and we now have a broad and successful body of theory and observation dealing with these features of male animals (Andersson, 1994). The possession of similar extravagant traits by females is by no means uncommon, but until recently these have generally been interpreted simply as the consequence of genetic correlations with the sexually selected traits of males (Lande, 1980; Amundsen, 2000) except in those cases where there is clear sex-role reversal (Andersson, 1994, 2000). Recent work has demonstrated that, in addition to these cases, there may be selective advantages for females with extravagant traits in systems without sex-role reversal. In the two-spotted goby, for example, *Gobiusculus flavescens*, males perform more courtship displays when presented with females with more brightly colored bellies, indicating that female ornamentation in this species is associated with male mate choice (Amundsen and Forsgren, 2001; see also Amundsen [2000] for examples from birds and Bonduriansky [2001] for a review of male choice in insects). Ornaments of

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female animals may also play a role in intrasexual or intraspecific competition between females for resources or for access to mates. Immature females of the hummingbird *Heliangelus amethysticollis*, for example, possess a brightly colored gorget, which is lost when the bird molts to adulthood. This gorget is believed to act as a badge of competitive status in competition between juveniles of this species, which aggressively defend nectar-rich feeding territories (Bleiweiss, 1992; other examples from birds reviewed in Amundsen [2000]).

In the insects there has been an increasing amount of interest in male choice for females (Bonduriansky, 2001) but there has been little attention paid to the role of female ornaments in intrasexual contests. One family where such ornaments are found is the "stalk-eyed flies" of the family Diopsidae. These are characterized by the possession of long protuberances on the sides of their heads, with the eyes and the antennae located at the ends of these eyestalks. Most species are sexually dimorphic for eyestalk length, with males having relatively longer eyestalks than females (Wilkinson and Dodson, 1997). There has been considerable interest in the role of these eyestalks in the mating behavior of these animals, and in particular, there is evidence from the species *Cyrtodiopsis dalmanni* that male eyespan (the length of the eyestalks) is important in both female choice and intrasexual competition, such that males with longer eyestalks enjoy enhanced mating success (Burkhardt and de la Motte, 1988; Wilkinson *et al.*, 1988; Hingle *et al.*, 2001) and relative eyespan is the best predictor of victory in aggressive interactions with other males (Panhuis and Wilkinson, 1999). Although, unlike most other Diptera with enlarged heads or eyestalks (Wilkinson and Dodson, 1997), female diopsids also carry the trait, there has been very little attention paid to the eyestalks carried by the females. Burkhardt and de la Motte (1983) noted that female *C. dalmanni* appear to use their eyestalks in aggressive encounters with conspecifics, and Knell *et al.* (1999) suggested that females of the species *Diasemopsis aethiopica* might also use their eyestalks in intrasexual encounters during competition for resources such as oviposition sites or food. Here, we report the results of a laboratory experiment to investigate whether female *C. dalmanni* benefit from the possession of longer eyestalks during encounters with other females of the same species.

## METHODS

All flies used were taken from a large laboratory colony of *C. dalmanni*, originally collected in Malaysia some years ago. Adult flies were housed in 30 × 20 × 25-cm boxes with a netting sleeve attached, at a constant temperature of 25°C and a 14:10-h LD cycle. Homogenized maize was provided for

food for adults and as an oviposition medium, with 25 ml of a 10% (w/v) solution of methyl paraben in 100% ethanol added per liter to inhibit fungal growth. Food was replaced twice a week and then kept moist to allow larvae to develop and pupate. Once the adults emerged they were kept for 4 weeks to allow them to become sexually mature before being used in experiments.

The experimental design for assessing the outcome of contests between female flies was similar to that used by Panhuis and Wilkinson (1999) to assess the importance of eyespan in contests between male flies of the species *Cyrtodiopsis dalmanni*, *C. whitei*, and *C. quinqueguttata*. Individual female flies were taken from the mass culture, marked with a small spot of coloured paint on the thorax, and kept individually in Petri dishes for 24 h with no food but a small ball of wet tissue paper to maintain humidity. After this two females were introduced to a further petri dish containing a small dab of homogenized maize in the center, and the timing, nature, and outcome of any aggressive interactions between the two female flies over the next 20 min were recorded. Aggressive encounters were divided into “assessments” and “fights.” In encounters flies confront each other head-on in a manner very reminiscent of similar encounters between males of the same species (Fig. 1), usually resulting in one (the loser) turning away and retreating (an assessment). Occasionally these aggressive encounters resulted in the flies grappling with their forelegs (a fight), in which case the loser would generally end up disengaging and fleeing, often being chased by the victor. Overall, the fly that won the most encounters was designated as the winner, and if both flies won the same number of encounters, a draw was recorded.

Immediately after each contest was staged, both flies were anaesthetized in a refrigerator and photographed under a dissection microscope with a Nikon Coolpix 950 digital camera. Body length (tip of head to tip of abdomen) and eyespan (distal end of left eye to distal end of right eye) were measured using NIH image version v.1.62 image analysis software.

## RESULTS

Overall, 28 contests were staged, of which 4 resulted in draws and the remaining 24 provided a clear winner. The number of aggressive encounters between flies over the 20-min observation period ranged from 1 to 18, with a mean of 10.18 (SE = 0.884). Of these aggressive encounters, between 0 and 8 ended in fights, with a mean number of fights of 2.89 (SE = 0.379). The mean proportion of encounters that ended in fights was 0.276 (SE = 0.034). We found no statistically significant correlation between either the difference in eyespan or the difference in body length between the two female flies in each contest and the number of encounters, the number of fights,

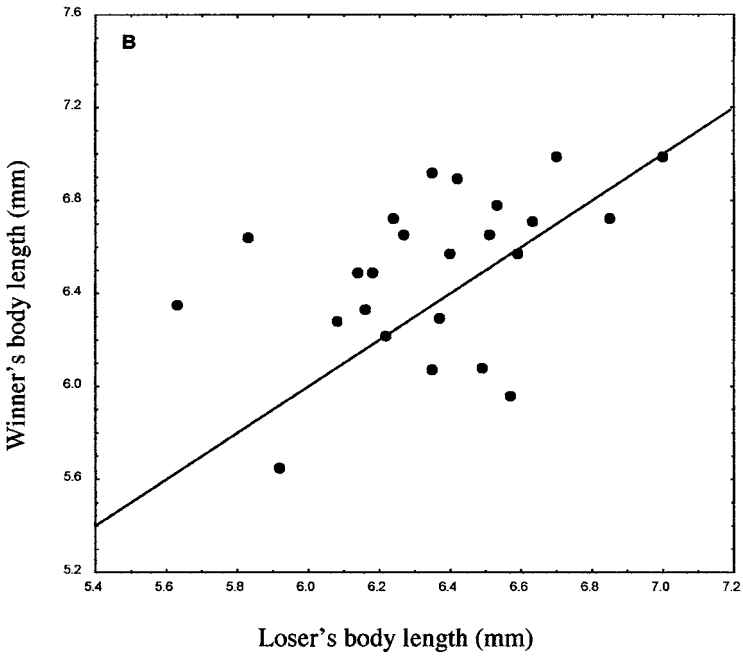
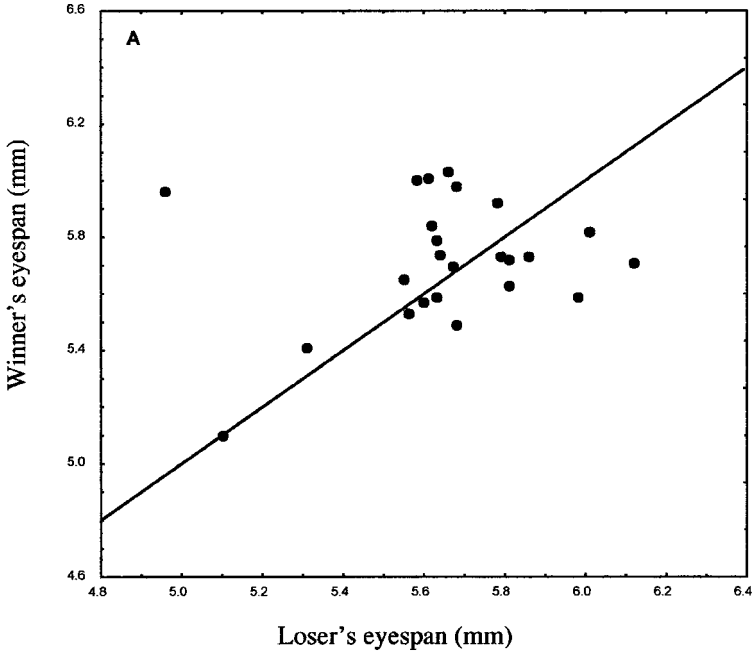


**Fig. 1.** Photograph of two female *C. dalmanni* encountering each other.

or the proportion of encounters that became fights (difference in eyespan and number of encounters,  $r = -0.233$ ,  $t = 1.22$ ,  $P = 0.232$ , and number of fights,  $r = -0.170$ ,  $t = 0.880$ ,  $P = 0.387$ , and proportion of encounters that escalated to fights,  $r = -0.007$ ,  $t = 0.034$ ,  $P = 0.972$ ; difference in body length and number of encounters,  $r = 0.060$ ,  $t = 0.308$ ,  $P = 0.760$ , and number of

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**Fig. 2.** (A) Eyespan of winning flies plotted against the eyespan of the losing fly from the same pair. If flies with longer eyestalks won contests more often, we would see more data points above the line of  $y = x$  plotted on the graph than below. Instead, we see what appears to be a random distribution of data points about the line. (B) Body lengths of winning and losing flies plotted against each other. Here we can see more data points above the line of unity than below, indicating that flies with long bodies are more likely to win contests.



fight,  $r = -0.119$ ,  $t = 0.613$ ,  $P = 0.545$ , and proportion of encounters that escalated to fights,  $r = -0.824$ ,  $t = 0.421$ ,  $P = 0.676$ ).

When attributes of winners and losers were compared, no significant difference was found in eyestalk length (Fig. 2a) (paired sample  $t$ -test,  $t = 1.10$ , 23 df,  $P = 0.283$ ), but the body length of winners was found to be significantly larger than that of losers (Fig. 2b) (paired sample  $t$ -test,  $t = 2.11$ , 23 df,  $P = 0.046$ ).

## DISCUSSION

We find that eyespan is not important in contest resolution in female *Cyrtodiopsis dalmanni* and that body size is the only important variable that we measured in determining the outcome of intrasexual encounters and fights in this species. This is a surprising result, given the apparent similarity between the aggressive encounters between males and females in this species. Obviously our laboratory contests were in an entirely artificial situation, but given that Panhuis and Wilkinson (1999) obtained very clear results when using males of the same species, we can at least be confident that there are important differences between males and females in eyestalk function, with the eyestalks of females playing a much reduced role in conflict resolution. This leaves us with the interesting question of whether the eyestalks of female *C. dalmanni* do indeed convey some form of selective advantage or if they are indeed simply there as the result of a genetic correlation with the heavily ornamented males.

That there is an element of genetic correlation between male and female eyestalk length is not in doubt. Wilkinson (1993) demonstrated a correlated response of female eyespan to artificial selection for male eyespan in *C. dalmanni*, and Baker and Wilkinson (2001) found a strong correlation between male and female eyespan in a comparative study of 33 species from the Diopsidae. Nonetheless, there was considerable variation in the male:female eyespan ratio and in the differences between male and female allometric slopes for eyespan, suggesting that the linkage between male and female eyespan is not particularly strict. Given that there are definite costs of possessing eyestalks (e.g., Swallow *et al.*, 2000), we might expect selection for female eyespans which are as short as possible, yet this does not appear to have taken place.

Two studies have suggested that one role for female eyespan could be in enabling females to assess male eyespan. First, Wilkinson and Reillo (1994) investigated female choice in *C. dalmanni* that had previously been subjected to artificial selection for either longer or shorter male eyestalks. Females from the line selected for longer male eyestalks showed a greater

preference for males with longer eystalks and, also, themselves showed an increase in eyespan as the result of selection on the males. These data can be interpreted as demonstrating a genetic correlation between male eyespan and female preference, or alternatively the change in female preference could be due to longer eystalks allowing females to assess male eyespan more accurately. Further to this, Hingle *et al.* (2001) showed that female *C. dalmanni* mate preference is related to eyespan when flies with long or short eystalks are produced experimentally varying rearing density. Neither of these two studies conclusively demonstrates that it is female eyespan that determines the extent of female preference, but they are certainly suggestive. If this were the case, then a selective advantage of female eystalks could arise from the ability to assess male eyespan and, therefore, quality. Nonetheless, we must also note that there are many species of Diptera where the male possesses eystalks, and in at least some cases there are known female preferences for eyespan (Wilkinson and Dodson, 1977; Boake *et al.*, 1997), but the Diopsidae is the only taxon where both males and females have eystalks, indicating that female eystalks are not essential for assessment of males with eystalks.

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