Genetica **117:** 37–46, 2003.
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Phylogeography of *sex ratio* and multiple mating in stalk-eyed flies from southeast Asia

Gerald S. Wilkinson[1], John G. Swallow[2], Sarah J. Christensen[1] & Kevin Madden[1]
[1]*Department of Biology, University of Maryland, College Park, MD 20742, USA (Phone: +1-301-405-6942; Fax: +1-301-314-9358; E-mail: gw10@umail.umd.edu);* [2]*Department of Biology, University of South Dakota, Vermillion, SD, USA*

Key words: coevolution, *Cyrtodio psis*, meiotic drive, sex chromosomes, suppression

Abstract

The factors maintaining sex chromosome meiotic drive, or *sex ratio* (SR), in natural populations remain uncertain. Coevolution between segregation distortion and modifiers should produce transient SR distortion while selection can result in a stable polymorphism. We hypothesize that if SR is maintained by selection, then phylogenetically related populations should exhibit similar SR frequency and intensity. Furthermore, when drive is present, females should mate with multiple males more often both to insure fertility and to increase the probability of producing male progeny. In this paper we report on variation in SR frequency and multiple mating among seven populations and three species of stalk-eyed flies, genus *Cyrtodiopsis*, from southeast Asia. Using a phylogenetic hypothesis based on 1100 bp of mtDNA sequence we find that while sex chromosome meiotic drive is present in all populations of *C. whitei* and *C. dalmanni*, the frequency and intensity of drive only differs between populations or species with greater than 4.8% sequence divergence. The frequency of females mating with multiple males is higher in populations with SR. In addition, SR males mate less often, possibly to compensate for sperm depletion. Our results suggest that sex chromosome drive is maintained by balancing selection in populations of *C. whitei* and *C. dalmanni*. Nevertheless, coevolution between drive and suppressors deserves further study.

Introduction

Meiotic drive, the nonrandom segregation of chromosomal regions into gametes, requires the action of at least two loci-drive and sensitivity, that must be linked to avoid self-destruction. Autosomal drive is predicted to be less common than sex chromosome drive because an autosomal driver can only invade if it arises near a sensitivity locus. In contrast, reduced recombination between the sex chromosomes facilitates linkage disequilibrium between sensitivity and drive loci (Frank, 1991; Hurst & Pomiankowski, 1991; Wu & Hammer, 1991; Pomiankowski & Hurst, 1993). The distribution of known meiotic drive elements in flies matches this prediction (Jiggins, Hurst & Majerus, 1999). Sex chromosome meiotic drive, often referred to as *sex-ratio* (SR), has been described in 15 species of *Drosophila* (Jaenike, 1996), while autosomal meiotic drive has only been described for *D. melanogaster* (Lyttle, 1991).

If a driving sex chromosome invades and spreads through a population, three outcomes are possible. Either one sex disappears, which causes the population to go extinct (Hamilton, 1967), the drive element fails to reach fixation because carriers suffer reduced fitness, or modifiers that suppress drive appear and spread. Considerable evidence indicates that genetic modifiers of meiotic drive rapidly evolve and often differ between populations. Variation in X-chromosome sensitivity to Y-linked drive has been found among populations of the mosquito, *Aedes aegypti* (Wood & Newton, 1991; Owusu-Daaku, Wood & Butler, 1997). Geographic variation in X chromosome drive and Y chromosome suppression has also been reported for *D. paramelanica* (Stalker, 1961), *D. mediopunctata* (Carvalho, Vaz & Klaczko, 1997) and *D. simulans*

(Atlan et al., 1997). In *D. simulans*, recently colonized populations have low frequencies of X-linked drive elements or suppressors, while populations from East Africa, the supposed origin of the species, have high frequencies of distorters (Atlan et al., 1997) but lack sex ratio distortion due to strong suppression caused by Y-linked and autosomal factors (Cazemajor, Landre & Montchamp-Moreau, 1997). This result has led to the suggestion that sex chromosome meiotic drive may be widespread but cryptic (Carvalho & Vaz, 1999).

If modifiers fail to suppress drive completely, then a stable SR polymorphism can be maintained either by differential fitness of female genotypes (Edwards, 1961; Carvalho & Vaz, 1999) or by frequency-dependent selection operating via male fertility (Jaenike, 1996). The latter scenario requires that SR males have less than half the fertility of Standard (ST) males (Jaenike, 1996). In *D. pseudoobscura*, one of the few cases where modifiers of SR have not been identified, frequency of the SR inversion has been stable for many years in the field (Dobzhansky, 1958) and the inversion has been associated with reduced fecundity in the lab (Wallace, 1948) and field (Beckenbach, 1996), as well as reduced male fertility (Wu, 1983).

An SR polymorphism can influence selection on female mate choice, male display traits, and promiscuity. When the sex chromosomes are not transmitted in equal proportions, the population sex ratio will be distorted. Consequently, females that preferentially mate with males that produce more offspring of the rare sex have an evolutionary advantage over randomly mating females (Fisher, 1958). Evolution of ornaments that indicate males lacking drive elements will then be favored by Fisherian sex ratio selection until sex ratio unity is restored in the population (Wilkinson et al., 1998b; Lande & Wilkinson, 1999). If drivers and suppressors evolve and go to fixation sequentially, as some have proposed (Jaenike, 1996), then heritable variation in sex ratio could be transient and allow for punctuated evolution of sex-linked indicator traits. Under this scenario male display traits should exhibit sex linkage, a result recently reported for a variety of species (Reinhold, 1998; Ritchie, 2000; Wolfenbarger & Wilkinson, 2001) and isolated populations would be expected to differ in sex ratio as a consequence of independent coevolution between drive and suppressors.

On the other hand, if sex chromosome meiotic drive produces persistent sex ratio distortion in a population and indicator traits are absent or unreliable, females should be under selection to remate (Haig & Bergstrom, 1995). Mating with multiple males can benefit a female directly by increasing her fertility and indirectly by increasing the probability she will produce progeny of the rare sex. Because meiotic drive operates by differential survival of sperm (Lyttle, 1993; Cazemajor, Joly & Montchamp-Moreau, 2000), SR males are expected to produce half as many fertilizable sperm as ST males. Consequently, females should receive more sperm from ST than SR males. By mating with multiple males, females increase the chance that at least one of their mates does not carry the SR chromosome. In accordance with this prediction, fertility of SR males is lower than ST males after repeated mating in *D. pseudoobscura* (Wu, 1983), *D. recens*, *D. quinaria* (Jaenike, 1996), *D. simulans* (Atlan, pers. Comm.) and *Cyrtodiopsis whitei* (Wilkinson & Fry, 2001).

If sperm is limiting for SR males and the SR chromosome is maintained in a balanced polymorphism, then sexual conflict should arise over mating frequency with SR males preferring to mate less often than females. The extent to which mating rate can differ between the sexes depends on the population sex ratio and the degree to which multiple mating in males is correlated with that in females (cf. Halliday & Arnold, 1987). When the sex ratio is unity, multiple mating by males and females must be the same. In this situation, promiscuity is unlikely to differ between the sexes unless males or females can detect and avoid their previous mates. In addition, multiple mating should influence the invasion of a population by an SR element. If promiscuity is high, SR males will be at a disadvantage relative to ST males as a consequence of sperm competition. The relatively high rate of multiple mating by female *D. melanogaster* (Markow, 1996) could explain, therefore, why this species appears to be devoid of SR chromosomes, while the sister species, *D. simulans*, has them (Atlan et al., 1997).

In this paper we evaluate the possibility that evolution of sex chromosome drive and suppressors of drive in isolated populations results in geographic variation in SR frequency. We use three species and seven populations of stalk-eyed flies from southeast Asia in the genus *Cyrtodiopsis* (family Diopsidae). Diopsid stalk-eyed flies provide an attractive system for this study because a well-supported phylogeny is available for many species in the family (Baker, Wilkinson & Desalle, 2001) and two closely related species, *C. whitei and C. dalmanni*, are known to exhibit X chromosome meiotic drive (Presgraves, Severence

& Wilkinson, 1997). Although the range of these species is not well known, populations of *C. dalmanni* can be found in peninsular Malaysia and adjacent islands (Steyskal, 1972), all of which were connected above water during the last glacial maxima. Consequently, populations on islands have been isolated for at least 11,000 years (Hannebuth, Stattegger & Grootes, 2000) barring long-range migration events. The two species that carry SR exhibit extreme sexual dimorphism in eye stalk length while their congener, *C. quinqueguttata*, is sexually monomorphic for eyespan and does not show distorted progeny sex ratios. Females of the two dimorphic species, but not the monomorphic species, prefer to mate with males possessing long eyespan (Wilkinson, Kahler & Baker, 1998a), a trait that exhibits linkage to meiotic drive (Wilkinson, Presgraves & Cŕymes, 1998b). Males and females mate promiscuously in the field (Lorch, Wilkinson & Reillo, 1993) and lab (Wilkinson, Kahler & Baker, 1998a) without elaborate courtship. The cytological basis of SR in *Cyrtodiopsis* resembles that described for *D. simulans* (Montchamp-Moreau & Joly, 1997). The proportion of spermatocyst bundles exhibiting incompletely elongated spermatids in a male's testis predicts the SR of his progeny independent of his age (Wilkinson & Sanchez, 2001). When mated singly to females, SR males father 74% as many progeny as ST males (Wilkinson & Sanchez, 2001). However, when a female mates with both an SR and ST male, the SR male sires less than 10% of the progeny independent of mating order, indicating that sperm competitive ability, in addition to sperm number, is compromised in SR males (Wilkinson & Fry, 2001).

To interpret change in the frequency and intensity of sex chromosome drive we develop a phylogenetic hypothesis for seven populations using partial sequences of two mtDNA genes. We then estimate SR frequency and intensity by obtaining progeny sex ratios from a large sample of males reared from field-captured females. To quantify the frequency of multiple mating by males and females we conduct observations of repeated copulations using three individually marked females from each population housed with either one or two males. We hypothesize that if sex chromosome meiotic drive is maintained in a stable polymorphism, then closely related populations should exhibit similar frequency and intensity of drive whereas distantly related populations should differ in drive frequency or intensity. Alternatively, if suppressors and drive frequently coevolve, then drive frequency and intensity should vary between isolated populations. We further hypothesize that drive frequency should influence the tendency of males and females to mate repeatedly.

Methods

Population samples

We used four populations of *C. dalmanni*, two populations of *C. whitei* and one population of *C. quinqueguttata* for this study (Figure 1). *C. dalmanni* were captured in peninsular Malaysia near Ulu Gombak, (3°9′N, 101°43′E) in August 1999 and at three sites in Indonesia: near the Soraya field station, northern Sumatra (3°12′N, 97°54′E) in August 1999, near Bukit Lawang, northern Sumatra (3°35′N,98°6′E) and at a forestry research station in Bogor, Java (6°34′S,106°50′E) in September 2000. *C. whitei* were collected near Ulu Gombak and near Chieng Mai, Thailand (19°9′N,98°7′E) in January 1996. *C. quinqueguttata* were collected near Ulu Gombak in January 1996. At each site we hand-netted at least 50 individuals of each species and fed them instant *Drosophila* food (Ward's Biological Supplies) until they were returned to the lab.

Species designations were assigned after comparing external morphological characters of collected flies with type specimens at the National Museum of Natural History in Washington, DC. Populations

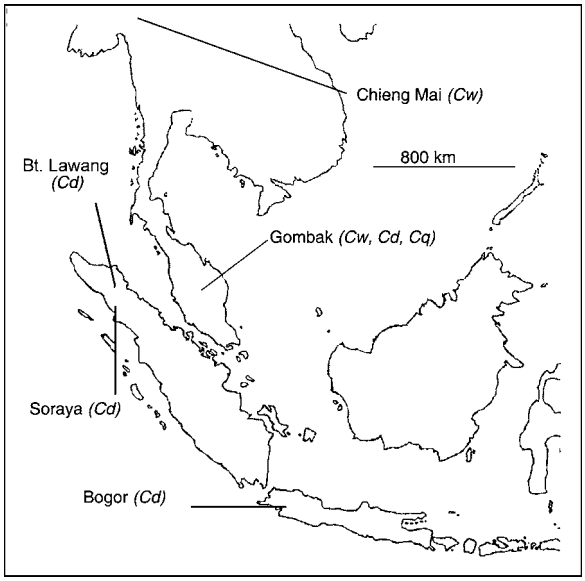

Figure 1. Locations where flies were collected in southeast Asia.

of *C. whitei* differ in body color but are interfertile (Wilkinson & Fry, 2001). In contrast, populations of *C. dalmanni* are indistinguishable using external morphological characters, but exhibit variation in fertility when flies are mated across populations (S. Toll & G. Wilkinson, unpublished data).

In the lab we have kept flies from each population in large plexiglass cages ($40\times40\times40\,cm^3$) at $25\pm2°C$ with at least 75% relative humidity on a 12 h light–dark cycle. We provide pureed yellow corn treated with 10% methylparaben as a mold inhibitor for feeding and oviposition twice each week. Newly eclosed flies are returned to each cage as necessary to keep populations at or above 100 individuals.

Phylogeography

We developed a phylogenetic hypothesis for the relationships among five individuals from each population using 1100 bp of mitochondrial DNA sequence. We amplified and sequenced DNA from two loci: 535 bp of cytochrome oxidase II and 565 bp of the 16S subunit of ribosomal RNA using conserved primers (Baker, Wilkinson & Desalle, 2001). Tree topologies created with PAUP* v. 4.0b10 (Swofford, 2001) using distance, parsimony, and maximum likelihood optimization criteria did not differ in branch arrangement. We present, therefore, an unrooted neighbor-joining tree based on uncorrected genetic distances and indicate bootstrap values on each branch.

SR comparisons

To maximize the number of independent X chromosomes assayed from each population we paired male progeny from wild-caught females with three or more virgin females from the same population and then scored the sex ratio of their progeny. We allowed females to mate and oviposit in ventilated plastic cages ($12\times16\times13.5\,cm^3$) lined with moist blotting paper and cotton. All flies were at least 4 weeks past eclosion and, therefore, sexually mature (De La Motte & Burkhardt, 1983) when mated. In each cage we provided 50 ml of pureed corn twice a week for at least 4 weeks for feeding and oviposition. For *C. dalmanni*, we mated 93 Bt. Lawang, 93 Gombak, 90 Soraya and 83 Bogor males. For *C. whitei* we mated 64 Gombak and 62 Chieng Mai males and for *C. quinqueguttata* we scored sex ratios from 103 males. We tested sex ratios for deviation from 1:1 using chi-squared tests corrected for continuity. Males with significantly ($\alpha = 0.05$) female-biased progeny sex ratios were designated as carrying a SR, rather than ST, X chromosome. We did not adjust alpha for multiple tests because Bonferroni-correction results in an unacceptable increase in Type II errors, as indicated by significantly female-biased average sex ratios for ST males in all populations. We used a contingency test to determine if populations differ in SR frequency. We then used the angular transformation (arcsin(squareroot)) of male progeny sex ratios in a nested analysis of variance (ANOVA) to determine if populations or species differ in progeny sex proportion produced by all males, as well as by only SR males. We also used nested ANOVA to compare the number of progeny produced by each male between populations and species.

Mating observations

To determine if mating multiple males by females is influenced by the presence of sex chromosome meiotic drive, for each population we conducted observations of copulations by two males housed with three virgin females in replicate plastic cages ($12\times16\times13.5\,cm^3$) at $25\pm2°C$ and at least 75% RH. Each cage contained a tray of pureed corn and was lined with moist blotting paper and cotton. We observed 12 cages for each population of *C. dalmanni* and *C. whitei* and 21 cages containing *C. quinqueguttata*. We aspirated males into cages before the lights came on in the morning, and then for *C. dalmanni* and *C. whitei* observed all copulations during the subsequent 4 h. Because *C. quinqueguttata* flies mated much less frequently, we observed their cages for at least 6 h on each of 5 successive days (average total observation time $\pm$ SE $= 26.4 \pm 0.8$ h). *C. quinqueguttata* flies remained in the cages between observation sessions. We marked all flies with a unique color of paint on the thorax and recorded the time of every copulation on audio tape. Short duration copulations, typically lasting 15 s or less, were not counted, as these do not result in sperm transfer in *C. whitei* (Lorch, Wilkinson & Reillo, 1993), which has the shortest copulation duration of the three species (Wilkinson, Kahler & Baker, 1998a). To determine if the frequency of mating multiple males by females differed between populations we conducted a logistic regression in which we entered mating rate, that is, the number of copulations observed per hour by each female, as a covariate to control for differences in activity between cages.

In addition, we observed matings in cages containing single males housed with three individually marked females from each population. These

observations were conducted under identical conditions as above except that they lasted 2.5 h and 16 cages were observed for each population. We then used nested ANOVA to determine if mating rates in each population depend on species, population or the number of males present. Furthermore, because some of the males used in these observations carried the SR chromosome, we also used ANOVA to determine if either females or males alter mating rates as a function of SR genotype.

Results

Phylogeography

Phylogenetic analysis indicates that multiple populations within species form monophyletic units with complete bootstrap support (Figure 2). Gene flow between populations must also be extremely rare as the five sequences sampled from each population exhibit monophyly with high bootstrap support in most cases. The magnitude of genetic distance and bootstrap support between populations reflects both species identity and geographic separation (Figure 2). For example, between *C. quinqueguttata* and the four *C. dalmanni* populations the average genetic distance is 11.2% while between *C. quinqueguttata* and the two *C. whitei* populations it is 10.9%. In contrast, the genetic distance between all *C. whitei* and *C. dalmanni* populations is 7.6%. Within *C. dalmanni*, the Java site shows 4.8% sequence divergence from the other three sites, while the peninsular Malaysia site is 2.2% different from the northern Sumatran sites and the two Sumatran sites, Soraya and Bt. Lawang, exhibit 0.3% divergence. Despite having greater geographic separation than any pair of *C. dalmanni* populations, the two *C. whitei* sites show only 0.9% sequence divergence.

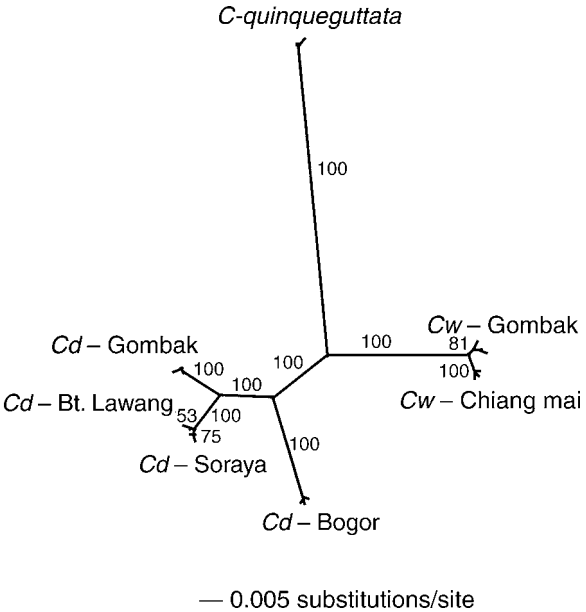

Figure 2. Unrooted neighbor-joining phylogram illustrating the uncorrected genetic distances between five individuals sampled from each population.

SR variation

The distribution of progeny SRs for males from each population is shown in Figure 3. Nested ANOVA on the angular transformation of the proportion of male progeny reveals significant differences between species ($F_{2,4} = 14.50$, $P = 0.015$ but not between

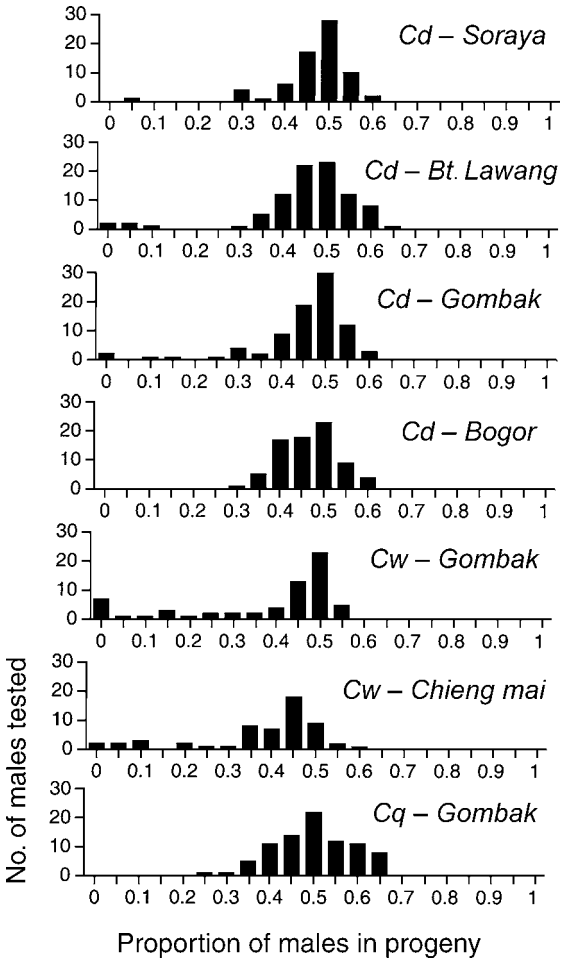

Figure 3. Proportion of males in progeny from first generation lab-reared males in each population. Only males producing more than 12 progeny are included.

populations within species ($F_{4,581} = 1.61$, $P = 0.17$). A Fisher's protected least squares difference (PLSD) post-hoc test indicates that *C. whitei* differs from the other two species in the proportion of male progeny produced (*Cd–Cw*, $P = 0.0093$; *Cw–Cq*, $P = 0.0070$; *Cd–Cq*, $P = 0.106$). The average ± SE proportion of male progeny produced by males from each species was 0.373 ± 0.015 for *C. whitei*, 0.453 ± 0.007 for *C. dalmanni*, and 0.502 ± 0.011 for *C. quinqueguttata*.

Contingency tests also reveal significant heterogeneity in the frequency of SR males between species ($\chi^2 = 34.1$, $P < 0.0001$) and among populations of *C. dalmanni* ($\chi^2 = 10.2$, $P = 0.017$) but not between populations of *C. whitei* ($\chi^2 = 0.19$, $P = 0.66$). Within *C. dalmanni*, Bogor had the highest frequency of SR males (25.3%), followed by Bt. Lawang (19.4%), Gombak (15.1%) and Soraya (7.8%). Combining both *C. whitei* populations, 43 of 126 (34.1%) males exhibited biased sex ratios. For *C. quinqueguttata*, five of 103 males produced sex ratios which deviated from unity. However, the average sex ratio of those five males was not significantly female-biased, as expected given an $\alpha = 0.05$ criterion and absence of SR.

In contrast, a nested ANOVA on the proportion of male progeny produced by SR males reveals no difference between species ($F_{2,4} = 1.58$, $P = 0.31$) but a significant difference between populations within species ($F_{4,101} = 3.38$, $P = 0.012$). ANOVA of sex proportions between populations within each species shows that this effect is caused by Bogor SR males having a sex proportion (0.42 ± 0.03, $n = 21$) which is greater than that of SR males from the other three *C. dalmanni* populations (Bt. Lawang: 0.29 ± 0.04, $n = 18$, $P = 0.012$; Gombak: 0.25 ± 0.04, $n = 14$, $P = 0.002$; Soraya: 0.22 ± 0.07, $n = 7$, $P = 0.005$). The two *C. whitei* populations do not differ from each other (Gombak: 0.19 ± 0.03, $n = 23$; Chieng Mai: 0.20 ± 0.03, $n = 20$).

While the differences in sex ratios observed between species are not likely to be influenced by progeny production given the sample sizes involved, the number of progeny per male might influence the SR designation of a male when there is weak sex ratio distortion. ANOVA reveals that this issue could be relevant in some cases because species differ in the number of progeny produced per male ($F_{2,585} = 43.95$, $P < 0.0001$). A Fisher's PLSD post-hoc test indicates that *C. quinqueguttata* males produce significantly ($P < 0.0001$) fewer progeny (34.5 ± 2.3) than either *C. dalmanni* (118.6 ± 4.5) or *C. whitei* males (140.8 ± 12.0), and *C. dalmanni* differs from *C. whitei* ($P = 0.019$). ANOVA on progeny production between populations within each species further show that the two *C. whitei* populations differ (F_1, $124 = 96.7$; $P < 0.001$; Chieng Mai: 50.7 ± 4.3 progeny per male; Gombak: 228 ± 17.2 progeny per male) but the four *C. dalmanni* populations do not differ ($F_{3,355} = 0.33$, $P = 0.80$; Bogor: 122.5 ± 7.6; Bt. Lawang: 113.9 ± 6.9; Gombak: 114.7 ± 7.8; Soraya: 123.7 ± 12.3). Thus, for *C. whitei* the frequency of SR males that cause weakly distorted SRs may be underestimated for Chieng Mai relative to Gombak. In contrast, our ability to estimate SR frequency and intensity is comparable for all four *C. dalmanni* populations. Similarly, the absence of any directional bias in the population SR for *C. quinqueguttata* indicates that low progeny production is unlikely to bias SR frequencies in this species.

Multiple mating and SR

Logistic regression analysis revealed that the frequency of multiple mating by females depends on the population ($\chi^2_6 = 14.7$, $P = 0.022$) and on the mating rate of each female ($\chi^2_1 = 71.7$, $P < 0.0001$). If *C. quinqueguttata* females are excluded from this analysis, population is no longer significant ($\chi^2_5 = 5.6$, $P = 0.35$). Similarly, logistic regression on multiple mating frequency using species as a factor and female mating rate as a covariate reveals a significant effect of species ($\chi^2_2 = 68.1$, $P < 0.0001$), but no difference between the frequency of multiple mating observed in *C. dalmanni* versus *C. whitei* females ($\chi^2_1 = 0.4$, $P = 0.52$). The frequency of mating multiple males by females is higher in the two species which carry SR (60.2% of 244) than in the species which lacks SR (42.6% of 47). This result mirrors the pattern seen in mating rates between species, that is, male and female *C. whitei* and *C. dalmanni* mate more frequently than female *C. quinqueguttata* (Figure 4). However, nested ANOVA reveals that male and female mating rate also differ between populations within species and depend on whether one or two males are present in a cage (Table 1). Male mating rate also exhibits an interaction between species and number of males caused by a higher mating rate by single male *C. whitei* than *C. dalmanni* (Figure 4).

ANOVA on the rate of male mating when housed alone with females reveals a significant effect of drive

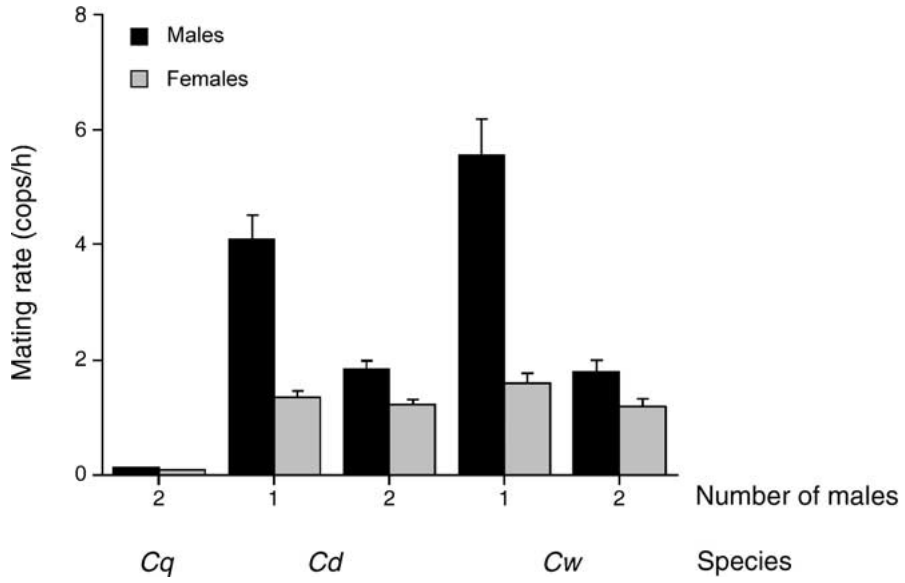

Figure 4. Mating rates for males and females of each species when either one or two males is caged with three females and observed for 4 h (*C. whitei* and *C. dalmanni*) or 26 h (*C. quinqueguttata*).

Table 1. Nested ANOVA on female and male mating rates with species, population and number of males mating (one or two) as factors

Source	df	Mean squares	*F*-ratio	*P*
Females				
Species	2	29.2	21.7	0.0001
Population (species)	4	15.4	11.4	0.0001
Number of males	1	4.33	3.22	0.07
Species × no. of males	1	1.91	1.42	0.23
Residual	512	1.35		
Males				
Species	2	50.77	12.6	0.0001
Population (species)	4	40.43	10.04	0.0001
Number of males	1	385.64	95.74	0.0001
Species × no. of males	1	26.43	6.56	0.0110
Residual	258	4.03		

($F_{1,112} = 6.32$, $P = 0.013$) but not of species. Among the flies we observed mating, there were five of 39 *C. dalmanni* and 26 of 75 *C. whitei* classified as SR. SR males of both species mated less often than ST males (Figure 5). In contrast, females did not appear to discriminate between drive genotypes as an ANOVA on female mating rate revealed no effect of drive ($F_{1,112} = 2.48$, $P = 0.12$) or species.

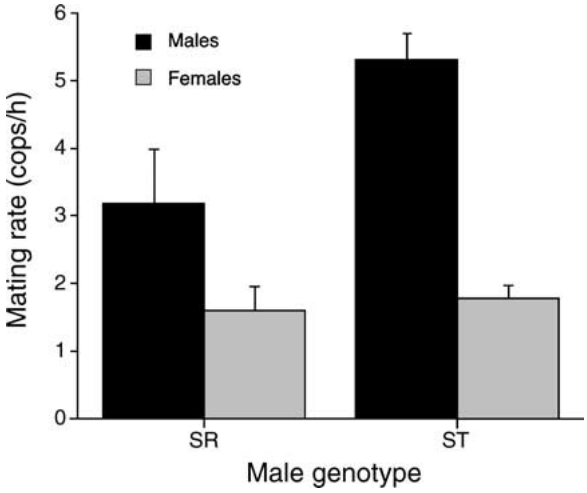

Figure 5. Mating rates for males and females when either an SR or ST male is housed with three females and observed for 2.5 h.

Discussion

Evolution of SR frequency and intensity

We hypothesized that if sex chromosome meiotic drive is maintained in a stable polymorphism, then closely related populations should exhibit similar frequency and intensity of drive. Comparison of average progeny SRs, as well as the frequency of drive and the female-bias exhibited by drive-carrying males, across

populations is largely consistent with this prediction. Sex chromosome meiotic drive appears to be present in all six populations of *C. dalmanni* and *C. whitei*, but either absent or completely suppressed in the sexually monomorphic species, *C. quinqueguttata*. The frequency of SR males (34%) and the proportion of male progeny produced by SR males (19% males) did not differ between the two populations of *C. whitei.* In addition, the three most closely related populations of *C. dalmanni* (Bt. Lawang, Soraya and Gombak) exhibited similar SR male frequencies (8–19%) and SR male progeny SRs (22–29% males). The most genetically divergent *C. dalmanni* population, Bogor, differed from the other *C. dalmanni* populations in having more SR males (25%) with less female-biased progeny (42% males).

While the similarity in SR frequency and intensity among closely related populations is consistent with a balanced SR polymorphism, differences in the magnitude of drive between species, as well as between populations of *C. dalmanni*, requires rapid evolution either in modifiers that suppress the effect of SR on progeny sex ratios or in SR chromosomes. Suppressors could be more abundant or effective in *C. dalmanni* flies from Bogor than in either population of *C. whitei.* Alternatively, novel SR chromosomes may have recently arisen in *C. whitei*. The relatively low genetic distance between the two *C. whitei* populations (0.9%), compared to that between Bogor and the other *C. dalmanni* populations (4.8%), is compatible with both possibilities. Detailed genetic mapping of drive and suppressor loci in each population would help clarify the evolution of this meiotic drive system. Unfortunately, crosses between populations that differ by more than 4% in their mtDNA sequence do not produce fertile progeny (S. Toll & G. Wilkinson, unpublished data). Consequently, it has not yet been possible to compare male progeny SRs from hybrid males carrying alternative SR chromosomes from divergent populations. Nevertheless, comparison of the fertility of flies produced from crosses involving more closely related populations provides indirect evidence that the sex chromosome drive system is evolving rapidly. Male F1 hybrids produced by crosses between most pairs of *C. dalmanni* populations, even those separated by only 0.3% sequence divergence, such as Soraya and Bt. Lawang, are sterile (S. Toll & G. Wilkinson, unpublished data) while F1 females from the same crosses are fertile. Male hybrid sterility has been proposed to be caused by mismatched sex chromosome meiotic drive and suppressor of drive loci, which would be expected if drive and suppressor loci rapidly coevolve within populations (Frank, 1991; Hurst & Pomiankowski, 1991).

Although a causal connection between sex chromosome meiotic drive and hybrid male sterility (or Haldane's rule) has been doubted (Coyne, Charlesworth & Orr, 1991; Johnson & Wu, 1992; Coyne & Orr, 1993), several recent studies provide support for this hypothesis. For example, crosses between strains of *Drosophila subobscura* resulted in the production of sterile males and males with distorted SRs (Hauschteck-Jungen, 1990). Although this study did not demonstrate the genetic basis of male sterility, two recent studies have shown that male sterility and SR distortion are genetically linked. Analysis of introgressions between *D. simulans* and *D. mauritiana* indicate that a suppressor of sex chromosome drive lies within 80 kb of a male sterility factor (Tao, Hartl & Laurie, 2001). Similarly, flies produced from crosses between *D. pseudoobscura* from the United States and Columbia are normally sterile, but genetic regions on the X chromosome that restore fertility also cause distorted SRs (Orr & Presgraves, 2000). Since the *D. pseudoobscura* populations diverged within the last 250,000 years, these results indicate that male sterility can evolve rapidly. Direct evidence for rapid evolution of drive and suppressor loci in stalk-eyed flies must await population comparisons of sequence information from genomic regions near drive and suppressor loci, as has been demonstrated for the autosomal drive system, segregation distorter (SD), in *D. melanogaster* (Palopoli & Wu, 1996).

A potential criticism of our interpretations is that our phylogenetic hypothesis is based solely on variation in mitochondrial DNA sequences. In some cases, such topologies do not match those predicted by variation in nuclear DNA sequences. For example, mtDNA haplotypes in *D. simulans* are inconsistent with phylogenetic relationships predicted by nuclear, morphological and behavioral traits and may be influenced by past selection on cytoplasmic factors, such as *Wolbachia* (Ballard, Chernoff & James, 2002). While we cannot unequivocably address this possibility until we have evidence from nuclear markers, genetic distances between *Cyrtodiopsis* populations estimated from mtDNA sequences correlates strongly with levels of fertility exhibited in interpopulation crosses and with frequencies of matings between flies from different populations (S. Toll & G. Wilkinson, unpublished data). We suspect, therefore, that relationships among populations of *Cyrtodiopsis* species predicted

by mtDNA information will be largely consistent with those predicted by autosomal DNA information.

Multiple mating and SR

Species differences in drive frequency correspond to species differences in the frequency of multiple mating and mating rate. Females from all populations of the two species which carry SR exhibit higher frequencies of multiple mating than females from the species which lacks SR. Similarly, the mating rate for females and males increases as the frequency of drive increases across species. Given that male, but not female, mating rate differs depending on the number of males in a cage, females appear to control mating in *C. dalmanni* and *C. whitei*. These results suggest, therefore, that the rate of female mating in each species increases when there is a risk of producing female-biased offspring. However, mating rate varies between populations independently of variation in SR frequency. Thus, other factors, which were not measured in this study, are likely to be important in explaining population variation in mating rate. Because SR male sperm precedence is less than 10% when a female mates with both SR and ST males (Wilkinson & Fry, 2001), multiple mating by females appears to be an effective strategy to reduce the effect of segregation distortion.

From the male perspective, the SR chromosome reduces the number of fertilization competent sperm a male can produce (Wilkinson & Sanchez, 2001). Consequently, SR males should be more prone to sperm depletion than ST males (Jaenike, 1996). Our results show that SR males may compensate for having fewer competent sperm by mating less frequently. Given that *C. whitei* males have been observed mating up to 24 times in 30 min (Lorch, Wilkinson & Reillo, 1993), this behavior should reduce the likelihood that males become sperm depleted in female-biased populations.

Females would not be expected to remate more often if SR males could be reliably identified and avoided during mating. While eyespan exhibits X linkage in *C. dalmanni*, the X chromosome only explains about 30% of the variation in eyespan (Wolfenbarger & Wilkinson, 2001). Furthermore, male eyespan is a condition-dependent trait (Wilkinson & Taper, 1999; David et al., 2000) and the effect of drive on condition-dependent expression has not yet been determined. Consequently, eyespan may not always be an unambiguous indicator of drive. Comparison of eyespan to body length allometry between populations reinforces this possibility. Even though SR frequency and intensity differ between species and populations of *Cyrtodiopsis* as noted above, no difference in male eyespan to body length allometry can be detected between those populations (J. Swallow & G. Wilkinson, unpublished data). However, one population of *C. dalmanni* from northern Sumatra does exhibit dramatically longer eye stalks than any other population. Whether the frequency and intensity of drive covaries with this morphological change must await further study.

Acknowledgements

We greatly appreciate the logistical support provided by the staff of the Leuser Management Unit and the Indonesian Institute of Sciences. Dr Katherine Monk, Purnama Hidayat and Professor H.S. Yong assisted our efforts to find flies. We are grateful to Anne Atlan for helpful comments on the manuscript. This work was supported by National Science Foundation graduate fellowships to SJT, a National Research Service Award from the National Institutes of Health and a grant from the American Philosophical Society to JGS and National Science Foundation grants DEB-9807937 and DEB-0077878 to GSW.

References

Atlan, A., H. Mercot, C. Landre & C. Montchamp-Moreau, 1997. The sex-ratio trait in *Drosophila simulans*: Geographical distribution of distortion and resistance. Evolution 51: 1886–1895.

Baker, R.H., G.S. Wilkinson & R. Desalle, 2001. The phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). Syst. Biol. 50: 87–105.

Ballard, J.W.O., B. Chernoff & A.C. James, 2002. Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. Evolution 56: 527–545.

Beckenbach, A.T., 1996. Selection and the 'sex-ratio' polymorphism in natural populations of *Drosophila pseudoobscura*. Evolution 50: 787–794.

Carvalho, A.B. & S.C. Vaz, 1999. Are *Drosophila* SR chromosomes always balanced? Heredity 83: 221–228.

Carvalho, A.B., S.C. Vaz & L.B. Klaczko, 1997. Polymorphism for Y-linked suppressors of *sex-ratio* in two natural populations of *Drosophila mediopunctata*. Genetics 146: 891–902.

Cazemajor, M., C. Landre & C. Montchamp-Moreau, 1997. The sex-ratio trait in *Drosophila simulans*: genetic analysis of distortion and suppression. Genetics 147: 635–642.

Cazemajor, M., D. Joly & C. Montchamp-Moreau, 2000. *Sex-ratio* meiotic drive in *Drosophila simulans* is related to equational nondisjunction of the *Y* chromosome. Genetics 154: 229–236.

Coyne, J.A. & H.A. Orr, 1993. Further evidence against meiotic-drive models of hybrid sterility. Evolution 47: 685–687.

Coyne, J.A., B. Charlesworth & H.A. Orr, 1991. Haldane rule revisited. Evolution 45: 1710–1714.

David, P., T. Bjorksten, K. Fowler & A. Pomiankowski, 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature 406: 186–188.

De La Motte, I. & D. Burkhardt, 1983. Portrait of an Asian stalk-eyed fly. Naturwiss 70: 451–461.

Dobzhansky, T., 1958. Genetics of natural populations. XXVII. The genetic changes in populations of *Drosophila pseudoobscura* in the American Southwest. Evolution 12: 385–401.

Edwards, A.W.F., 1961. The population genetics of 'sex-ratio' in *Drosophila pseudoobscura*. Heredity 16: 291–304.

Fisher, R.A., 1958. The Genetical Theory of Natural Selection. Dover, New York.

Frank, S.A., 1991. Divergence of meiotic drive-suppression systems as an explanation for sex-biased hybrid sterility and inviability. Evolution 45: 262–267.

Haig, D. & C.T. Bergstrom, 1995. Multiple mating, sperm competition and meiotic drive. J. Evol. Biol. 8: 265–282.

Halliday, T. & S. Arnold, 1987. Multiple mating by females – a perspective from quantitative genetics. Anim. Behav. 35: 939–941.

Hamilton, W.D., 1967. Extraordinary sex ratios. Science 156: 477–488.

Hannebuth, T., K. Stattegger & P.M. Grootes, 2000. Rapid flooding of the Sunda shelf – a late-glacial sea-level record. Science 288: 1033–1035.

Hauschteck-Jungen, E., 1990. Postmating reproductive isolation and modification of the sex-ratio trait in *Drosophila subobscura* induced by the sex chromosome gene arrangement $A_{2+3+5+7}$. Genetica 83: 31–44.

Hurst, L. & A. Pomiankowski, 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation of Haldane's rule and related phenomenon. Genetics 128: 841–858.

Jaenike, J., 1996. Sex-ratio meiotic drive in the *Drosophila quinaria* group. Am. Nat. 148: 237–254.

Jiggins, F.M., G.D.D. Hurst & M.E.N. Majerus, 1999. How common are meiotically driven sex chromosomes? Am. Nat. 154: 481–483.

Johnson, N.A. & C.-I. Wu, 1992. An empirical test of the meiotic drive models of hybrid sterility: sex-ratio data from hybrids between *Drosophila simulans* and *Drosophila sechellia*. Genetics 130: 507–511.

Lande, R. & G.S. Wilkinson, 1999. Models of sex-ratio meiotic drive and sexual selection in stalk-eyed flies. Genet. Res. 74: 245–253.

Lorch, P., G.S. Wilkinson & P.R. Reillo, 1993. Copulation duration and sperm precedence in the Malaysian stalk-eyed fly, *Cyrtodiopsis whitei* (Diptera: Diopsidae). Behav. Ecol. Sociobiol. 32: 303–311.

Lyttle, T.W., 1991. Segregation distorters. Ann. Rev. Genetics 25: 511–557.

Lyttle, T.W., 1993. Cheaters sometimes prosper: distortion of Mendelian segregation by meiotic drive. Trends Genet. 9: 205–208.

Markow, T.A., 1996. Evolution of Drosophila mating systems. Evol. Biol. 29: 73–106.

Montchamp-Moreau, C. & D. Joly, 1997. Abnormal spermiogenesis is associated with the X-linked *sex-ratio* trait in *Drosophila simulans*. Heredity 79: 24–30.

Orr, H.A. & D.C. Presgraves, 2000. Speciation by postzygotic isolation: forces, genes and molecules. Bioessays 22: 1085–1094.

Owusu-Daaku, K.O., R.J. Wood & R.D. Butler, 1997. Selected lines of *Aedes aegypti* with persistently distorted sex ratios. Heredity 79: 388–393.

Palopoli, M.F. & C.-I. Wu, 1996. Rapid evolution of a coadapted gene complex: evidence from the *segregation distorter* (SD) system of meiotic drive in *Drosophila melanogaster*. Genetics 143: 1675–1688.

Pomiankowski, A. & L.D. Hurst, 1993. Genomic conflicts underlying Haldane's rule. Genetics 133: 425–432.

Presgraves, D.C., E. Severence & G.S. Wilkinson, 1997. Sex chromosome meiotic drive in stalk-eyed flies. Genetics 147: 1169–1180.

Reinhold, K., 1998. Sex linkage among genes controlling sexually selected traits. Behav. Ecol. Sociobiol. 44: 1–7.

Ritchie, M.G., 2000. The inheritance of female preference functions in a mate recognition system. Proc. R. Soc. Lond. B 267: 1–6.

Stalker, H.D., 1961. The genetic systems modifying meiotic drive in *Drosophila paramelanica*. Genetics 46: 177–202.

Steyskal, G.C., 1972. A catalogue of species and key to the genera of the family Diopsidae. Stuttg. Beitr. Naturkd. Ser. A 1–20.

Swofford, D.L., 2001 PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0b8. Sinauer, Sunderland, MA.

Tao, Y., D.L. Hartl & C.C. Laurie, 2001. Sex-ratio segregation distortion associated with reproductive isolation in Drosophila. Proc. Natl. Acad. Sci. USA 98: 13183–13188.

Wallace, B., 1948. Studies on 'sex-ratio' in *Drosophila pseudoobscura*. I. Selection and 'sex-ratio'. Evolution 2: 189–217.

Wilkinson, G.S. & C.L. Fry, 2001. Meiotic drive alters sperm competitive ability in a stalk-eyed fly. Proc. R. Soc. Lond. B 268: 2559–2564.

Wilkinson, G.S. & M.I. Sanchez, 2001. Sperm development, age and sex chromosome meiotic drive in the stalk-eyed fly, *Cyrtodiopsis whitei*. Heredity 87: 17–24.

Wilkinson, G.S. & M. Taper, 1999. Evolution of genetic variation for condition dependent traits in stalk-eyed flies. Proc. R. Soc. Lond. B 266: 1685–1690.

Wilkinson, G.S., H. Kahler & R.H. Baker, 1998a. Evolution of female mating preferences in stalk-eyed flies. Beh. Ecol. 9: 525–533.

Wilkinson, G.S., D.C. Presgraves & L. Crymes, 1998b. Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. Nature 391: 276–278.

Wolfenbarger, L.L. & G.S. Wilkinson, 2001. Sex-linked expression of a sexually selected trait in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. Evolution 55: 103–110.

Wood, R.J. & M.E. Newton, 1991. Sex-ratio distortion caused by meiotic drive in mosquitoes. Am. Nat. 137: 379–391.

Wu, C.-I., 1983. Virility deficiency and the sex-ratio trait in *Drosophila pseudoobscura*. II. Multiple mating and overall virility selection. Genetics 105: 663–679.

Wu, C.-I. & M.F. Hammer, 1991. Molecular evolution of ultraselfish genes of meiotic drive systems. pp. 177–203 in Evolution at the Molecular Level, edited by R.K. Selander, A.G. Clark & T.S. Whittam. Sinauer, Sunderland, MA.