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Genetic divergence does not predict change in ornament expression among populations of stalk-eyed flies

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Abstract

Stalk-eyed flies (Diptera: Diopsidae) possess eyes at the ends of elongated peduncles, and exhibit dramatic variation in eye span, relative to body length, among species. In some sexually dimorphic species, evidence indicates that eye span is under both intra- and intersexual selection. Theory predicts that isolated populations should evolve differences in sexually selected traits due to drift. To determine if eye span changes as a function of divergence time, 1370 flies from 10 populations of the sexually dimorphic species, *Cyrtodiopsis dalmanni* and *Cyrtodiopsis whitei*, and one population of the sexually monomorphic congener, *Cyrtodiopsis quinqueguttata*, were collected from Southeast Asia and measured. Genetic differentiation was used to assess divergence time by comparing mitochondrial (cytochrome oxidase II and 16S ribosomal RNA gene fragments) and nuclear (*wingless* gene fragment) DNA sequences for *c.* five individuals per population. Phylogenetic analyses indicate that most populations cluster as monophyletic units with up to 9% nucleotide substitutions between populations within a species. Analyses of molecular variance suggest a high degree of genetic structure within and among the populations; > 97% of the genetic variance occurs between populations and species while < 3% is distributed within populations, indicating that most populations have been isolated for thousands of years. Nevertheless, significant change in the allometric slope of male eye span on body length was detected for only one population of either dimorphic species. These results are not consistent with genetic drift. Rather, relative eye span appears to be under net stabilizing selection in most populations of stalk-eyed flies. Given that one population exhibited dramatic evolutionary change, selection, rather than genetic variation, appears to constrain eye span evolution.

Keywords: *Cyrtodiopsis*, genetic diversity, secondary sexual traits, sexual selection, stalk-eyed flies

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Introduction

Sexual selection, through mechanisms of either female choice or male–male competition, has been implicated in the evolution of elaborate secondary sexual traits in many species (Andersson 1994). Secondary sexual characteristics often show conspicuous differences between closely related species (Panhuis *et al*. 2001), leading to the suggestion that sexual selection, especially in populations susceptible to genetic drift, can result in rapid morphological divergence and potentially speciation (Lande 1981). For example, among different clades of birds, sexual selection by female choice has been implicated in morphological diversity (Barraclough *et al*. 1995; Mitra *et al*. 1996; Prum 1997; Møller & Cuervo 1998; Owens *et al*. 1999). The explosive radiations of African cichlid fish have been attributed, at least in part, to sexual selection (Galis & Metz 1998), as a result of differences in female colour preferences (van Doorn *et al*. 1998; Lande *et al*. 2001).

Given the degree of interspecific variation in secondary sexual characters, evidence of morphological differences among isolated populations might be expected. Indeed, examples of population-level variation in traits putatively under sexual selection have been reported (e.g. Young *et al*. 1994; Endler & Houde 1995; Wilcox *et al*. 1997; Emerson & Ward 1998; Irwin 2000; Uy & Borgia 2000; Panhuis *et al*. 2001; Masta & Maddison 2002). However, because

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population-level variation can arise from a variety of sources, including local adaptation and migration, variation alone is insufficient to ascribe cause of change. If drift is responsible for population divergence, then male display traits should show as much variability among populations as neutral genetic markers (Questiau 1999; Panhuis *et al*. 2001). In such a situation, one would expect a positive correlation between morphological and molecular divergence between populations. Empirical studies that quantify the correlation between morphological and genetic variation are rare. While some recent molecular studies confirm this prediction (e.g. Masta & Maddison 2002), others have revealed an unexpected degree of molecular divergence among populations of morphologically indistinguishable sexually dimorphic species (Zeh & Zeh 1994; Wilcox *et al*. 1997; Emerson & Ward 1998), as might be expected for traits under net stabilizing selection.

Stalk-eyed flies are emerging as a model system for understanding the evolution of male ornamental traits (Burkhardt & de la Motte 1988; Wilkinson *et al*. 1998b; David *et al*. 2000). All flies in the family Diopsidae have eyes displaced laterally on elongated peduncles. In some species, extreme sexual dimorphism in eye stalk length is indicated by the slope of the regression of eye span on body length; in dimorphic species, males have a steeper slope than females resulting in greater eye span for a given body length (Wilkinson & Dodson 1997). Evidence from the laboratory and field indicate that in dimorphic species, such as *Cyrtodiopsis dalmanni* and *Cyrtodiopsis whitei*, eye span is under strong sexual selection that involves both female choice and male–male competition. These flies form nocturnal mating aggregations on exposed rootlets and male mating success is highly correlated with eye span (Wilkinson & Reillo 1994). Females preferentially mate with males possessing long eye span (Wilkinson *et al*. 1998a), and this preference changes in response to artificial selection for eye span (Wilkinson 1993; Wilkinson & Reillo 1994). In addition, males compete for access to and control of prime breeding sites (Lorch *et al*. 1993); the outcome of competition is affected by relative male eye span (Panhuis & Wilkinson 1999).

Phylogenetic analysis reveals that sexual dimorphism for eye-span allometry is evolutionarily labile with relatively rapid, recurrent losses and gains of sexual dimorphism among 30 extant diopsid species (Baker & Wilkinson 2001). Evolutionary change in sexual dimorphism strongly correlates with change in male, but not female, eye-span allometry (Wilkinson & Taper 1999; Baker & Wilkinson 2001), as expected if sexual selection acts on male eye span. Given these results, we hypothesized that sexual selection should generate morphological differentiation among populations in proportion to genetic divergence. To test this prediction we quantify and compare the degree of divergence in morphological and molecular characters among populations of stalk-eyed flies, genus *Cyrtodiopsis*, collected from 10 locations on four major land masses in the Sunda Shelf region of Southeast Asia. We use mitochondrial and nuclear DNA sequence data to estimate levels of genetic variation within populations and the degree of genetic differentiation among populations. Additionally, we evaluate the utility of both mitochondrial and nuclear sequence data sets in reconstructing the evolutionary history of these populations. Lastly, we use genealogical methods (Nielsen & Wakeley 2001) to estimate migration, effective population sizes and genetic divergence times among extant and ancestral populations (Rosenberg & Nordborg 2002).

Materials and methods

Population samples

Using published (e.g. Steyskal 1972) and museum collection information (National Museum of Natural History, Washington, D.C.) for guidance, we visited and attempted to collect stalk-eyed flies from replicate sites on the major land masses in the Sunda Shelf region. This area covers the known range of the most well-studied *Cyrtodiopsis* species, *Cyrtodiopsis dalmanni*. Over a 5-year period we collected 1370 stalk-eyed flies by hand net near streams from nine sites in Thailand, peninsular Malaysia, and the islands of Java, Sumatra, and Borneo (Fig. 1, Table 1). These animals exhibited obvious morphological similarities to three species: *quinqueguttata*, *whitei*, and *dalmanni*. In January 1996, we captured *C. dalmanni* near Cameron Highlands, Malaysia (4°15′N, 101°21′E). In August 1999 we captured *C. dalmanni* at Ulu Gombak, Malaysia (3°12′N, 101°42′E),

Fig. 1 Collection localities in the Sunda Shelf region of Southeast Asia. For site details and species collected, see text.

Table 1 Morphological measurements (mean ± SE mm) and sample sizes (*N*) of field-collected flies

Site	Sex	*N*	Eye span	Body length	Thorax width	Allometric slope*
Cyrtodiopsis dalmanni						
1. Gombak, Malaysia	M	90	7.0 ± 0.1	6.1 ± 0.1	1.73 ± 0.02	1.99 ± 0.06
	F	89	5.1 ± 0.1	5.7 ± 0.1	1.69 ± 0.02	1.01 ± 0.03
2. Langat, Malaysia	M	135	7.1 ± 0.1	6.1 ± 0.1	1.66 ± 0.02	2.17 ± 0.05
	F	106	5.1 ± 0.1	5.8 ± 0.1	1.62 ± 0.02	1.04 ± 0.03
3. Bt. Lawang, Sumatra	M	72	7.2 ± 0.2	6.4 ± 0.1	1.87 ± 0.03	2.23 ± 0.06
	F	49	5.2 ± 0.1	6.0 ± 0.1	1.76 ± 0.04	0.99 ± 0.03
4. Soraya, Sumatra	M	24	7.9 ± 0.3	6.3 ± 0.1	1.70 ± 0.03	2.02 ± 0.13
	F	43	5.1 ± 0.1	5.7 ± 0.1	1.60 ± 0.03	1.07 ± 0.07
5. Brastagi, Sumatra	M	27	10.6 ± 0.7	7.3 ± 0.2	1.83 ± 0.05	3.26 ± 0.13
	F	38	6.0 ± 0.1	6.8 ± 0.1	1.82 ± 0.04	1.23 ± 0.04
6. Bogor, Java	M	74	7.5 ± 0.2	6.5 ± 0.1	1.81 ± 0.03	2.04 ± 0.05
	F	39	5.6 ± 0.1	6.4 ± 0.1	1.81 ± 0.03	1.01 ± 0.04
7. Belalong, Borneo	M	19	7.7 ± 0.3	6.7 ± 0.1	1.85 ± 0.04	1.92 ± 0.11
	F	14	5.2 ± 0.2	6.0 ± 0.2	1.66 ± 0.08	0.94 ± 0.09
8. Cameron, Malaysia	M	14	10.2 ± 0.2	7.3 ± 0.1	2.12 ± 0.04	1.94 ± 0.18
	F	12	6.3 ± 0.2	6.8 ± 0.2	1.94 ± 0.07	1.06 ± 0.09
Cyrtodiopsis whitei						
9. Gombak, Malaysia	M	78	7.1 ± 0.2	6.0 ± 0.1	1.46 ± 0.03	2.24 ± 0.05
	F	184	4.7 ± 0.1	5.6 ± 0.1	1.37 ± 0.01	1.15 ± 0.02
10. Chiang Mai, Thailand	M	41	7.3 ± 0.2	6.2 ± 0.1	1.61 ± 0.04	2.06 ± 0.10
	F	51	4.7 ± 0.1	5.9 ± 0.1	1.46 ± 0.03	1.04 ± 0.05
Cyrtodiopsis quinqueguttata						
11. Bt. Ringit, Malaysia	M	80	4.3 ± 0.1	7.0 ± 0.1	1.91 ± 0.02	0.69 ± 0.03
	F	57	4.3 ± 0.1	7.1 ± 0.1	2.02 ± 0.02	0.67 ± 0.04

*Least squares slope of the eye span on body length regression line.

Ulu Langat, Malaysia (3°5′N, 101°47′E), Brastagi, Sumatra (3°11′N, 98°28′E), the Soraya field station, Sumatra (2°52′N, 97°54′E), and the Kuela Belalong Field Station in Brunei, Borneo (4°30′N, 115°10′E). In September 2000 we collected *C. dalmanni* near Bukit Lawang, Sumatra (3°35′N, 98°6′E), and at a forestry research station in Bogor, Java (6°34′S, 106°50′E). We captured *Cyrtodiopsis whitei* near Chiang Mai, Thailand (19°9′N, 98°7′E), in January 1996 and at Ulu Gombak in August, 1999. *Cyrtodiopsis quinqueguttata* were collected near Bukit Ringit, Malaysia (3°42′N, 102°8′E), in January 1996. We classified flies to species based on morphological comparisons to specimens housed at the National Museum of Natural History, Washington, D.C. At each site individuals of each species were either preserved in ethanol or returned live to the laboratory to establish breeding populations.

Molecular analyses

To estimate genetic variation within and between populations we sequenced DNA from five field-collected flies from each population using QIAamp tissue extraction kits (QIAGEN). DNA sequence data were generated from fragments of two mitochondrial gene regions, cytochrome oxidase II (COII) and 16S ribosomal RNA (16S), and one nuclear gene region, *wingless*. We amplified the three gene fragments using primers and polymerase chain reaction (PCR) protocols optimized for diopsid flies (Baker *et al*. 2001). Single polymerase chain reaction (PCR) products were visualized on 1% agarose gels stained with ethidium bromide. Using amplifying primers, both strands of the products were sequenced using BigDye cycle sequencing chemistry (PE Applied Biosystems) on an ABI 310 or 3100 automated genetic analyser. Chromatographs were imported into SEQUENCHER (Gene Codes Corp.) for visual inspection and editing of the chromatographs. Individual alleles of heterozygous genotypes for *wingless* were determined using the haplotype subtraction method of Clark (1990). Complete sequences were then aligned manually; alignment was trivial given the low number of indels. The COII and 16S sequences were combined into a single data set and analysed together since the mitochondrial genome is nonrecombining and inherited as a single unit. Sequence data for *Diopsis apicalis* (COII, AF304777; 16S, AF304742; *wingless*, AF304811), *Diopsis fumipennis* (COII, AF304778; 16S, AF30743; *wingless*, AF304812) and *Eurydiopsis argentifera* (COII, AF304764; 16S, AF304729; *wingless*, AF304799) were obtained from GenBank (Baker *et al*. 2001) and included as outgroups to root the tree in the phylogenetic analyses. All COII (AY876495–AY876545, DQ098024–DQ098033), 16S (AY876546–AY876595, DQ098014–DQ098023), and *wingless*

(DQ098034–DQ098091) sequence data are accessible from GenBank.

Genetic polymorphism and population structure

For each population and species we determined the total number of distinct haplotypes and average number of nucleotide differences per site between sequences (π) for COII + 16S and *wingless* using the program DNASP version 4.0 (Rozas & Rozas 1999). The *wingless* data were checked for the possibility of recombinant haplotypes using split decomposition (Fitch 1971). This analysis was undertaken using the SPLITSTREE program (Huson 1998) based on the Kimura 3-ST method (Kimura 1981). No evidence of recombination was detected. Tajima's *D* (Tajima 1989) was calculated to test for selective neutrality of the COII + 16S and *wingless* sequences.

Analyses of molecular variance (AMOVA) based on haplotype data were used to compare relative levels of genetic variation among the populations and species (Excoffier *et al*. 1992). Our groupings were first by species and then by population within species. Additionally, we analysed the data for the *C. dalmanni* populations to the exclusion of the other species. The analyses of molecular variance used pairwise differences based on Tamura & Nei's (1993) model with a correction for substitution rate heterogeneity among sites ($\Gamma = 3.1667$ for COII + 16S; $\Gamma = 0.568$ for *wingless*) suggested by the best-fitting model identified by MODELTEST (see Results). The software ARLEQUIN version 2.0 (Schneider *et al*. 2000) was used for the analyses of molecular variance.

Phylogenetic analysis

Phylogenetic hypotheses based on the COII + 16S and *wingless* sequences were generated using neighbour-joining (NJ) and maximum-likelihood (ML) in PAUP* 4.0b10 (Swofford 1998). Optimal parameters of DNA substitution rates for genetic distances in the NJ and ML searches were obtained using MODELTEST 2.0 (Posada & Crandall 1998). Gaps were treated as missing data. NJ and ML analyses of each of the data sets resulted in very similar topologies, and only the NJ trees are presented here. Statistical support for nodes within the NJ tree was assessed using a bootstrap analysis of 10 000 replicate trees performed in PAUP* 4.0b10 (Swofford 1998). The parameters of the most likely model were used in the bootstrap analyses.

Coalescent estimation of population divergence times and migration rates

In order to determine if the *C. dalmanni* populations diverged in the presence of gene flow or in isolation, we conducted coalescent analyses using the *wingless* sequences to simultaneously estimate the parameters theta ($\theta = 4N_e\mu$), migration ($M = 2N_em$), divergence time ($T = t/2N_e$), and time to most recent common ancestor (TMRCA), where N_e is the effective population size, t is generation time, and μ is the per-locus mutation rate, between all conspecific populations and the larger groups by combining clades. We conducted similar analyses on the mtDNA sequences, taking into consideration that the mitochondrial genome is haploid in the calculation of each of the parameters; thus, $\theta = 2N_{ef}\mu$, $M = N_{ef}m$, and $T = t/N_{ef}$. Coalescent analyses were conducted using the software MDIV (Nielsen & Wakeley 2001). We used a HKY model of DNA substitution, and ran the analyses for 3×10^6 Markov chains with a 10% burn-in period as recommended by the authors. After repeated analyses with varying priors for M and T, we set maximum values of $M = 2$, and $T = 50$ for COII + 16S. For the *wingless* sequences, M was set to 3 and T was set to 30. We ran duplicate chains with different random seeds for all analyses to ensure convergence of the chains. Mutation rate, migration rate and divergence time were estimated from the maximum posterior probability of each distribution. Where possible, 95% credibility intervals were also estimated for each parameter. Values for N_e, t, and TMRCA for the COII + 16S data were estimated using a mutation rate, μ, of 2.018×10^{-5} substitutions per sequence per year, which is based on 2.3% sequence divergence per million years (Myr) calibrated for COI and COII of other insects (Brower 1994). For *wingless*, we used a mutation rate of 1.681×10^{-6} substitutions per sequence per year based on $D = 2Kt$, where D is the mean number of substitutions, K is the mutation rate, and t is the time of divergence. We used the data set of Baker *et al*. (2001) to calculate a mean pairwise genetic distance between *Sphyracephala* and diopsid species in the ingroup ($P = 0.2566$). The mean pairwise distance (p) is related to D by $D = -0.75 \ln(1 - 4p/3)$ (Hartl & Clark 1997). The time of divergence between these two groups was estimated at 75 million years ago (Ma) based on the fossil record (Lewis 1971; Feijen 1983).

Morphological comparisons

In order to determine the extent to which populations varied with regard to male ornaments and body size, we measured eye span, body length, and thorax width from field-captured flies (CO_2 anaesthetized or ethanol preserved) to the nearest 0.01 mm. NIH IMAGE version 1.59 software was used to measure digitized video images of flies placed under a dissecting microscope on their orbital and thoracic spines. Eye span was measured from the outer edge of the ommatidia, body length from face to wingtip, and thorax width from the widest point on each animal (Wilkinson 1993). See Table 1 for sample sizes and average measurements of each sex in each population.

Because adult body size is influenced by the amount of resources acquired during larval development, body size

can vary due to environmental factors. However, eye span varies linearly with body size in stalk-eyed flies and the slope of this allometric relationship often differs between sexes and species (Baker & Wilkinson 2001). Consequently, we report the least squares regression of eye span on body length for males and females and use mixed model analysis of covariance (ANCOVA) to compare the allometric slopes among populations by sex. In these analyses population is treated as a random effect using JMP version 5.0 (SAS 2003). To provide a second method for measuring change in eye span, we conducted a principal components analysis using all three morphological measurements. The first unrotated factor from this analysis quantifies overall body size while the second factor measures shape, which in this case is predominantly influenced by eye span, independent of size. Consequently, we compare second factor scores among populations and species by sex. We quantified morphological divergence between populations by computing the Euclidean distance between pairs of populations when female allometric slope is plotted against male allometric slope.

Because measures of divergence involve pairs of populations, they are not independent; therefore, the significance of any pattern of concordance cannot be determined by parametric statistics. Instead, we use Mantel tests to assess the degree to which morphological diversity correlates with genetic diversity, expressed as the uncorrected percent sequence divergence as described above. Genetic distances based on each of the two molecular data sets were compared separately against distances derived from morphological characters. For each comparison, the observed product–moment correlation between the two measures is compared to a distribution of 1000 correlation values computed by randomizing, without replacement, the morphological distance relative to genetic distance for each population pair.

Results

Genetic polymorphism

Haplotype diversity differed among the populations and the data sets. Based on the mitochondrial genes, a total of 30 unique haplotypes was identified among the 55 individuals surveyed. The 30 haplotypes were distinguished by 186 polymorphic sites, including five indels, 75 transitions, and 106 transversions (aligned data set available from author for correspondence upon request). Generally, mitochondrial haplotypes were unique to populations, except that the most common haplotype in Cameron is identical to a haplotype in the Langat population. The *wingless* sequences are less variable as only 18 unique haplotypes were found among the 48 individuals surveyed. Eleven individuals were identified as heterozygotes. The total data set contained 85 polymorphic sites, including 29 indels, 35 transitions, and 21 transversions. Tajima's D was not significantly different from zero for either the COII + 16S ($D = 1.17$; $P > 0.05$) or *wingless* data ($D = -0.16$; $P > 0.05$), suggesting selective neutrality of these regions and their suitability for making phylogeographical inferences.

Table 2 Summary of genetic diversity in each population and species for 889 bp of mitochondrial DNA sequence (COII + 16S) and 614 bp of nuclear DNA sequence (*wingless*). For each gene region, the number of individuals genotyped (N), number of haplotypes observed (n), and nucleotide diversity per site (π) per population are reported

	COII + 16S			*Wingless*		
Population	N	n	π	N	n	π
Cyrtodiopsis dalmanni						
1. Gombak, Malaysia	5	4	0.0016	4	4	0.0076
2. Langat, Malaysia	5	5	0.0032	5	7	0.0047
3. Bt. Lawang, Sumatra	5	2	0.0009	5	3	0.0027
4. Soraya, Sumatra	5	3	0.0011	3	4	0.0027
5. Brastagi, Sumatra	5	2	0.0004	4	2	0.0006
6. Bogor, Java	5	4	0.0016	5	4	0.0024
7. Belalong, Borneo	5	2	0.0004	3	3	0.0037
8. Cameron, Malaysia	5	2	0.0023	5	2	0.0007
All *C. dalmanni*	40	21	0.0436	34	13	0.0131
Cyrtodiopsis whitei						
9. Gombak, Malaysia	5	3	0.0029	4	1	0.0000
10. Chiang Mai, Thailand	5	3	0.0016	4	4	0.0033
All *C. whitei*	10	6	0.0059	8	4	0.0023
Cyrtodiopsis quinqueguttata						
11. Bt. Ringit, Malaysia	5	3	0.0082	6	3	0.0022

The number of haplotypes observed in populations ranged from two to five for the COII + 16S data set and one to seven for the *wingless* data set (Table 2). Based on the COII + 16S data, overall nucleotide diversity (π) among *Cyrtodiopsis dalmanni* populations ranged from a low of 0.0004 in the Brastagi and Belalong populations to a high of 0.0032 in the Langat population, whereas values ranged from 0.0006 in Brastagi to 0.0076 in Gombak for *wingless* sequences. For both data sets *C. dalmanni* exhibited the greatest amount of haplotype diversity at the species level, but at the population level, neither haplotype diversity nor nucleotide diversity differed significantly among the three species ($P > 0.05$; Kruskal–Wallis test).

Population structure

Analysis of molecular variance (AMOVA) on the COII + 16S data set indicated substantial structure among the species and populations. Interspecific differences accounted for 54.7% of the observed variation, and an additional 43.2% resided among populations within species. Remarkably, within *C. dalmanni*, 97% of the variation occurs among

populations. The *wingless* sequences indicated a similar pattern with 79.3% of the variation distributed among species and 18.3% occurring among the populations. Considering *C. dalmanni* alone, 88.7% of the variation in *wingless* is partitioned among the populations. All variance components were significant ($P < 0.05$).

Average genetic distance between individuals in the eight populations of *C. dalmanni* based on the proportion of nucleotide differences between individual sequences of COII + 16S was 0.045 ± 0.0008 whereas it was 0.006 ± 0.0005 between the two populations of *Cyrtodiopsi whitei.* In comparison, mean genetic distance among individuals across the three described species of *Cyrtodiopsis* is 0.064 ± 0.00087. Again, less divergence among populations and species was found within the *wingless* data set. Among the eight populations of *C. dalmanni* the average genetic distance was 0.023 ± 0.0004, and it was 0.002 ± 0.0004 for the two *C. whitei* populations. Across the three species, average genetic distance was 0.047 ± 0.0010.

Phylogenetic relationships

The COII + 16S and *wingless* data sets consisted of 889 characters and 614 characters of which 215 and 217 were phylogenetically informative, respectively. Hierarchical likelihood ratio tests and the Akaike information criterion implemented in MODELTEST (Posada & Crandall 1998) indicated the GTR model (Lanave *et al*. 1984) with invariable sites (I = 0.6487) and gamma-distributed rates with α = 3.1667 as the best fit for the COII + 16S sequences. Base frequencies used in the analyses were A = 0.3895, C = 0.1069, G = 0.1049, T = 0.3987, whereas substitution rates were A-C = 2.431, A-G = 7.729, A-T = 9.110, C-G = 3.727, C-T = 68.578, G-T = 1.00. The HKY (Hasegawa *et al*. 1985) model with gamma-distributed rates (α = 0.5608) was determined to be the best fit for the *wingless* sequences. Base frequencies used in the analyses were A = 0.3343, C = 0. 1810, G = 0.1629, T = 0.3219 with a transitions/transversion ratio of 1.6889.

Phylogenetic analysis of the two data sets resulted in very similar topologies, but populations were more genetically distinct for COII + 16S sequences than for *wingless* sequences (Fig. 2). For example, based on the COII + 16S sequences, all populations except Cameron and Langat are monophyletic, and all but one population (*C. whitei* Gombak) has significant bootstrap support (> 50%). By contrast, in the *wingless* data set only six populations are monophyletic, and only five of these (Bogor, Bt. Lawang, Brastagi, and Bt. Ringit) have high bootstrap support. While the COII + 16S sequences support the monophyly of the three species with at least 68% bootstrap support, the *wingless* sequences show a polytomy between *C. whitei* and *C. dalmanni* with *Cyrtodiopsi quinqueguttata* as sister to this group. Within *C. dalmanni*, the eight populations form three distinct clades, with Brastagi sister to the remaining populations. Both the COII + 16S and *wingless* sequences indicated a close relationship among the individuals of the Cameron and Langat populations, and the clade encompassing these two populations is strongly supported for both sequence sets (97–100%; Fig. 2).

Estimates of migration rate and population divergence time

Coalescent analysis of both data sets indicated very low levels of gene flow between populations as estimates of *M*

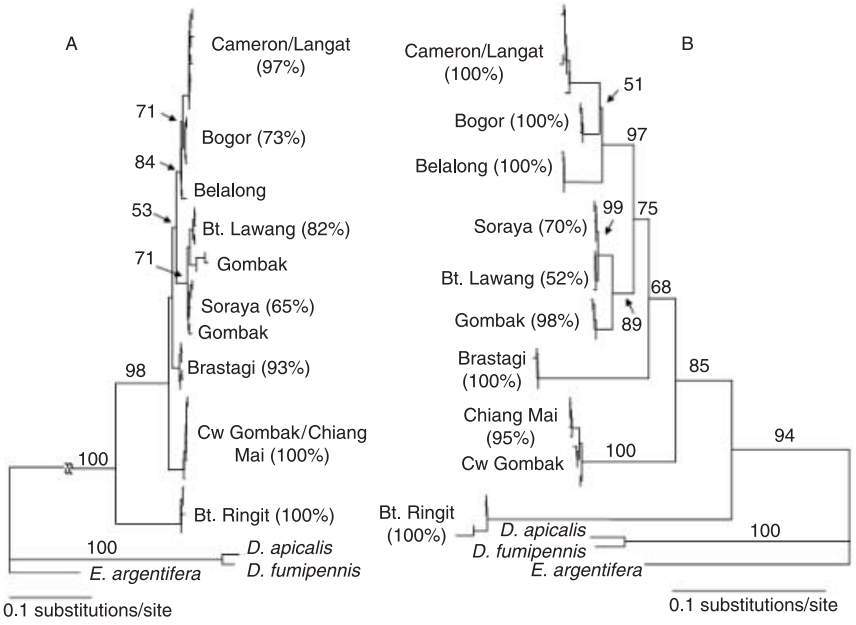

Fig. 2 Neighbour-joining tree based on maximum-likelihood distances for 614 base pairs of the nuclear gene, *wingless* (A) and 889 base pairs of mitochondrial fragments of cytochrome oxidase II and 16S ribosomal subunit genes (B). Genetic distances for the *wingless* sequences are based on a HKY model with gamma-distributed rates while genetic distances for COII + 16S sequences are based on a GTR model with invariable sites and gamma-distributed rates (see text for details). For both data sets, the tree topologies are congruent with phylogenetic hypotheses generated by maximum-parsimony and maximum-likelihood analyses. Bootstrap support for each branch is provided above each node or after the population name. In (A), the branch leading to *Cyrtodiopsis* is longer than depicted.

Table 3 Coalescent-based joint maximum-likelihood estimates for effective population size (N_e), migration rate (M), and time of genetic divergence (TMRCA) based on COII + 16S or *wingless* DNA sequences for all pairwise comparisons using the method of Nielsen & Wakeley (2001). TMRCA is expressed in millions of years ago (Ma) and is based on a generation time of 2 months. Credibility intervals of 95% are given in brackets for estimates of M. Undefined bounds (udf) were those for which the likelihood estimate did not reach zero. The mutation rate (μ) used to determine N_e is 2.018×10^{-5} substitutions per sequence per year for the COII + 16S data and 1.681×10^{-6} substitutions per sequence per year for the *wingless* data. See Fig. 2 for clade descriptions

	COII + 16S			*Wingless*		
Comparison	N_e	M	TMRCA	N_e	M	TMRCA
Brastagi vs. Belalong	235 385	0.004 [0–0.23]	1.21	1 599 036	0.03 [0–1.60]	3.15
Brastagi vs. Soraya	256 789	0.004 [0–0.32]	1.16	1 387 223	0.006 [0–1.15]	4.07
Brastagi vs. Bt. Lawang	234 779	0.004 [0–0.26]	1.10	1 467 612	0.006 [0–0.94]	5.14
Brastagi vs. Cd Gombak	232 027	0.004 [0–0.39]	0.09	1 272 038	0.06 [0–1.56]	2.00
Brastagi vs. Cameron	278 178	0.004 [0–0.35]	1.14	944 950	0.006 [0–0.86]	3.68
Brastagi vs. Langat	453 964	0.012 [0–0.66]	1.42	2 980 906	0.048 [0–1.20]	5.08
Brastagi vs. Bogor	242 116	0.004 [0–0.34]	1.01	1 276 320	0.006 [0–0.98]	3.66
Belalong vs. Soraya	189 536	0.004 [0–0.32]	0.79	2 125 268	0.06 [0–1.81]	3.37
Belalong vs. Bt. Lawang	183 410	0.004 [0–0.308]	0.78	2 150 874	0.018 [0–1.86]	4.28
Belalong vs. Cd Gombak	223 162	0.004 [0–0.50]	0.84	1 475 286	0.036 [0–2.24]	2.37
Belalong vs. Cameron	256 789	0.008 [0–0.87]	0.64	1 329 497	0.048 [0–2.12]	1.69
Belalong vs. Langat	406 572	0.016 [0–0.91]	0.82	3 088 865	0.102 [0–1.79]	2.29
Belalong vs. Bogor	173 321	0.004 [0–0.66]	0.53	1 566 827	0.096 [0–2.15]	1.45
Soraya vs. Bt. Lawang	150 404	0.068 [0–1.52]	0.08	1 400 339	0.078 [0–1.69]	1.85
Soraya vs. Cd Gombak	299 582	0.008 [0–0.97]	0.46	955 657	0.162 [udf]	1.05
Soraya vs. Cameron	365 610	0.008 [0–0.64]	1.04	1 544 165	0.012 [0–1.27]	4.51
Soraya vs. Langat	464 658	0.008 [0–0.95]	0.99	3 073 876	0.078 [0–1.16]	4.54
Soraya vs. Bogor	304 468	0.004 [0–0.8]	0.85	1 995 896	0.012 [0–1.05]	4.71
Bt. Lawang vs. Cd Gombak	272 067	0.02 [0–0.992]	0.42	965 114	0.38 [0.17-udf]	1.11
Bt. Lawang vs. Cameron	315 480	0.004 [0–0.56]	0.98	1 415 418	0.006 [0–1.242]	4.95
Bt. Lawang vs. Langat	348 487	0.008 [0–0.72]	0.79	3 164 258	0.042 [0–1.17]	5.65
Bt. Lawang vs. Bogor	265 653	0.004 [0–0.692]	0.78	1 900 785	0.018 [0–1.02]	5.52
Cd Gombak vs. Cameron	326 477	0.008 [0–0.57]	1.06	1 430 585	0.072 [0–1.95]	2.32
Cd Gombak vs. Langat	518 464	0.016 [0–0.77]	1.07	1 177 016	0.054 [0–1.45]	1.94
Cd Gombak vs. Bogor	385 169	0.008 [0–0.708]	0.92	1 579 140	0.042 [0–1.458]	2.42
Cameron vs. Langat	271 462	1.83 [0.11–1.92]	0.12	1 520 967	1.78 [0.48-udf]	0.96
Cameron vs. Bogor	386 696	0.036 [0–1.024]	0.64	1 169 700	0.048 [0–1.52]	1.86
Langat vs. Bogor	487 893	0.04 [0–1.112]	0.62	2 753 212	0.108 [0–1.43]	2.30
Cw Gombak vs. Chiang Mai	329 533	0.072 [0–1.39]	0.19	893 201	2.93 [0.41-udf]	0.90
Brastagi vs. other *C. dalmanni*	2 506 443	0.06 [0–1.536]	1.73	2 573 519	0.120 [0–0.99]	2.31
Bogor vs. Cameron/Langat	482 718	0.032 [0–0.82]	0.57	2 315 935	0.102 [0–0.918]	2.18
Belalong vs. Bogor/Cameron/Langat	883 641	0.064 [0–1.34]	0.74	4 581 817	0.120 [0–1.37]	2.83
Belalong/Bogor/Cameron/Langat vs. Bt. Lawang/Gombak/Soraya	1 399 409	0.032 [0–0.45]	1.18	1 477 248	0.048 [0–0.43]	2.15
Cd Gombak vs. Bt. Lawang/Soraya	363 044	0.028 [0–0.656]	0.47	NA	NA	NA
C. quinqueguttata vs. *C. dalmanni*/*C. whitei*	3 878 481	0.172 [0–1.42]	2.56	4 756 156	0.018 [0–0.62]	11.30
C. dalmanni vs. *C. whitei*	2 800 879	0.148 [0–1.24]	1.89	3 399 714	0.072 [0–0.46]	3.45

are typically less than 0.05 with a credibility interval encompassing zero (Table 3). Estimates of N_{ef} derived from the mitochondrial data are two to nine times smaller than estimates of N_e based on the nuclear data. Estimated effective population sizes range from *c*. 200 000–500 000 for the COII + 16S sequences to more than 3 million for the *wingless* sequences (Table 3). Consequently, the times of population and genetic divergence were estimated to be much older for the *wingless* sequences than for the COII + 16S sequences. For many of the comparisons, population divergence was estimated to be older than genetic divergence, providing little confidence in our ability to estimate exactly when these populations began to diverge from one another. Thus, these findings are consistent with a scenario of very old divergence times and very little gene flow since these populations began to diverge. There is one notable

Table 4 Results of mixed model ANCOVAs for eye span using population as a random effect and body length (BL) as a covariate for each sex

Source	d.f.	Mean square	*F*	*P*
Males				
Body length	1	555.2	4399.2	< 0.0001
Population	10	46.9	3.5	0.031
Body length × Population	10	8.1	64.2	< 0.0001
Residual	634	0.13		
Females				
Body length	1	171.9	4681.9	< 0.0001
Population	10	2.4	4.2	0.021
Body length × Population	10	0.3	9.0	< 0.0001
Residual	660	0.04		

exception found in both data sets. Two populations, Cameron and Langat, shared haplotypes and exhibited the lowest genetic distances observed for both data sets, suggesting that they have either maintained genetic contact through migration or retained ancestral polymorphisms, making them appear genetically similar. Migration rates for Cameron and Langat were estimated to be 1.83 individuals per generation based on COII + 16S sequences and 1.78 individuals per generation based on *wingless* sequences.

Morphological variation among populations

Mixed model ANCOVA explained 97.1% and 94.6% of the variation in eye span for males and females, respectively. Body length was a highly significant predictor of eye span and exhibited a highly significant interaction with population in both sexes (Table 4), indicating that changes in eye span allometry have occurred in both males and females (Fig. 4). Differences in eye span attributable to population were weakly significant. Tukey HSD tests revealed that the only significantly different population was Bt. Ringit, the sole population of *C. quinqueguttata*. Re-analysis of the data by ANCOVA after excluding this population resulted in highly significant effects of body length and body length by population interaction, but no effect of population, for both sexes. The source of the interaction between body length and population can be attributed to significant change in eye span on body length allometry for the Brastagi population (Fig. 4). In this population male eye span scales linearly with more than a threefold increase in body length (cf. Fig. 3).

The first two factors from the principal component analysis of all 1370 flies explained 96.3% of the variation in the three morphological measurements. Factor 1 explained 78.9% and was calculated as 0.513 (eye span) + 0.619 (body length) + 0.595 (thorax width) while factor 2 was calculated as 0.847 (eye span) – 0.253 (body length) – 0.467 (thorax

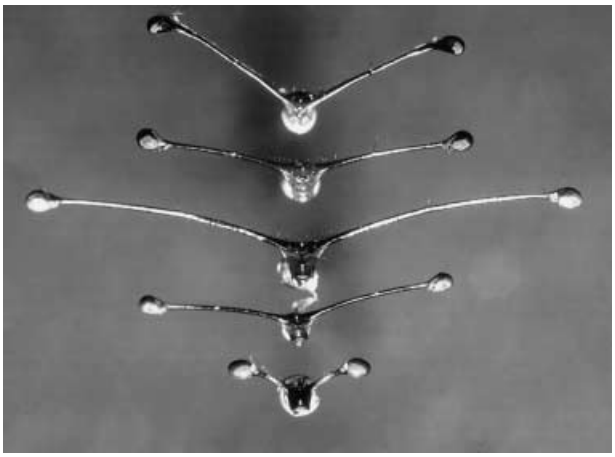

Fig. 3 Heads of large males from the following populations, top to bottom: *Cyrtodiopsis dalmanni* from Belalong, Gombak, and Brastagi, *Cyrtodiopsis whitei* from Gombak, and *Cyrtodiopsis quinqueguttata* from Bukit Ringit.

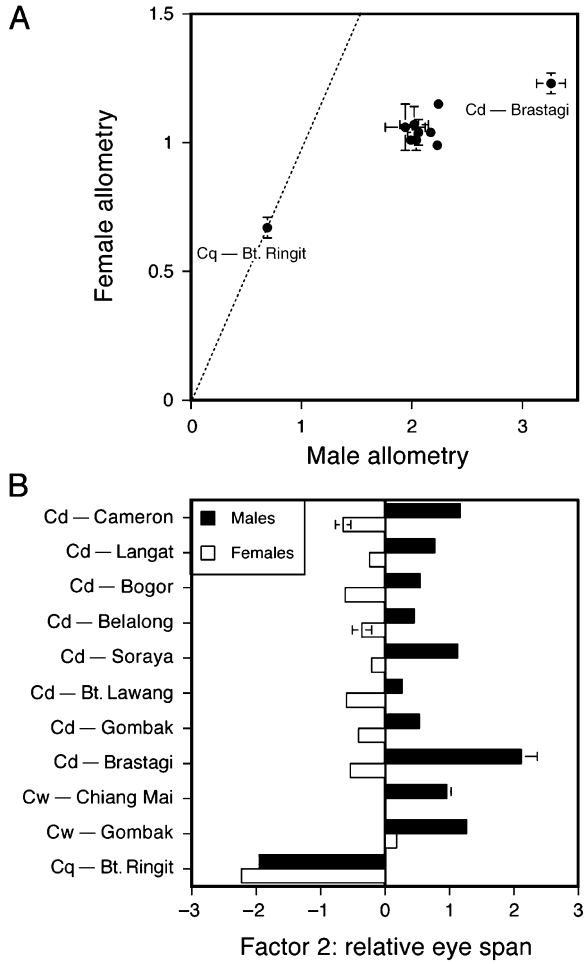

Fig. 4 (A) Slope ± SE for the least squares regressions of eye span on body length for females and males from each population. The dashed line indicates sexual monomorphism for eye span on body length allometry. (B) Mean ± SE for the second principal component scores for flies of each sex from each population.

Table 5 Nested ANOVAs for each principal component by sex using species (*Cyrtodiopsis dalmanni* and *Cyrtodiopsis whitei*) and populations within species as factors

Source	d.f.	Mean square	*F*	*P*
Factor 1: Body size — Females				
Species	1	120.35	6.4	0.0358
Population (Species)	8	10.70	12.8	< 0.0001
Residual	615	1.25		
Factor 2: Eye span — Females				
Species	1	14.73	17.9	0.0031
Population (Species)	8	0.70	11.2	< 0.0001
Residual	615	0.06		
Factor 1: Body size — Males				
Species	1	114.76	4.6	0.0633
Population (Species)	8	27.50	13.4	< 0.0001
Residual	564	2.05		
Factor 2: Eye span — Males				
Species	1	2.20	0.4	0.5227
Population (Species)	8	5.49	57.1	< 0.0001
Residual	564	0.10		

width). Factor 1 is most strongly correlated with body length ($r = 0.95$, $P < 0.0001$) while factor 2 is most strongly correlated with eye span ($r = 0.61$, $P < 0.0001$) across all individuals. After excluding *C. quinqueguttata*, nested ANOVAS revealed significant effects of species and populations within species for both factors in females, but only significant effects of populations within species for both factors in males (Table 5). Figure 4(B) illustrates the variation for males and females among populations and species in the second factor. The most divergent populations are male and female *C. quinqueguttata*, which have much shorter relative eye span and male *C. dalmanni* from Brastagi, which have much longer relative eye span than any other populations.

Comparison of morphological divergence, as measured by the geometric difference in allometric slopes, to genetic divergence between populations reveals significantly positive relationships for both genetic data sets (Fig. 5; $r = 0.68$, $P < 0.001$ for COII + 16S; $r = 0.69$, $P < 0.05$ for *wingless*, Mantel tests). However, the significance of these relationships depends entirely on the inclusion of two populations — *C. quinqueguttata* from Bt. Ringit and the Brastagi population of *C. dalmanni*. When these two populations are excluded, morphological distance is uncorrelated to genetic distance ($r = 0.02$, $P > 0.10$ for both COII + 16S and *wingless*, Mantel tests).

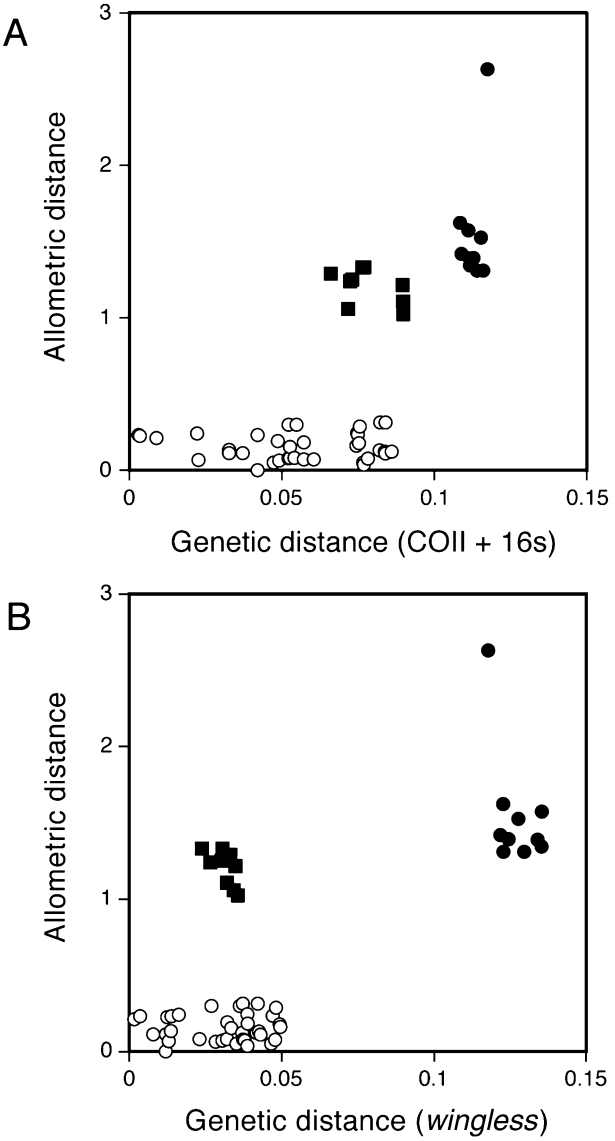

Fig. 5 Euclidean distance for allometric slopes of males and females (cf. Fig. 4A) plotted against the proportion of nucleotide substitutions between all pairs of populations for 889 base pairs of mitochondrial fragments of cytochrome oxidase II and 16S ribosomal subunit (A) or 614 base pairs of the nuclear gene, *wingless* (B). Pairs involving *Cyrtodiopsis quinqueguttata* are indicated with filled circles and pairs involving *Cyrtodiopsis dalmanni* from Brastagi are indicated with filled squares.

Discussion

Genetic and morphological divergence

Our analyses indicate that populations of sexually dimorphic stalk-eyed flies exhibit a high degree of genetic structure despite, in some instances, close geographical proximity (cf. Figs 1 and 2). For example, over 80% of the genetic variation in either nuclear or mitochondrial sequences observed across individuals within *Cyrtodiopsis dalmanni* is due to differences among populations. In the phylogenetic analysis of COII + 16S sequences, six of the eight putative *C. dalmanni* populations, as well as the Chiang Mai (*Cyrtodiopsis whitei*) and *Cyrtodiopsis quinqueguttata* populations, were unambiguously identified as monophyletic

units with significant bootstrap support. Within *C. dalmanni*, both data sets also support the presence of three distinct clades among the eight populations, with the Brastagi population sister to the remaining populations (Fig. 2). The monophyly of each of these clades was strongly supported by bootstrap analysis of the mtDNA sequences. In comparison, the degree of genetic divergence among these clades (5.3–7.3% for the COII + 16S sequences) is comparable to that observed among sister species of *Drosophila*. Average genetic distances among all species in a variety of *Drosophila* species groups [e.g. *obscura* (Beckenbach *et al*. 1993), *saltans* (O'Grady *et al*. 1998), *melanogaster*, *willistoni* (O'Grady & Kidwell 2002), *buzzatii* (Spicer 1995), *quinaria* (Spicer & Jaenike 1996)] falls between 5% and 12% for the same COII sequence. In contrast, sibling species and population samples typically display lower genetic distances. For example, in the *Drosophila saltans* species group COII sequence divergence for sibling species and populations fell between 2% and 3% and < 2%, respectively (O'Grady *et al*. 1998).

In contrast to the high amount of genetic divergence we found between most populations, our analyses of eye span allometry show that morphology has exhibited relatively little change among populations of both sexually dimorphic species with one notable exception: the population of *C. dalmanni* from Brastagi. Males from this population exhibit a much steeper eye span allometry, which for large individuals results in an eye span twice that of males from any other population of either species (Fig. 3). This extreme eye span allometry is not due to unusual rearing conditions as the relative eye span of Brastagi flies raised in the lab does not differ from flies of similar body size collected in the field (G.S.W., personal observation). While differences in relative eye span were also detected among other populations using principal components, these differences were quantitatively much smaller. The only other conspicuous difference involved Belalong flies, which display eye stalks that project upwards (cf. Fig. 3).

If sexual selection has driven speciation among species of stalk-eyed flies, we would expect morphological diversification to accompany genetic diversification. Instead, genetic differentiation appears to be largely decoupled from morphological change (cf. Fig. 5). Evolutionary change in relative eye span is clearly not constrained by lack of genetic variation as the Brastagi population has much longer relative eye span than any other *C. dalmanni* or *C. whitei* population despite evolving within the clade. This conclusion is reinforced by experimental data, which show that eye span allometry responds to artificial selection (Wilkinson 1993). Our results are consistent with recent reports that interpopulation morphological uniformity in secondary sexual traits can mask underlying genetic diversity in pseudoscorpions (e.g. Wilcox *et al*. 1997). Zeh & Zeh (1994) proposed that sexual selection may oscillate between favouring small and then large males, maintaining a high level of morphological variation within populations. This explanation seems less likely for stalk-eyed flies because female choice appears to favour large males consistently in sexually dimorphic species (Burkhardt & de la Motte 1988; Wilkinson *et al*. 1998b).

Alternatively, Emerson & Ward (1998) suggested that absence of a correlation between morphological and genetic distance in fanged ranid frog populations results from a balanced equilibrium between natural and sexual selection. The idea that eye stalks might impose a handicap (e.g. Zahavi 1975) is consistent with measurements of flight performance (Swallow *et al*. 2000) which show that male *C. quinqueguttata* with short eye stalks are more manoeuvrable and faster fliers than male *C. whitei* with long eye stalks. Thus, increased eye span seems likely to reduce flight performance. If flight performance influences predation success, then sexual selection for long eye span could be balanced by natural selection against long eye span. If natural selection does balance sexual selection, then the *C. dalmanni* population in Brastagi must experience a different selective environment than the other *C. dalmanni* or *C. whitei* populations. Further ecological study of this and related populations is clearly warranted.

Comparisons between nuclear and mitochondrial genomes

Mitochondrial gene regions have been widely used to study phylogeographical patterns in animals (e.g. Avise 1994), but evolutionary inferences based on mitochondrial markers can have limitations. For example, Ballard & Whitlock (2004) point out that inconsistencies have been found between gene trees and species trees as a result of the nonrecombining nature of the mitochondrial genome, introgression that masks other historical evolutionary events, and the potential for natural selection to affect mitochondrial genes, either directly or indirectly, through cytonuclear interactions. To date relatively few studies have jointly evaluated population genetic variation and historical demography within natural species using markers from multiple genomes. In contrast to other studies in which discrepancies in evolutionary inferences have been suggested by nuclear and mitochondrial data sets (e.g. Powell 1983; Bernatchez *et al*. 1995; Lu *et al*. 2001; Shaw 2002; Sota 2002; Rognon & Guyomard 2003), our results from analysis of mitochondrial sequences are largely consistent with inferences drawn from the nuclear sequences. Both gene regions indicate that most populations have achieved reciprocal monophyly. Furthermore, coalescent analyses using both data sets suggest that monophyly is the result of long divergence times with little or no migration between populations.

Placing exact dates on evolutionary divergence events is still controversial despite methodological advances. In

theory, the time to the most recent common ancestor for nuclear genes is four times that of mitochondrial genes due to their haploid and maternal inheritance. However, we estimate that the date of genetic divergence between *C. dalmanni* populations based on nuclear DNA sequences is nearly three times older than that estimated for the mitochondrial sequences while the effective population sizes based on the mitochondrial markers differ in many cases from those estimated from *wingless* sequences by more than four-fold (Table 3). Several factors likely contribute to these results. The expected fourfold difference between nuclear and mitochondrial DNA assumes autosomal inheritance, equal sex ratios, and constant mutation rates. Evidence for X chromosome meiotic drive has been found in most of these populations (Wilkinson *et al*. 2003) and likely causes female-biased adult sex ratios. In combination with high levels of polygyny and X-linked inheritance, the effective population size of a nuclear marker will approach that of a mitochondrial marker (Wright *et al*. 2004). Furthermore, mutation rate is necessary for determination of N_e, and differences in branch lengths between the two trees (Fig. 2) suggest that the two markers used in this study have evolved at different rates. Additionally, Markovtsova *et al*. (2000) have shown that heterogeneity in mutation rates across sites may cause estimates of TMRCA to be overestimated. Nevertheless, it is noteworthy that genetic divergence times for all population comparisons within *C. dalmanni* are positively correlated ($r = 0.61$, $P < 0.01$, Mantel test) across the two data sets, thereby suggesting congruent evolutionary patterns that only differ in rate. In addition to these differences, credibility intervals of population divergence time are quite large, being undefined at the upper end for all comparisons and for both data sets. Independent estimates of mutation rates for each marker, as well as additional genetic markers, are likely to provide refined estimates of population divergence times.

Demographic history of C. dalmanni *populations*

Despite close geographical proximity between several of the *C. dalmanni* populations (cf. Fig. 1), coalescent analyses indicate that almost all of the populations have been isolated for long periods of time. For many population comparisons, genetic divergence is estimated to have occurred more recently than population divergence. This is expected if population divergence time is long and low levels of gene flow continued between the populations for some period of time after the divergence began (R. Nielsen, personal communication.). Analyses of the two data sets agree that most populations diverged some time during the Pleistocene. Additionally, for both data sets, migration rates are estimated at less than one migrant every generation between all populations except Cameron and Langat and the two populations of *C. whitei* (Table 3).

The geological history of Southeast Asia is consistent with a genetic structure among populations that has been determined by range expansion through long distance colonization. The flies collected for this study came from northern Sumatra, Java, peninsular Malaysia, and northern Borneo. Although these regions are now separated by water, geological data indicate that they have periodically formed a contiguous land mass, referred to as the Sunda Shelf, since the Miocene (Heaney 1991), with the last period of connection occurring approximately 11 000 years ago at the end of the Pleistocene (Hanebuth *et al*. 2000). This region is characterized by extensive and recurrent volcanic activity. Volcanic eruptions could have destroyed local populations that were subsequently recolonized. For example, Mount Toba in northern Sumatra underwent an enormous eruption around 70 000 years ago and may have caused extirpation of regional flora and fauna (Ambrose 1998). This eruption resulted in the formation of Lake Toba, which is currently situated directly between the Soraya and Bt. Lawang collection sites. The genetic divergence time we estimated of approximately 80 000 years ago based on COII + 16S sequences suggests that these populations were either split by the eruption of Mount Toba or colonized afterwards from a similar source population, such as peninsular Malaysia. Presumably, the Brastagi population, a close geographical neighbour to Soraya and Bt. Lawang, was also colonized after this time but from a different source population. Southern Sumatra is a likely source for such colonists. Additional specimens of *C. dalmanni* with extremely long eye stalks are present in the collections at the US National Museum of Natural History (Washington, D.C.) and were collected from southern Sumatra sites.

Traditionally, evolutionary inference has been made by quantifying genetic variation at the phenotypic or genotypic level in species without explicit hypotheses about the demography or biogeography of the populations. At the species level and above, phylogenetic methods are generally good at depicting patterns of species descent, which are assumed to have dichotomous relationships. Phylogenetic methods are, however, often not suitable for studying genealogical patterns within species because demographic factors, such as migration, are not considered in tree construction. Because phylogenetic methods were generally good at depicting patterns of descent among *Cyrtodiopsis* populations, we suggest that phylogenetic methods are likely to be a useful tool in identifying cryptic species in this group.

Evidence in support of cryptic *Cyrtodiopsis dalmanni* species has recently been obtained by a series of cross-population mating studies, which indicate that most of the populations used in this study exhibit some form of reproductive isolation (Christianson *et al*. 2005). For example, crosses between the two *C. dalmanni* clades (Belalong-Bogor-Cameron-Langat and Bt. Lawang-Gombak-Soraya;

Fig. 4) either fail to produce any offspring or produce sterile hybrids while crosses between populations within clades tend to produce sterile males. Furthermore, in contrast to the results of this study, measurements of internal reproductive traits indicate that all populations of *C. dalmanni* exhibit significant differences in sperm length and female sperm storage organ size (E. Amitin & G. Wilkinson, unpublished). Thus, evolutionary change among *C. dalmanni* populations can be detected for traits that are involved in determining fertilization success after mating. Speciation in stalk-eyed flies may therefore be influenced more strongly by postcopulatory than precopulatory sexual selection, as has been suggested for other taxa (Arnqvist *et al*. 2000; Martin & Hosken 2003; Pitnick *et al*. 2003). This inference leads us to conclude that the dramatic differences in male head shape that characterize many diopsid species (e.g. Baker & Wilkinson 2001) likely evolved in most cases after reproductive isolation of fragmented populations, at least in Southeast Asia. The absence of variation in eye stalk allometry among most populations of *C. dalmanni* indicates that stabilizing selection, rather than genetic variation, constrains eye span evolution. We expect that similar patterns will be found in a variety of other species where both precopulatory and postcopulatory sexual selection operate.

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This study began when John Swallow was an NIH postdoctoral fellow working with Jerry Wilkinson at the University of Maryland. John is now an Assistant Professor of Biological Science at the University of South Dakota. He is an evolutionary physiologist interested in how behavior coadapts with morphology and physiology. He has recently begun investigating how evolution driven by sexual selection conflicts with locomotor performance in stalk-eyed flies. Lisa Wallace is a Research Assistant Professor at the University of South Dakota. She is interested in using molecular data to address systematic and population genetic questions in plants and animals. Sarah Christianson is a graduate student and is investigating the mechanisms underlying speciation in stalk-eyed flies for her dissertation. Philip Johns is a postdoctoral researcher who uses molecular tools to study social evolution in insects, including sexual selection in stalk-eyed flies. Jerry Wilkinson is a Professor at the University of Maryland. He has spent over 15 years developing stalk-eyed flies as a model experimental system for studying the process and consequences of sexual selection. For this study, John, Jerry and Sarah collected flies, John, Sarah and Philip obtained sequence data, and John, Lisa and Jerry conducted the analyses reported in this paper.
