# Evolution of female mating preferences in stalk-eyed flies

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Sensory exploitation predicts that female mate preferences exist before the evolution of exaggerated male ornaments. We tested this prediction by estimating female preference functions, remating intervals, and copulation durations for three species of stalk-eyed flies. Two species, *Cyrtodiopsis whitei* and *C. dalmanni*, exhibit extreme sexual dimorphism in eye span, with eye stalks exceeding body length in large males. In contrast, *C. quinqueguttata* of both sexes possess short eye stalks. Maximum parsimony analysis of 437 basepairs of the 16S mitochondrial ribosomal RNA gene from 6 Malaysian diopsids reveals that short, sexually monomorphic eye stalks are plesiomorphic in *Cyrtodiopsis*. Observations of multiple copulations by females in paired-choice mating chambers indicated that female *C. whitei* and *C. dalmanni* exhibit relative preferences for longer eye stalks such that preference intensity increases linearly with the difference in eye stalk length between males. Females from the sexually monomorphic species showed no detectable preference for male eye stalk length. Female mating preferences of both sexually dimorphic species exhibited significant repeatability, as expected if genetic variation underlies the preference. In addition, female *C. whitei* and *C. dalmanni* exhibited shorter copulations, mated more frequently, and rejected fewer mating attempts than female *C. quinqueguttata*. Thus, opportunities for sperm competition have increased with acquisition of female preferences. We conclude that female sensory bias for males with long eye span did not exist in a common ancestor to these species. Instead, female preference and remating propensity either coevolved with eye span dimorphism or evolved after male eye stalks elongated. *Key words:* Diopsidae, mate choice, sensory bias, sexual selection. *[Behav Ecol 9:525–533 (1998)]*

Female mating preferences for ornamented males have been documented by field and laboratory experiments in many species of insects, fish, frogs, and birds (see Andersson, 1994, for a recent review). These studies support Darwin's (1871) proposition that female mating behavior has been important in the evolution of male ornaments and courtship behaviors. However, whether female preferences evolve in concert with male display traits by a Fisherian (Fisher, 1915, 1930; Kirkpatrick, 1982; Lande, 1981) or good genes (Hamilton, 1982; Iwasa et al., 1991; Pomiankowski, 1987; Zahavi, 1975) process has been questioned. Male display traits could instead have evolved and currently be maintained to exploit preexisting female sensory biases (Basolo, 1990, 1996; Burley, 1985; Endler, 1992; Endler and McLellan, 1988; Ryan, 1990; Ryan and Keddy-Hector, 1992). Evidence in support of sensory exploitation and preexisting biases has been obtained from studies on female choice for chuck calls in *Physalaemus* frogs (Ryan and Rand, 1993; Ryan et al., 1990), for swordtails in some *Xiphophorus* fish (Basolo, 1990, 1995), for large body size in a gynogenetic *Poecilia* fish (Marler and Ryan, 1997), and for preylike water vibrations produced by male water mites (Proctor, 1991, 1993).

Two methods have been proposed for examining the sensory exploitation hypothesis. The comparative approach involves reconstructing the origin of male display traits and female preferences on a dichotomously branching phylogenetic tree (Basolo, 1996; Ryan and Rand, 1993; Shaw, 1995). Presence of a preexisting bias is concluded if ancestral species can be inferred to have acquired preferences prior to ornaments. This method requires a well-supported phylogenetic hypothesis as well as evidence of preferences for derived characters.

As with any comparative analysis, conclusions depend on the accuracy of the phylogeny. For example, preexisting preferences for swordtails were inferred initially from a phylogeny based on morphological characters (Basolo, 1990). This inference was rejected by a phylogeny based on mtDNA sequences (Meyer et al., 1994) but has recently been resurrected by evidence of a preference for swordtails in a swordless outgroup (Basolo, 1995). Whether female preferences predate swordtail evolution remains controversial because developmentally labile tail protrusions may be characteristic of all poeciliid fish (Meyer, 1997; Meyer et al., 1994).

In addition to affecting the acquisition of male traits, sensory exploitation could also influence the maintenance and evolution of sexually selected traits. For example, a female sensory bias could favor unlimited exaggeration of a male trait. Such bias need not lead to coevolution between male trait and female preference if all females possess similarly biased preferences (Sherman and Wolfenbarger, 1995). Although a sensory bias does not preclude variable preferences (Basolo and Endler, 1995), net directional selection on a male trait is likely to be diminished if females differ in their preferences. In contrast, genetic variation for female preferences will increase the potential for coevolution between a male trait and preference. Estimation of preference variability can also be used, therefore, to evaluate the importance of sensory exploitation relative to coevolutionary processes in maintaining male display traits.

Applying either of these methods requires quantifying and statistically comparing female preference behavior within and between species. A variety of approaches have been used to measure female preferences. The two most common experimental paradigms involve either sequential or paired presentations of males (or their traits) to females. In sequential tests, preference is measured as either the frequency with which females exhibit a mating preference when offered a particular male (Gilburn and Day, 1994) or the latency to mate (Con-

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nolly et al., 1974). Although this design can determine acceptability of a mate, it may or may not indicate a female's preference when offered a choice of possible mates, and it ignores the possibility that females exhibit postcopulatory preferences by altering mating latency to influence sperm competition (Eberhard, 1996). Paired-choice designs more directly demonstrate a preference, but they do so only for the particular male exemplars displayed. Even though male ornaments are often characterized as discrete traits (e.g., presence or absence of a sword), high levels of phenotypic and genetic variation are typical of male secondary sexual characters (Meyer, 1997; Pomiankowski and Møller, 1995). Consequently, in the wild, females of many species must choose between a continuous range of possible male phenotypes. The need to measure female preference functions, rather than to simply test female responses to fixed differences among males, has begun to be recognized, and several recent studies have quantified female preferences over a range of male phenotypes (Basolo, 1995; Gerhardt, 1995; Gerhardt et al., 1996; Ritchie, 1996; Wagner et al., 1995). Such estimates are essential for assessing the magnitude of selection caused by female preferences and their potential effect on male trait evolution.

The approach we adopted in this study has four steps. First, we used mitochondrial DNA sequence data to construct a phylogenetic hypothesis for closely related species with and without an exaggerated male ornament. Second, we estimated preference functions by observing repeated copulations of individual females for each species using paired male presentations that span a biologically relevant range of male phenotypes. We also compared copulation durations and remating intervals between species because these behaviors may influence sperm competition and reflect female postcopulatory preferences (Eberhard, 1996). Third, we estimated repeatability (Boake, 1989) of female preferences to assess preference variability and hence the opportunity for coevolution between preference and trait. And fourth, we used our phylogenetic hypothesis to test if female preferences and remating behaviors evolved before male ornament exaggeration.

The study system includes several sympatric species of diopsid stalk-eyed flies in the genus Cyrtodiopsis. In all diopsids, eye stalk expression increases linearly with body size (Burkhardt and de la Motte, 1985; Wilkinson and Dodson, 1997). In the two sexually dimorphic species, C. whitei and C. dalmanni, the slope of the regression of eye stalk on body length is much greater in males than in females, whereas it is indistinguishable between the sexes in the monomorphic species, C. quinqueguttata (Wilkinson and Dodson, 1997). Large male C. whitei and C. dalmanni possess eye stalks 1.5 times their body length, whereas both sexes of C. quinqueguttata have eye stalks less than half their body length. Previous work (Burkhardt and de la Motte, 1988; Wilkinson and Reillo, 1994) has indicated that female C. whitei and C. dalmanni prefer to roost with males possessing long eye stalks. Female mate choice has been inferred because mating in C. whitei (Lorch et al., 1993) and C. dalmanni (Wilkinson and Reillo, 1994), but not C. quinqueguttata (Kotrba, 1996), occurs predominantly in nocturnal aggregations on exposed rootlets. If eye stalk monomorphism is plesiomorphic in Cyrtodiopsis, then the sensory exploitation hypothesis predicts that all three species should possess female preferences for long eye stalks.

To determine the phylogenetic relationships among these three species, we included three additional sympatric diopsids: Teleiopsis quadriguttata, Eurydiopsis subnotata, and Sphyracephala detrahens. These genera have traditionally been considered outgroups of Cyrtodiopsis on the basis of morphological characters (Feijen, 1989; Steyskal, 1972). In all three of these species, both sexes possess short eye stalks (Burkhardt and de la Motte, 1985). Comparison of the allometric rela-

tionships between eye span and body length between the sexes reveals that slight sexual dimorphism exists in S. detrahens (Wilkinson GS, unpublished data), but both T. quadriguttata and E. subnotata are sexually monomorphic with regression slopes indistinguishable from C. quinqueguttata (Wilkinson and Dodson, 1997).

## METHODS

### Collections

We found flies by searching leaf litter and vegetation along streams (or rocks in streams for S. detrahens) in forested ravines 20–30 km north of Kuala Lumpur, Malaysia, in January 1989 and 1996. Individuals were netted by hand, placed in moist containers with instant Drosophila media, and transported to Maryland, USA. In the laboratory, we maintained all six species on pureed corn media (Lorch et al., 1993) at 25°C with a 12 h light:dark cycle in large, plexiglass population cages lined with moist blotting paper and cotton. Flies to be used for sequencing were placed in 95% ethanol and shipped overnight to the American Museum of Natural History (AMNH), where they were kept frozen at −70°C until used for DNA extractions. Voucher specimens for all species are stored at the AMNH.

### DNA manipulation and analysis

We extracted DNA from single flies using a small-scale preparation (DeSalle et al., 1993). A region of the 16S mitochondrial ribosomal RNA gene was amplified from single flies of each species using polymerase chain reaction (PCR) primers (5'-AATTTATTGCAC TAATCTGCC-3' [12747] and 5'-GCTGGAATGAATGGTTGGACG-3' [13269]). PCR conditions consisted of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, for a total of 35 cycles. Amplified DNA was sequenced directly either by manual dideoxy sequencing using $^{35}$S or on an ABI 373 automated sequencer using the flourescent dideoxy terminator mix. In addition to the PCR primers, a set of internal primers (5'-TAATCCAACATCGAG GTCGC-3' and 5'-TATAATTTTGGGTGTAGCCG-3') was used for sequencing. Automated sequencing outputs were transferred to Sequencher (Gene Codes Corp.) and verified by visual inspection of the chromatographs produced from each run. Amplified products ranged in size from 486 to 492 basepairs.

We aligned sequences with MALIGN (Wheeler and Gladstein, 1994) using several gap:change costs (2:1, 4:1, 6:1, 8:1, 10:1) in order to identify regions of stable sequence alignment (Gatesy et al., 1993). The MALIGN options used included build, alignmultiswap 4, score 4, treemultiswap 3, and extragaps 1. Deletion of alignment-ambiguous regions was performed using the CULL option in MALIGN and resulted in a final data matrix of 437 basepairs. We used the exhaustive search option in PAUP 3.1 (Swofford, 1993) with all characters equally weighted to find the maximum parsimony tree. Clade stability was estimated using two different methods: bootstrap analysis (Felsenstein, 1985) and Bremer support analysis (Bremer, 1994). Bootstrap values were generated in PAUP from 200 replicates of simple-addition heuristic searches. For Bremer support indices, we used PAUP to search for trees 1–10 steps longer than the most parsimonious tree.

### Measuring mate preferences

To obtain animals for mate-choice experiments, we reared larvae on pureed corn media (Lorch et al., 1993) and isolated eclosing flies by sex and age. After males reached at least 1 month of age, (i.e., were sexually mature; de la Motte and Burkhardt, 1983), we measured eye span and body length to the nearest 0.01 mm using digitized microscope images of $CO_2$ anesthetized flies. We then sorted males of the same age

**Table 1**

Mean (SD) measurements (mm) for males used in the mate choice experiments

| Species | N | All males | | Difference between pairs | |
|---|---|---|---|---|---|
| | | Eye span | Body length | Eye span | Body length |
| C. quinque-guttata | 62 | 4.52 (0.18) | 7.29 (0.26) | 0.24 (0.13) | 0.14 (0.26) |
| C. dalmanni | 40 | 8.86 (0.74) | 7.27 (0.42) | 1.01 (0.87) | 0.54 (0.45) |
| C. whitei | 56 | 9.89 (0.93) | 7.27 (0.48) | 1.31 (0.75) | 0.61 (0.47) |

by size and paired them to maximize differences in eye span while minimizing differences in body length (Table 1). We combined each male pair with three or four reproductively mature female C. quinqueguttata or five female C. dalmanni or C. whitei in 14 × 24 cm polycarbonate cages. We marked all flies in each cage with a unique color on the thorax using a paint pen and allowed flies 3 days to acclimate to the mating chambers before beginning observations.

To measure female choice in the absence of male competition in the two sexually dimorphic species, we separated males within a cage using a transparent acetate partition punched with 7-mm diameter holes (Figure 1). This perforated partition allowed females, but not males, to move unimpeded between sides of the cage (Wilkinson and Reillo, 1994). Food was always available on both sides of the partition. Occasionally, small males succeeded in moving across the barrier. Whenever this occurred, we aspirated and returned the intruding male to his side and resumed observations. To balance any potential side bias, we switched males between sides half-way through observations on each sexually dimorphic species. Because males and females do not differ in eye span in the sexually monomorphic species, we could not use partitions to separate male C. quinqueguttata. Attempts to tether males using Manduca sexta cocoon silk failed because tethered males became entangled or were unable to move normally. Consequently, for C. quinqueguttata we observed matings in cages without partitions. Although any nonrandom mating observed among female C. quinqueguttata might be due either to male-male interactions or female preferences, females can dislodge males (Wilkinson GS, personal observation), and size does not influence access to food among male C. quinqueguttata (Panhuis T, Wilkinson GS, unpublished data).

The majority of copulations in C. dalmanni and C. whitei occur in nocturnal aggregations on root threads during the half hour preceding dusk or following dawn (Lorch et al., 1993; Wilkinson and Reillo, 1994). In contrast, the sexually monomorphic species, C. quinqueguttata, mates throughout the day (Kotrba, 1996). Therefore, to compare female mate choice among all three species, we observed copulations directly and scored a female's preference as the proportion of copulations with the male exhibiting larger eye span. In addition, we recorded all instances of males attempting to mount but failing to establish genital contact before being dislodged by females.

For each species, we observed between 5 and 20 cages every day for 2 weeks. We systematically conducted observations during the first 3 h after dawn (lights on) or the last 3 h before dusk (lights off) and opportunistically during other hours. In total, we observed 159 copulations in 26 cages containing 74 female C. quinqueguttata during 1289 cage-h, 683 copulations in 20 cages containing 72 female C. dalmanni over 490 cage-h, and 791 copulations in 30 cages containing 102 female C. whitei during 393 cage-h.
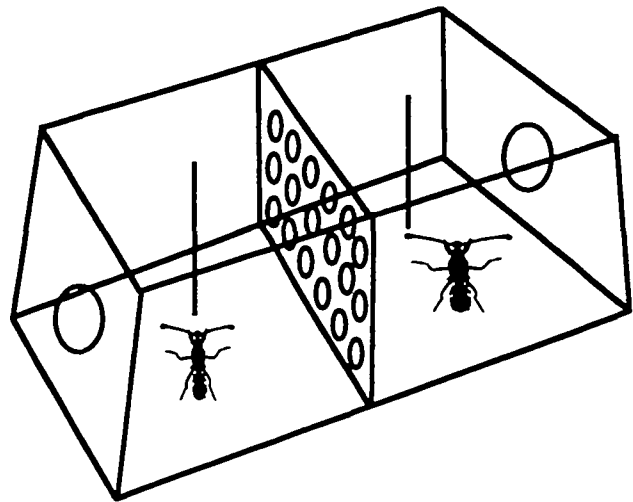


**Figure 1**
Schematic diagram of mate-choice chamber used for the two sexually dimorphic species, C. dalmanni and C. whitei. Chambers without perforated partitions were used for the sexually monomorphic species, C. quinqueguttata.

*Statistical analyses of preferences*

A cage represents the unit of replication because all females in a cage were exposed to the same pair of males. Therefore, to avoid pseudoreplication (Hurlbert, 1984; Lombardi and Hurlbert, 1996), we calculated mean female preferences for a cage as the mean proportion of copulations with the male with the larger eye span. Then, to quantify preference functions for each species, we plotted mean female preferences against the difference between males in eye span. We express eye span difference as the percentage of the larger male eye span in order to compare all three species on a standardized scale (Wilkinson and Dodson, 1997). We estimated nonparametric preference functions using cubic splines (Schluter, 1988; Schluter and Nychka, 1994). Cubic spline estimation fits nonlinear curves by joining a collection of cubic polynomial pieces end to end. We chose this technique to allow the data to determine the form of the preference function (cf. Ritchie, 1996). To fit a preference function we used the minimum generalized cross-validation score to estimate the smoothing parameter, λ, for each species. We then estimated 95% confidence limits for each predicted female preference function from 100 bootstrapped replicates of the data, assuming λ remained constant (Schluter, 1988).

We estimated mate preference repeatability (Boake, 1989) within a species by restricting analysis to those females that copulated at least 10 times. We chose 10 as the minimum sample to divide because five represents the smallest sample that can detect significant departure from unbiased binomial sampling. We then divided the mating sequence into two equal replicates to obtain two estimates for a female's preference during the 2-week observation period.

Unless otherwise noted, parameter estimates are presented as means ± SE. The standard error for repeatability was estimated according to Becker (1975).

**RESULTS**

*Phylogeny*
Maximum parsimony analysis of the 16S ribosomal mitochondrial sequences produced a single tree with 123 steps, a consistency index (excluding uninformative characters) of 0.71, and a retention index of 0.57 (Figure 2). A clade consisting
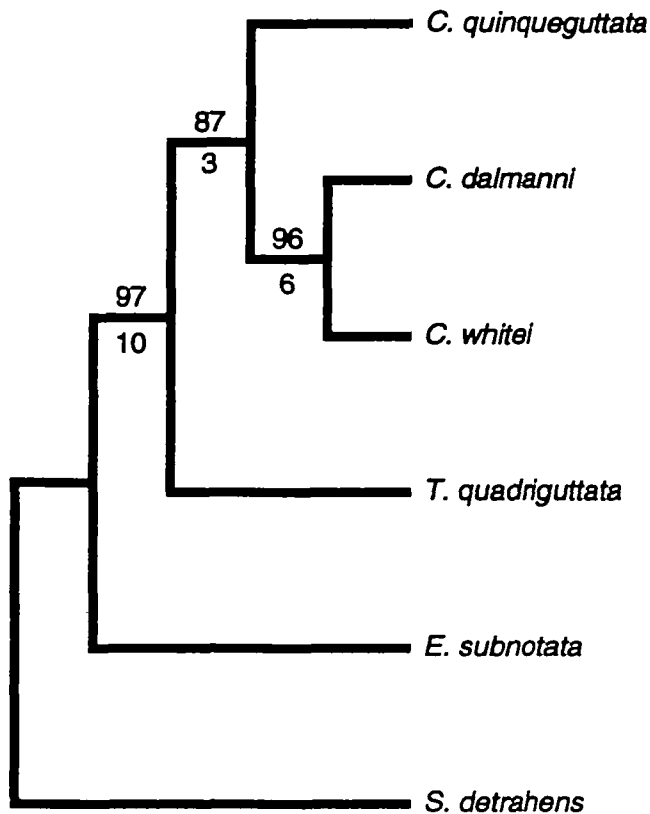
**Figure 2**
Maximum parsimony cladogram for 16S ribosomal mitochondrial DNA sequences constructed with PAUP. For each node bootstrap values are indicated above the line and Bremer support indices below the line.



**Figure 3**
Mean female remating intervals (a) and copulation durations (b) for each of the three species. Error bars indicate 1 SE.

of the two sexually dimorphic *Cyrtodiopsis* species is strongly supported with high bootstrap and Bremer support values. Boostrap and Bremer support indices also support monophyly of *Cyrtodiopsis*. Thus, this tree indicates that short, sexually monomorphic eye stalks are plesiomorphic in *Cyrtodiopsis*.

### Copulation intervals

Females of all three species routinely mated multiple times and often crossed the partition, when it was present, to mate with both males in a cage. However, males and females of the two sexually dimorphic species copulated more frequently than males and females of the sexually monomorphic species. On average, we observed a mating by each female *C. quinqueguttata* every 19.3 h, by each female *C. dalmanni* every 2.6 h, and by each female *C. whitei* every 1.7 h. As expected given the female-biased sex ratios in the mating chambers, average copulation intervals for males were shorter: each *C. quinqueguttata* male mated every 16.2 h of observation time, each *C. dalmanni* male every 1.4 h, and each *C. whitei* male every 1.0 h.

We tested for differences between species in remating behavior by comparing the interval between matings for marked females observed on the same day. Because a few individuals of each species had relatively long intervals between matings, we first transformed intervals by natural logarithms to normalize the data. Then, to control for potential differences among cages, we nested cage within species in an analysis of variance (ANOVA) and used the mean square associated with the cage effect as the error term to test for a difference between species. This analysis revealed significant differences among species ($F_{2,48}$ = 10.3, $p$ = .0002), as well as among cages ($F_{48,893}$ = 5.5, $p$ < .0001) and explained 43% of the
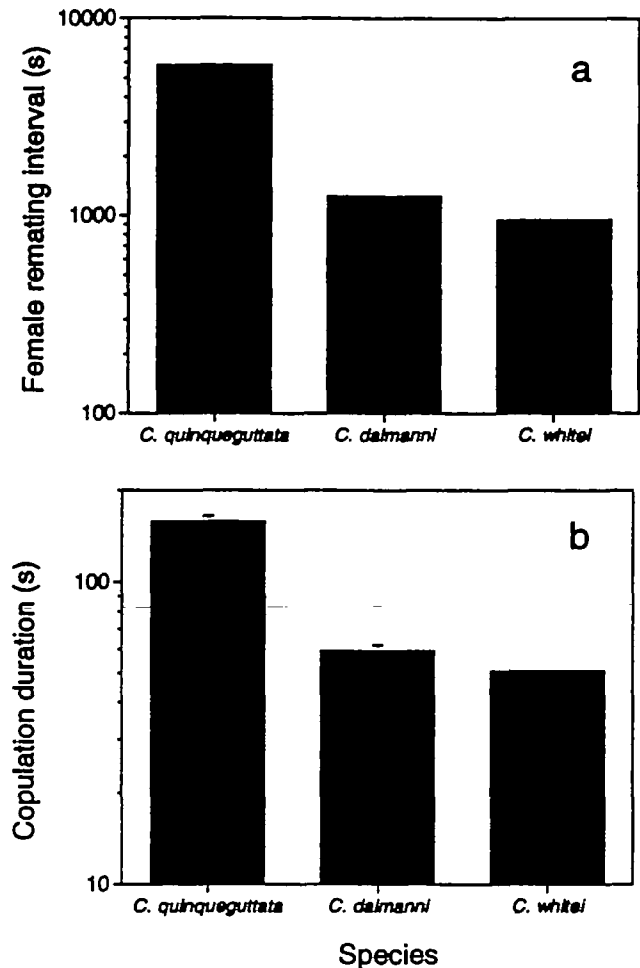
variation in remating interval. *C. quinqueguttata* females exhibited the longest remating times (Figure 3a), which were significantly different from females of either sexually dimorphic species according to Fisher's protected least squares difference (PLSD) post-hoc tests ($p$ < .0001). A Fisher's PLSD test also revealed that remating intervals differed between *C. dalmanni* and *C. whitei* ($p$ = .006). The most sexually dimorphic species, *C. whitei*, remated faster than *C. dalmanni* (Figure 3a).

These three species also differed in the frequency with which females permitted intromission when mounted by a male ($\chi^2$ = 37.5, $p$ < .0001). Female *C. quinqueguttata* rejected 17.2% of male copulation attempts, while female *C. whitei* rejected 8.1% and female *C. dalmanni* rejected 4.2%. Females of the sexually monomorphic species rejected copulations more often than females of the two sexually dimorphic species combined ($\chi^2$ = 28.8, $p$ < .0001). The frequency of female rejections did not depend on whether the male was relatively large or small for any of the three species ($\chi^2$ contingency tests, $p$ > .05).

### Copulation durations

Copulation duration differed among species (ANOVA: $F_2$, 1044 = 216.4, $p$ < .0001). Fisher's PLSD tests indicated significant differences exist among all three species ($p$ < .01 for each paired comparison); however, *C. quinqueguttata* copulations lasted, on average, over twice as long as copulations of either

sexually dimorphic species (Figure 3b), and differed highly from them ($p$ < .0001 for both paired comparisons). Furthermore, both of the sexually dimorphic species exhibited short copulations lasting 30 s or less. In a previous study on *C. whitei*, Lorch et al. (1993) found that copulations lasting less than 40 s failed to transfer sperm. The two dimorphic species differed in the frequency of short copulations ($\chi^2$ = 13.2, $p$ = .0003), with 12.1% of *C. dalmanni* and 6.9% of *C. whitei* copulations classified as short. In contrast, the shortest *C. quinqueguttata* copulation we recorded lasted 50 s.

The size of the copulating male influenced the frequency of short copulations in one species. *C. whitei* females displayed more short copulations (8.9% of 348 copulations) when mating with smaller males than when mating with larger males (3.9% of 335 copulations; $\chi^2$ = 7.2, $p$ = .008). The frequency of short copulations did not differ among female *C. dalmanni* mating with smaller males (13.8% of 275 copulations) than mating with larger males (10.9% of 402 copulations; $\chi^2$ = 1.3, $p$ = .26). Because we could not compare frequencies of short and long copulations in *C. quinqueguttata*, for this species we compared copulation duration estimates directly between large and small males and found that copulation duration with the larger male (151.9 ± 7.8 s, $n$ = 71) did not differ from those with the smaller male (153.6 ± 10.8 s, $n$ = 86).

### Female preference functions

Bootstrap estimates of the 95% confidence limits for female preference functions did not deviate from linearity for any species (Figure 4). Consequently, we used analysis of covariance (ANCOVA) to compare the slopes of linear preference functions across species. The slopes of the female preference functions for the two sexually dimorphic species, *C. dalmanni* and *C. whitei*, do not differ ($F_{1,43}$ = 0.11, $p$ = .74). Combining the data for these two species yields a significant linear regression, $y$ = (0.465 ± 0.053) + (0.010 ± 0.003)$x$, of mean female preference on percent difference in male eye span ($F_{1,45}$ = 7.54, $r^2$ = .14; $p$ = .009). The slope of the combined preference function for the two dimorphic species differs from the monomorphic species (ANCOVA: $F_{1,74}$ = 4.93, $p$ = .03). Although the slope of the preference function for the monomorphic species is negative, it does not differ from zero ($F_{1,29}$ = 1.80, $p$ = .19). Thus, females from the two dimorphic species, but not the monomorphic species, prefer to mate with males with long eye spans.

To compare the potential consequences of these mate preferences for the evolution of exaggerated eye span, we calculated selection intensities (Falconer, 1981)—i.e., the standardized difference between mated individuals weighted by their copulation frequencies and the mean of the males used in each experiment. Because eye span correlates highly with body length, we calculated selection intensity for the ratio of eye span to body length to obtain a relative measure for how female mate choice could influence selection on relative eye span. Bidirectional artificial selection on this ratio results in rapid evolution of eye span with little change in body size (Wilkinson, 1993; Wilkinson et al., 1998). Consequently, positive selection on eye span/body length changes the allometric relationship between eye span and body length in the same direction as characterizes the difference between sexually monomorphic and dimorphic species. The potential selection intensity that would have occurred if females had consistently chosen the male with the longest eye stalks did not differ between any species (Figure 5). Thus, even though eye span differed among species, the potential for selection in our experimental choice cages was comparable. Nevertheless, only the two dimorphic species exhibited positive selection intensities for eye span/body length. The selection intensity for the monomorphic species was negative (Figure 5).
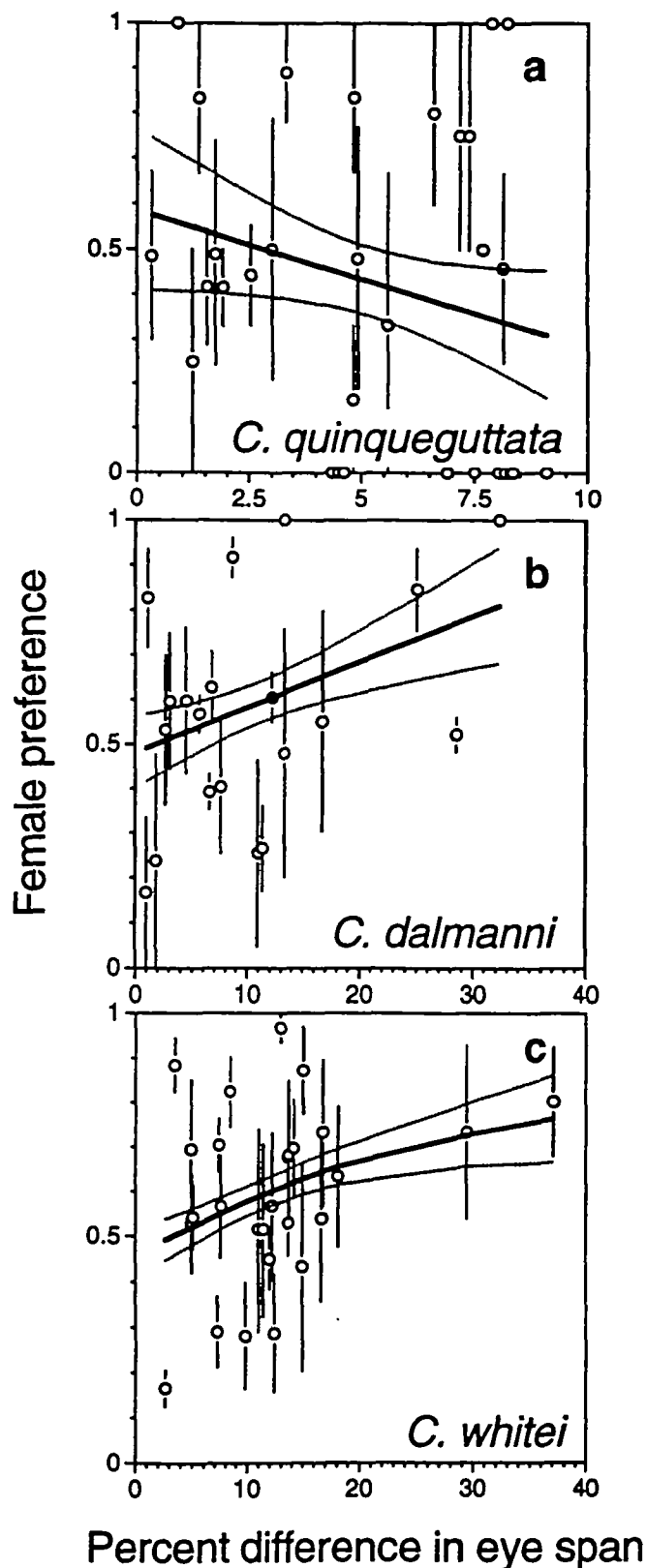


**C. quinqueguttata**

**C. dalmanni**

**C. whitei**

Female preference

## Percent difference in eye span

**Figure 4**
Mean (±SE) female preferences (i.e., the proportion of copulations with the larger male in a cage), plotted against the percent difference in eye span between each male pair for the sexually monomorphic species, *C. quinqueguttata* (a), and the two sexually dimorphic species, *C. dalmanni* (b), and *C. whitei* (c). Solid lines indicate cubic spline estimates of female preference functions. Dashed lines indicate bootstrap estimates of 95% confidence limits for those functions.
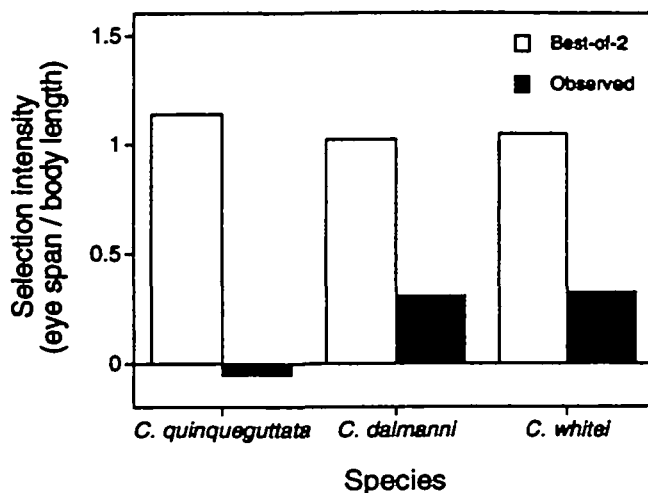
**Figure 5**
Selection intensities for the ratio eye span/body length, calculated for each of the three species. Open bars indicate expected values if females exhibited perfect best of two-mate choice. Filled bars indicate values observed in the experiments.

**Table 2**
Number of females with or without significant mating preferences for each species

| Species | Preference* | Eye span difference (%) | | |
|---|---|---|---|---|
| | | <10 | 10–20 | >30 |
| C. quinqueguttata | > 0.5 | 2 | | |
| | = 0.5 | 69 | | |
| | < 0.5 | 3 | | |
| C. dalmanni | > 0.5 | 5 | 4 | 3 |
| | = 0.5 | 33 | 13 | 9 |
| | < 0.5 | 1 | 4 | 0 |
| C. whitei | > 0.5 | 2 | 10 | 2 |
| | = 0.5 | 40 | 69 | 11 |
| | < 0.5 | 7 | 1 | 0 |

*The proportion of copulations with the larger of two males. Significant preferences for large (>0.5) or small (<0.5) males were determined by $\chi^2$ tests.

*Variation in female mating preferences*

The previous analyses focused on average preferences within and between species. Figure 4 indicates, however, that considerable variation in female preferences exists within species among cages, presumably due, in part, to uncontrolled differences among males. In addition, substantial standard errors on cage estimates reflect variation among females within cages. Some of the between-female variation reflects binomial sampling caused by variation in the number of copulations observed for each female. Variation beyond that expected by sampling reflects individual differences among females. Table 2 shows for each species the number of females exhibiting preferences, which did or did not differ significantly from 0.5 using a binomial test, according to the percent difference in eye span between the males. Although the two sexually dimorphic species exhibited more females with significant preferences for males with longer eye stalks, several females from both species preferred males with shorter eye stalks. In a few cases, female C. dalmanni and C. whitei in the same-cage exhibited significant, but opposite, preferences.

We tested if the variation among females in their mate preferences was significant by using females that mated 10 times or more and then dividing their copulation sequence in half to obtain two replicate estimates of preference for each female. This restriction yielded 23 C. whitei females with, on average, 14.6 copulations each and 28 C. dalmanni females with 15.4 copulations each. Both of these species exhibited significant variation among females in mate preference (C. whitei $F_{22,23}$ = 3.88, $p$ = .001; C. dalmanni $F_{27,28}$ = 1.98, $p$ = .039; ANOVA). The proportion of variation explained by the between-female variance component [i.e., the repeatability (±SE)], was 0.59 ± 0.14 for C. whitei and 0.33 ± 0.17 for C. dalmanni. Because we observed only two female C. quinqueguttata mate 10 times or more, we were unable to estimate repeatability for this species.

**DISCUSSION**

The results of this study are not consistent with a preexisting female mating bias for males possessing longer eye stalks. Females of the sexually monomorphic species, C. quinqueguttata, exhibited no preference to mate with males possessing longer eye spans. In contrast, females from both sexually dimor-

phic species preferred to mate with males possessing longer eye spans, and their preferences increased linearly with the difference in eye span between the males. In addition, female C. whitei appeared to exhibit a preference for males with long eye spans even after the initiation of a copulation. We observed more short copulations, which are unlikely to transfer sperm (Lorch et al., 1993), with small than with large male C. whitei. This observation is consistent with large C. whitei males having higher mating success than predicted based on copulation rates (Burkhardt et al., 1994). The concordance between female mating preferences and male display traits, combined with evidence for variation in female preferences, is consistent with coevolution of mating preferences and elongated male eye span. Similar support for coevolution between female preference functions and male display traits has recently been reported for bushcrickets (Ritchie, 1996).

Objections to our conclusion that preexisting preferences for long eye span are absent in C. quinqueguttata could be raised for at least three reasons. First, perhaps male–male competition interfered with female choice. This is a possibility because we did not separate males in C. quinqueguttata trials. However, for male interactions to obscure preferences, smaller males would have to prevent larger males from mating. Although we did sometimes observe aggressive interactions between two C. quinqueguttata flies, they typically involved two females feeding. In a study of fighting for food, body size did not predict fight outcome among male C. quinqueguttata (Panhuis T, Wilkinson GS, unpublished data). Thus, we have no evidence that small males actively excluded large males from mating. Female C. quinqueguttata also exhibited a higher frequency of mate rejection behavior than either of the two sexually dimorphic species. Females of this species can reject mates, but they did not do so on the basis of eye stalk length or body size. Therefore, we doubt that male–male interactions obscured mating preferences in this species.

A second potential concern is that the range of variation in eye span between paired males was less in trials with the sexually monomorphic species than in trials involving either sexually dimorphic species. Nevertheless, we believe our data are inconsistent with a preference for longer eye stalks in C. quinqueguttata for two reasons. First, the minimum difference in male eye span detectable by females of the sexually dimorphic species falls within the range of eye spans tested for the sexually monomorphic species (cf. Figure 4). If we assume that the just noticeable difference occurs where the minimum 95% confidence limit equals 0.5, then female C. whitei and C. dalmanni are able, on average, to detect a difference between

males differing in eye span by 7% (Figure 4). *C. whitei* and *C. dalmanni* have ommatidia divergence angles in their acoustic fovea close to 1° (Burkhardt and de la Motte, 1983). Divergence angles this small are typical of flies with excellent visual acuity (Land, 1981). In contrast, when male *C. quinqueguttata* differed by the same amount, females exhibited a trend toward preferring males possessing shorter, not larger, eye span (Figure 4). Thus, even if we restrict the data to the same eye-span range, a difference in female preferences between the sexually dimorphic and monomorphic species exists. Measurement of ommatidia divergence angles in *C. quinqueguttata* eyes is needed to determine if resolution has changed between these species.

Second, if a preexisting bias in an ancestral stalk-eyed fly species favored male eye span exaggeration, then that bias would have to influence mating among extant males. In other words, in the absence of a mutation causing a qualitative change in male eye stalk length, ancestral females must have chosen males from a continuous range of eye stalk lengths. A preexisting bias should, therefore, be sensitive to extremes of the phenotypic distribution. Mutations that qualitatively increase male eye span seem doubtful given that continuous, gradual bidirectional response to artificial selection on relative eye span has been observed in *C. dalmanni* (Wilkinson, 1993; Wilkinson et al., 1998).

Finally, perhaps a preexisting bias for long eye span existed in the joint ancestor of these three species, but *C. quinqueguttata* females lost this preference. This scenario is less parsimonious than joint or sequential evolution of eye span exaggeration and preference because female *Teleopsis quadriguttata*, a related (cf. Figure 2) sexually monomorphic species, also apparently lack a preference for long eye span males. Measurements of eye span from mated pairs in a population cage revealed that mated males did not differ from the cage mean (Wilkinson and Dodson, 1997). Thus, although we cannot rule out the possibility that female preferences for long eye span occur in other diopsids with sexually dimorphic eye spans (Wilkinson and Dodson, 1997), current evidence indicates that females from sexually monomorphic species lack preferences for long eye stalks.

In addition to exhibiting precopulatory preferences for males with long eye stalks, females from the two sexually dimorphic species also were more promiscuous (i.e., they mated more frequently, had shorter copulations, and rejected male mating attempts less often). Thus, in contrast to stasis, as predicted by the sensory exploitation hypothesis, behaviors that could potentially influence postcopulatory preferences have also changed in concert with eye span exaggeration. The direction of change appears counterintuitive: female *C. dalmanni* and *C. whitei*, which exhibit preferences for long eye span, took less time to assess and choose their mates than female *C. quinqueguttata*, which lack preferences for eye span. One possible explanation is that females that use a conspicuous male ornament for mate assessment can choose their mates more rapidly, as long as variation in ornament size persists in the population. While this explanation might explain differences in mating latencies, it fails to explain why females from the two sexually dimorphic species mated more often than females from the sexually monomorphic species. Thus, other selective factors are needed to explain the evolution of these mating behaviors.

Many alternative, although not necessarily exclusive, hypotheses for multiple mating by females have been proposed (Andersson, 1994; Keller and Reeve, 1995; Walker, 1980; Zeh and Zeh, 1996). Of these, three seem particularly worthy of further study in diopsids. First, females may mate multiple times to increase competition among sperm in response to meiotic drive (Haig and Bergstrom, 1995). Sex-linked meiotic

drive occurs in the two sexually dimorphic *Cyrtodiopsis* species (Presgraves et al., 1997). In support of this hypothesis, remating times decreased from *C. quinqueguttata* to *C. dalmanni* to *C. whitei*. This rank order matches the frequency of driving X chromosomes, which are undetectable in *C. quinqueguttata*, 16% in *C. dalmanni*, and 36% in *C. whitei* (Wilkinson et al., 1998). To further test the Haig and Bergstrom (1995) hypothesis, the number of males, on average, contributing sperm to a female's storage organs should be estimated for each species. Our data are inappropriate for such a comparison because all females were caged with two males. Nevertheless, the possibility that females from the sexually dimorphic species mate with multiple males to increase sperm competition also provides a plausible explanation for why most females mated with both males, even when they differed dramatically in eye stalk length.

A second possibility is that multiple mating by females improves fecundity either by seminal feeding or by fertility insurance (Ridley, 1988). Nutrient transfer via male accessory substances, as has been documented for several species of *Drosophila* (Pitnick et al., 1991), could occur. However, pilot studies in which female *C. whitei* were housed either with males or females failed to reveal an increase in female survival or rate of egg development in mated versus unmated females (Presgraves DC and Wilkinson GS, unpublished data). Further study is needed, therefore, to determine if male accessory substances are beneficial or possibly harmful (e.g. Chapman et al., 1995) to female diopsids. In contrast, fertility insurance seems likely, as male infertility exceeds 20% in the two sexually dimorphic species (Presgraves DC, Wilkinson GS, unpublished data). The causes of male infertility have not yet been identified but could be associated with sex chromosome drive (Hurst and Pomiankowski, 1991). In addition, sperm depletion in males from the sexually dimorphic species is a possibility because large males often mate many times in rapid succession while in an aggregation. For example, in the field one male *C. whitei* was observed mating with more than 20 females in a 30-min period after dawn (Lorch et al., 1993). Males that carry the X-linked meiotic drive allele should be more susceptible to sperm depletion because half of their sperm degenerate (Presgraves et al., 1997).

Finally, rapid female remating and decreased selectivity may represent a correlated response to selection for rapid mating in males (Halliday and Arnold, 1987). Mating speed seems likely to be under directional selection in males of the two sexually dimorphic species because matings are concentrated in crepuscular periods when the flies are in female-biased aggregations. Males that mate quickly should be able to inseminate more females. A decrease in spermatophore size and copulation duration in the two sexually dimorphic species, relative to *C. quinqueguttata*, is consistent with past selection for remating speed (Kotrba, 1996). Furthermore, the average aggregation size of *C. whitei* (Burkhardt and de la Motte, 1988) is larger than that of *C. dalmanni* (Wilkinson and Reillo, 1994), which is consistent with the results of this study showing faster remating in female *C. whitei* than in *C. dalmanni*. Whether genes that influence male mating speed also influence female remating behavior cannot be determined without direct genetic evidence. Artificial selection studies on male remating speed could be used to test this idea directly.

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