Sex-linked Correlated Responses in Female Reproductive Traits to Selection on Male Eye Span in Stalk-eyed Flies¹

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SYNOPSIS. Coevolution between male and female traits can result from correlated responses to selection or correlated selection on genetically independent traits. This study examines the possibility that traits involved in precopulatory sexual selection may influence the evolution of traits involved in postcopulatory sexual selection due to the existence of correlated selection or correlated responses to selection. Artificial selection on male eye span in Cyrtodiopsis dalmanni, a sexually dimorphic stalk-eyed fly, is used to test for correlated changes in reproductive traits of male and female flies. Flies from replicate lines that had been under selection for 57 generations were matched for age and genotyped at four X-linked microsatellite loci. Egg number and testis size increased with age, but did not differ among lines. Spermathecal areas and duct lengths differed among replicates, but not among selection treatments. Female relative eye span, size of the ventral receptacle and egg size exhibited significant correlated responses to selection on male relative eye span. The absence of any change in sperm length or testis size between lines indicates that changes in female traits are unlikely due to correlated selection mediated by sperm competition. Significant effects of X-linked microsatellite genotypes indicate instead that the correlated responses to selection were due, in part, to X-linked genes in linkage disequilibrium or that exhibit pleiotropy. The presence of nonadditive allelic effects on genetically correlated female traits combined with additive allelic effects on a male ornament provides a previously unrecognized mechanism by which genetic variation could be maintained despite strong sexual selection.

INTRODUCTION

Comparative studies in insects (Dybas and Dybas, 1981; Morrow and Gage, 2000; Pitnick et al., 1999; Pitnick et al., 2003; Presgraves et al., 1999) and birds (Briskie and Montgomerie, 1993; Briskie et al., 1997) have found evidence for correlated evolution between male and female reproductive traits while recent experimental studies on Drosophila melanogaster have shown that selection for longer sperm leads to changes in female sperm storage organ size (Miller and Pitnick, 2002). These examples of male-female coevolution could result from either correlated responses to selection, correlated selection or both. Correlated responses in female traits are expected under several models of sexual selection. For example, both runaway (Kirkpatrick, 1982; Lande, 1981) and good genes (Iwasa et al., 1991; Pomiankowski, 1987) models predict that linkage disequilibrium will accumulate as a result of female choice and cause coevolutionary change in male ornaments and female preferences. Evidence in support of a genetic correlation between precopulatory ornaments and preferences has been obtained from artificial selection (Houde, 1994; Wilkinson and Reillo, 1994) and breeding studies (Bakker, 1993; Gray and Cade, 2000).

Alternatively, correlated selection can lead to coevolution between genetically independent traits if change in one trait influences how selection operates on the other trait. For example, antagonistic coevolution due to sexual conflict (Gavrilets et al., 2001; Parker, 1979; Parker and Partridge, 1998) occurs when a trait that enhances male competitive ability leads to correlated selection on females to resist mating. Experimental evidence in support of antagonistic coevolution comes from studies on Drosophila melanogaster which show that when males are permitted to evolve greater offensive and defensive mating ability, females exhibit higher mortality (Rice, 1996). Selection on the reproductive tract of females could also produce correlated selection on sperm size or number to the extent that females create the arena in which sperm interact prior to fertilization (Eberhard, 1996).

Connecting the pattern of variation in reproductive traits observed among taxa to the process responsible for producing that pattern requires an understanding of both the pattern of selection and the genetic architecture. Disentangling effects of correlated selection from those due to correlated responses to selection is complicated by pleiotropy and genetic linkage because these factors can cause genetic correlations between traits involved in different biological functions. Given that most animals have thousands of genes, linkage might seem unlikely; however, the opportunity for linkage increases if selection favors gene clustering in the genome. For example, several scenarios could cause genes that influence reproductive traits to occur preferentially on the sex chromosomes. If a gene affects a trait that increases male competitive ability but is costly to females, then it will spread more rapidly when recessive and X-linked (Charlesworth et al., 1987; Rice, 1984). Alternatively, if selection fluctuates on males, then X-linked ornamental traits are expected to persist longer than autosomal traits (Reinhold, 1981; Morrow and Gage, 2000; Pitnick et al., 1999; Pitnick et al., 2003; Presgraves et al., 1999).

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In male heterogametic species, X chromosomes occur twice as often in females than males assuming an equal sex ratio. Consequently, X-linked traits experience stronger selection in females and weaker selection in males each generation. Under a good genes model of sexual selection X-linked inheritance of female preferences and male traits changes female preferences more than autosomal inheritance of preferences and traits (Kirkpatrick and Hall, 2004).

Evidence in support of X-linked inheritance of sexually selected traits is mixed. Reviews of the inheritance of sexually selected traits based on line crosses have found a bias towards X-linkage (Lindholm and Breden, 2002; Reinhold, 1998). Similarly, genes expressed in the testes tend to be X-linked in mice (Wang et al., 2001), as are genes associated with reproduction in humans (Saﬁ and Chandra, 1999). In contrast, in Drosophila melanogaster a survey of gene annotations found no evidence for an X-linked bias of genes involved in mating behavior, although such genes tend to have pleiotropic effects (Fitzpatrick, 2004), and genes with male-biased expression were signiﬁcantly less likely to be found on the X chromosome (Parisi et al., 2003; Ranz et al., 2003). However, the X chromosome contained the majority of genes with sexually antagonistic effects on ﬁtness (Gibson et al., 2002).

In this study we examine the possibility that traits involved in precopulatory sexual selection may inﬂuence the evolution of traits involved in postcopulatory sexual selection due to the existence of correlated selection or correlated responses to selection. We also evaluate the possibility that these traits exhibit linkage to regions of the X chromosome. After exerting selection on a trait known to inﬂuence mating success in males we measure male and female traits involved in determining fertilization success after copulation and test for evidence of correlated change. We use the stalk-eyed ﬂy, Cyrtodiopsis dalmani, because this species experiences intense precopulatory and postcopulatory sexual selection. Male relative eye span inﬂuences the outcome of male-male competition (Panhuis and Wilkinson, 1999) and female mate choice (Wilkinson et al., 1998a; Wilkinson and Reillo, 1994). Selection imposed by sperm competition is expected to be high due to extreme promiscuity. In C. dalmani males compete for access to groups of females at dusk and then mate with multiple females at dawn (Wilkinson and Reillo, 1994). Females routinely mate multiple times per day (Wilkinson et al., 1998a), do not reduce mating activity after copulation (Grant et al., 2002) and live for many months (Wilkinson and Reillo, 1994). Multiple mating does not appear to have deleterious effects on females (Reguera et al., 2004). Instead, multiply mated females have higher fecundity than single mated females (Baker et al., 2001) probably because they receive more sperm. Males transfer seminal material in a spermatophore (Kotrba, 1990) but pass few sperm. On average, only 65 sperm are found in the spermathecae of a C. dalmani female after a single copulation (C.L. Fry, personal communication). In the closely related species, C. whitei, sperm survival can also be reduced by seminal ﬂuid from competitors (Fry and Wilkinson, 2004). Sperm are stored in three spermathecae, but must move to individual chambers in the seminal receptacle before entering an egg and effecting fertilization.

Comparative studies have revealed that sexually dimorphic eye span has evolved rapidly and recurrently among species of stalk-eyed ﬂies (Baker and Wilkinson, 2001). Furthermore, male and female reproductive traits exhibit evidence of correlated evolution. A comparative analysis utilizing 17 species of diopsids revealed dramatic differences in sperm length, presence or absence of sperm heteroplasmy, and correlated changes in the size, shape, complexity and location of sperm storage organs in females (Presgraves et al., 1999).

Several lines of evidence indicate that the X chromosome inﬂuences sexually selected traits in this species. Analysis of line cross data indicates that genes on the X chromosome explain 30% of the variation in relative eye span (Wolfenbarger and Wilkinson, 2001). Furthermore, all populations tested from different islands in southeast Asia contain an X-linked factor that when present in males causes them to produce predominantly female progeny (Presgraves et al., 1997; Wilkinson et al., 2003) due to abnormal development of Y-bearing sperm (Wilkinson and Sanchez, 2001). Artificial selection on male eye span altered brood sex ratios suggesting that factors on the X chromosome jointly affect a male ornament and sperm development (Lande and Wilkinson, 1999; Wilkinson et al., 1998b). In many species of Drosophila, cases of X chromosome meiotic drive or sex ratio are associated with chromosomal inversions (Jaenike, 2001). Paracentric inversions restrict recombination between heterokaryotypes (Navarro et al., 1997) and recombination map length negatively correlates with the size of successful inversions in ﬂies (Caceres et al., 1999). Consequently, regions of linkage disequilibrium may be greater for X-linked genes found in inversions or under strong selection, such as is likely to occur with meiotic drive (Derome et al., 2004; Palopoli and Wu, 1996).

Materials and Methods

Selection regime

Artiﬁcial sexual selection was exerted on the ratio of eye span to body length in male Cyrtodiopsis dalmanii descended from animals captured in peninsular Malaysia in 1989. These ﬂies had been reared in the lab in mass culture for approximately seven generations prior to the onset of selection (Wilkinson, 1993). Beginning in March 1991, two lines each were selected for high and low ratios by taking 10 of 50 males and housing them with 25 females chosen at random from within each selected line in 40 × 40 × 40 cm cages. Two unselected control lines were maintained at the same time by randomly selecting 10 males and 25 females from each line. To produce offspring for

Correlated Responses in Stalk-eyed Flies

501

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the next generation, we collected eggs from each of the six cages twice each week in four cups containing 50 ml of food after allowing selected flies to mate for at least two weeks. Thus, larvae typically developed at low density and produced large-bodied adult flies. Every generation after eclosion we separated flies from each replicate line by sex and maintained them in single-sex cages as virgins until selection was imposed after at least four weeks of age. This selection regime was continued for 30 generations. At generation 31, selection was relaxed to reduce inbreeding such that 25 of 50 measured males were chosen for breeding each generation. Note that this change in selection intensity also altered the sex ratio of adult flies in each cage from 1:2.5 to 1:1 male:female, which could have potentially increased the intensity of sperm competition within each line. In this study we measured reproductive traits from male and female flies in each of the six lines after 57 generations of selection.

All cages are lined with moist cotton and blotting paper and kept at 25 ± 1 °C with at least 70% relative humidity on a 12 hr light-dark cycle. Twice each week pureed corn is provided in disposable dishes as food. Adding 5 ml of a 10% solution of methylparaben in 90% ethanol to each liter of corn pulp after autoclaving for 30 min inhibits mold growth. To rear flies food cups are placed inside larger 500 ml containers lined with damp cotton and plugged with foam stoppers to permit the larvae to climb out of the cups and pupate in the cotton. Any pupae remaining in cups are transferred to the cotton before eclosion.

Flies are measured under CO₂ anesthesia while resting on their orbital and thoracic spines. Using the program ImageJ we measure eye span from the outer edges of the ommatidia and body length from face to wing tip on each animal at 11(6 (resolution of 50 pixels/mm). The ratio of eye span to body length is referred to as relative eye span throughout this paper.

Reproductive trait measurements

Because these flies take two to three weeks to reach reproductive maturity, it is possible that some reproductive traits could change with age. Consequently, we dissected two or three males and females from each line every other day between 14 and 32 days of age. We supplemented these measurements of young flies with measurements of older females that were 40–48 days of age and older males that were 58–86 days of age. All flies were kept in single-sex cages prior to dissection.

To isolate reproductive structures, abdomens of anesthetized flies were removed with small scissors and placed in a single drop of phosphate buffered saline (PBS) on a clean glass slide. The remainder of the body was frozen and later measured for eye span, body length and thorax width as described above. For males, testes were removed into the PBS and the area of both testes, whenever possible, was measured at 100(6 (575 pixels/mm). The contents of the testes were then released into the PBS and the sperm tails of five fully elongated sperm bundles were measured at 400(6 (2.3 pixels/μm). Sperm heads were not included because of the possibility that sperm head length is modified in males that carry sex chromosome meiotic drive (Wilkinson and Sanchez, 2001).

For females, the reproductive tract (see Fig. 1) including ovaries was removed from the abdomen into a drop of PBS on a clean glass slide, and the rest of the abdomen was discarded. The number of mature eggs, defined as those at least 0.7 mm in length, were counted, if present, and moved to the side of the slide. The tract was oriented with the ventral sclerite (Kotrba, 1995) facing up and a cover slip was gently placed over the reproductive tract. The areas of all three spermathecae, the lengths of the two spermathecal ducts, the width of the ventral receptacle, and for a subset of the sample (n = 80), the number of chambers in the ventral receptacle were measured at 400(6 (2.3 pixels/μm). Egg size was measured for three mature eggs at 100(6 (575 pixels/mm).

Genotyping

We extracted DNA from all flies and then used the polymerase chain reaction (PCR) to amplify microsatellite alleles using four pairs of X-linked primers (Wright et al., 2004). These primer pairs were previously found to produce products that differ in size between high and low line flies and their relative positions on the X chromosome have been determined by linkage mapping using F2 progeny resulting from a cross between two selected lines (P.M.J. and G.S.W., unpublished data). The order of the loci and their recombination map intervals are: ms125–14.2 cM–ms244–8.2 cM–ms54–2.5 cM–ms 395. Unique alleles at three of the four loci have also been found to occur
in males that carry X chromosome meiotic drive and produce predominantly female offspring (P.M.J. and G.S.W., unpublished data).

We extracted DNA by grinding thorax and leg tissues before incubating at 65°C for at least 2 hr in 400 μl of 6.25% (w/v) Chelex 100 solution with 2 U proteinase K (Fisher Scientific). We then incubated samples at 95°C for 20 min and then centrifuged them for 3 min at 2,000 rpm on a microcentrifuge. PCR was performed in 10 μl reactions containing 1 μl extraction supernatant as template DNA, 0.125 U Taq DNA polymerase (Invitrogen, Carlsbad, CA), 1× PCR Buffer (Invitrogen), 0.2 mM of each dNTP, 2.5 mM MgCl₂ and 0.5 μM of each primer. Forward primers were labeled with NED (Applied Biosystems), HEX, or FAM fluorescent dyes. We used a touchdown cycle of 17 cycles with initial annealing at 63°C and decreases of 1°C per cycle followed by 20 cycles with annealing at 47°C. PCR products were separated on a 3100 DNA Analyzer (Applied Biosystems) using Pop-4 polymer and evaluated with Genescan 3.1.2 software (Applied Biosystems). Alleles were sized and scored using Genotyper 2.5 software (Applied Biosystems).

**Statistical analysis**

We used mixed model nested analyses of covariance (ANCOVA) to determine if age, body size, replicate or selection treatment influenced male and female traits. We nested replicate within selection treatment and assumed that replicate was a random effect. If selection on male relative eye span causes change in other characters due to pleiotropy or linkage, the magnitude of change in the other traits should correlate with the magnitude of change in male relative eye span. Therefore, we quantified the correlated response by fitting regressions to the least squares means of female traits that differed between selection treatments to male relative eye span for each replicate within each treatment. We tested for evidence of X chromosome linkage by conducting an ANCOVA on those traits that differed between selection treatment using microsatellite genotype as a main effect and body length as covariate. Age was not included in these analyses because it had not explained a significant fraction of the variation in these traits in the previous ANCOVAs.

**Results**

Artificial sexual selection resulted in rapid bidirectional response in male relative eye span (Fig. 2). Response to selection was consistent for 15 generations in the replicate high lines and for 23 generations in the replicate low lines. At generation 16 one of the high lines underwent a temporary reversal in response to selection, while at generation 24 one of the low lines reached a plateau. When selection was reduced in intensity at generation 30, all four lines stopped responding. Subsequently, each of the low lines and one of the high lines exhibited reversals at independent generations. Nevertheless, at generation 57 significant differences in relative eye span existed between selected lines and between replicates for both males (Table 1) and females (Table 2). Each replicate high line differed in relative eye span from each low line by at least 3.3 SD and by as much as 5.0 SD.

Differences in relative eye span between selection treatments are caused predominantly by changes in absolute eye span rather than by changes in body length. Selection produced consistent responses in eye span between low and high lines, as compared to control lines, for both males and females (Fig. 3). For male body length, no effect of selection treatment could be detected (F_{2,3} = 0.1, \( P = 0.9 \)) but significant differences among replicates occurred (F_{1,141} = 6.8, \( P = 0.0003 \)). In contrast, for female body length significant effects of line (F_{2,3} = 27.9, \( P = 0.012 \)), but not of replicate (F_{1,172} = 0.6, \( P = 0.6 \)), were found. However,
Table 2. F-ratios from nested analyses of covariance for female reproductive traits on selected line flies.

<table>
<thead>
<tr>
<th>Source (df)</th>
<th>Eye span</th>
<th>Egg size</th>
<th>Egg number</th>
<th>Ventral receptacle</th>
<th>Singlet spermatheca</th>
<th>Doublet spermatheca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Duct length</td>
<td>Area</td>
<td>Duct length</td>
</tr>
<tr>
<td>Log age (1,172)</td>
<td>0.0</td>
<td>0.0</td>
<td>12.3**</td>
<td>0.1</td>
<td>0.1</td>
<td>1.7</td>
</tr>
<tr>
<td>Body length (1,172)</td>
<td>213.6***</td>
<td>0.1</td>
<td>0.3</td>
<td>0.8</td>
<td>0.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Selected line (2,3)</td>
<td>17.6*</td>
<td>4.3</td>
<td>9.8*</td>
<td>0.3</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Replicate (3,172)</td>
<td>14.7***</td>
<td>1.8</td>
<td>2.8*</td>
<td>47.5***</td>
<td>5.1***</td>
<td>38.6***</td>
</tr>
<tr>
<td>Model (7,172)</td>
<td>129.2***</td>
<td>3.1**</td>
<td>2.4*</td>
<td>25.3***</td>
<td>3.4**</td>
<td>27.5***</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.0001.

the line effect in females is due to control line flies being smaller than flies from either selected line, which do not differ from each other (Fig. 3).

Two traits, average testis size in males and the number of mature eggs carried by females, showed significant effects of age but no effects of body length, selection line or replicate (Tables 1 and 2). Testis area exhibited a biphasic growth pattern (Fig. 4) with rapid growth occurring during the first three weeks of age followed by slow growth that appears to continue for five months or longer. The number of mature eggs in females also increased with age (Fig. 4), although egg number appears to plateau at four weeks of age and does not show subsequent change. The remaining trait in males, sperm length, was not influenced by body length, age, selection treatment or replicate (Table 1).

In females, nested ANCOVA explained significant variation in every reproductive trait (Table 2). Significant effects of replicate, but not selection treatment, were detected for the singlet and doublet spermathecal areas and duct lengths (Table 2, Fig. 5). All of these traits tended to increase with body length, but only for the average area of the doublet spermathecae was this effect significant (Table 2). The width of the ventral receptacle was independent of age and body length, but exhibited significant differences between selection treatments and replicates within treatments (Table 2). Selection line explained 29% of the variance in ventral receptacle width while replicate explained only 4%. Ventral receptacle width correlated with the number of chambers counted in the ventral receptacle ($r^2 = 0.55$, $P < 0.0001$). Because no significant effect of replicate could be detected for egg size, the ANCOVA was rerun after pooling replicates within selected lines. A significant effect of selection treatment on egg size ($F_{2,3} = 6.9$, $P = 0.0015$) was then detected and explained 16% of the variation in average egg size.

Differences between selection treatments are consistent with correlated responses to selection. The regression of female relative eye span on male relative eye span (Fig. 6a) was highly significant ($t = 10.2$, $P = 0.0005$) and explained 96% of the variation in female relative eye span. In addition, the regression between the number of chambers in the ventral receptacle and male relative eye span (Fig. 6b) was significant ($r^2 = 0.88$, $P = 0.006$). The regression between average egg size and male relative eye span approached significance ($r^2 = 0.61$, $P = 0.066$).

To determine if linkage between X-linked factors could be partly responsible for the correlated responses to selection, we compared trait values across genotypes at four X-linked microsatellite loci. Those traits that differed between selected line treatments also exhibited significant differences between genotypes at the X-linked loci (Table 3). For male eye span, all four X-linked loci exhibited significant genotype effects, although the effect of ms125 genotype was much weaker than for the other three loci. The effect of each of the six alleles at locus ms244 was nearly additive while for locus 244 only one of the four alleles appeared to influence male eye span (Fig. 7). All four X-linked loci also exhibited significant genotypic effects on female eye span (Table 3). However, because females are diploid at X-linked loci, rather than haploid, homozygous and heterozygous genotypes are present. Examination of mean female eye span for each genotype revealed strong dominance effects for some pairs of alleles at every locus. For example, at locus ms125 three different heterozygotes exhibited underdominance, while at locus ms244, ms54 and ms395, one heterozygous
genotype was overdominant (Fig. 8). For ventral receptacle width significant effects of genotype were detected at the same three loci that exhibited highly significant genotypic effects on male eye span (Table 3). Mean ventral receptacle width at each ms395 genotype showed the same pattern of overdominance as for female eye span while the homozygous genotypes at ms244 exhibited additivity (Fig. 9). The genotypic effects of ms54 on ventral receptacle width were significant, but exhibited less similarity to the pattern observed for female eye span.

Unique alleles at three of the four loci define a male haplotype that is always associated with highly female-biased sex ratios. We did not find any of these drive alleles in any male or female that was genotyped from the selected lines.

**DISCUSSION**

Long-term artificial sexual selection resulted in bidirectional change in male relative eye span in replicate selected lines as long as selection was intense. The strong correlated response in female relative eye span, which has been noted previously (Wilkinson, 1993; Wolfenbarger and Wilkinson, 2001), suggests that the genes that influence male eye span also influence female eye span. Direct support for this inference has recently been obtained from a QTL mapping study (P.M.J. and G.S.W., unpublished data), which has found four QTL in the same locations in both males and females and only one QTL unique to males. The correlated responses found for ventral receptacle size and egg size, both of which exhibit differences between selection treatments after adjusting for body size, have several possible explanations.

First, the expression of each of these traits, eye span, ventral receptacle size and egg size, may be under the influence of juvenile hormone (JH) and either JH titer, receptor distribution, or esterase concentration could have been changed by selection. Juvenile hormone has been implicated in the development of the imaginal discs that produce horns in beetles (Emlen and Nijhout, 1999, 2001). Juvenile hormone is also associ-
associated with reproductive development in insects and can influence ovulation and mating behavior in flies (Dubrovsky et al., 2002; Soller et al., 1999). However, a difficulty with any hormonal explanation is that the stage at which these traits develop is different, so hormone production would have to be altered for extended periods. Eyestalks form from eye-antennal discs in third instar larvae (Buschbeck et al., 2001) while the ventral receptacle forms from genital discs that differentiate late in pupal development in stalk-eyed flies. Recent work indicates that exogenous application of synthetic juvenile hormone on stalk-eyed fly third instar larvae increases relative eye span of small-bodied males and reduces testes growth (C.L. Fry, personal communication). However, no effect on female eye span, egg size or egg number has been observed when synthetic juvenile hormone is applied to larvae. Thus, the available evidence does not strongly support JH-mediation of the correlated responses observed in this study.

A second possibility is that the genes that influence eye span could have pleiotropic effects on the development of female reproductive traits. A recent survey of genes putatively under sexual selection in Drosophila melanogaster found that 73% of them exhibited pleiotropy (Fitzpatrick, 2004). Furthermore, one gene, icebox, influences both female courtship behavior and seminal receptacle development while another gene, ovarian tumor, influences both female courtship behavior and ovary development. While no genes have yet been found to influence a male ornament and a female reproductive trait, these examples show that differential expression of a single gene could alter traits involved in pre-copulatory and post-copulatory sexual selection. However, given that only 2 of 100 genes examined were found to influence both courtship behavior and female reproductive traits in Drosophila (Fitzpatrick, 2004), it seems unlikely that three out of four X-linked microsatellites, which were arbitrarily selected from a genomic library and do not occur in the coding regions of any known genes (Wright et al., 2004), would show pleiotropic effects on both male eye span and ventral receptacle size.

A third possibility is that genes that influence eye span may be in linkage disequilibrium with genes that influence egg and ventral receptacle size. For this to occur, genes that influence all three traits would need to lie on the same chromosome. In C. dalmanni, the X chromosome explained 30% of the variation in relative eye span between lines after 30 generations of selection (Wolfenbarger and Wilkinson, 2001) and a major QTL that affects male and female relative eye span has been identified on the X chromosome be-
Figure 7. Mean male relative eye span ordered from largest to smallest for each genotype at four X-linked microsatellite loci. Error bars represent one standard error and sample size is indicated next to each point. The length of each microsatellite allele is as follows. MS125: B~152 bp, F~156 bp, D~158 bp, K~174 bp; MS244: C~217 bp, D~220 bp, A~226 bp, F~230 bp; MS54: A~160 bp, B~162 bp, C~164 bp, G~166 bp; D~168 bp; E~170 bp, F~177 bp; MS395: D~195 bp, A~201 bp.

Figure 8. Mean female relative eye span for each genotype at four X-linked microsatellite loci. Error bars represent one standard error and sample size is indicated next to each point. Genotype order and allele sizes as in Figure 7.
Fig. 9. Mean female ventral receptacle width for each genotype at four X-linked microsatellite loci. Error bars represent one standard error and sample size is indicated next to each point. Genotype order and allele sizes as in Figure 7.

tween microsatellite markers ms244 and ms54 (P.M.J. and G.S.W., unpublished data). If linkage disequilibrium was responsible for the correlated responses, we would expect different trait means for flies that differ in X genotype, with this difference being greatest near the X-linked QTL for male relative eye span. Examination of Table 3 reveals that the genotypic effect on all three traits was greatest for the two loci, ms244 and ms54, that flank the eye span QTL and that significant genotypic effects on ventral receptacle size occur at three loci which span 10.7 cM of the X chromosome (P.M.J. and G.S.W., unpublished data). This evidence strongly supports linkage among genes on the X chromosome as the cause of the correlated changes in ventral receptacle and egg size.

Because strong selection, such as that imposed by long term artificial selection, can generate linkage disequilibrium, the most generalizable result of this study may be that genes which influence ventral receptacle and egg size are X-linked. Nevertheless, there is reason to consider the possibility that linkage may have influenced the correlated evolution of reproductive traits among species of diopsid stalk-eyed flies (Presgraves et al., 1999). Every population of *Cyrtodiopsis dalmanni* and *C. whitei* that has been examined to date contains appreciable frequencies of X chromosomes that exhibit meiotic drive (Presgraves et al., 1997; Wilkinson et al., 2003). Meiotic drive can increase the size of regions that persist in linkage disequilibrium because it prevents random assortment of alleles and reduces effective population size. Furthermore, drive chromosomes are often associated with paracentric inversions that reduce recombination (Jaenike, 2001; Jiggins et al., 1999). We have found that recombination is extremely reduced between drive and nondrive X chromosomes in *C. dalmanni* (P.M.J. and G.S.W., unpublished data). Recent studies on sex ratio X chromosomes in *Drosophila simulans* have found evidence of selective sweeps that reduce genetic variation near the meiotic drive factor (Derome et al., 2004). These observations suggest that a population genetic study designed to assess the degree of linkage disequilibrium across the X chromosome is warranted to determine the degree to which X-linked traits segregate independently in natural populations of stalk-eyed flies.

Correlated selection mediated by sperm competition seems to be the least likely explanation for the correlated responses we observed in this study, although it may explain the patterns of correlated evolution found between sperm and female reproductive traits among species of diopsids (Presgraves et al., 1999) and *Drosophila* (Pitnick et al., 1999; Pitnick et al., 2003). Even though our selection regime altered the breeding sex ratio and probably increased sperm competition midway through the experiment, all lines experienced the same regime and, therefore, sperm competition should not have differed between lines. The absence of any significant treatment effects on testis size supports this interpretation. However, in *Drosophila melanogaster* selection on seminal receptacle length resulted in
changes in sperm length, presumably due to correlated selection (Miller and Pitnick, 2002). If sperm length is heritable in diopsids, as it is in dung flies (Ward, 2000), dung beetles (Simmons and Kotiaho, 2002), crickets (Morrow and Gage, 2001), and drosophila (Joly et al., 1995; Pitnick and Miller, 2000), then sperm length should have changed due to selection exerted by the change in ventral receptacle size observed between treatments. The absence of any detectable differences in sperm length may be due to weak correlated selection, low heritability, or low experimental power. Comparison of sperm lengths and female reproductive tracts among recently isolated populations of Cyrtodiopsis is underway to determine the rate at which these traits evolve and coevolve.

The existence of genetic correlations between genes that influence a male ornament and female traits involved in fertilization success has potential consequences for the maintenance of genetic variation for sexually selected traits. How genetic variation can be maintained for traits under strong sexual selection is commonly known as the lek paradox (Borgia, 1979; Taylor and Williams, 1982). Possible solutions to the lek paradox include nonlinear selection with an accumulation of modifiers that increase phenotypic variation (Pomiankowski and Moller, 1995), genetic capture of condition dependence (Rowe and Houle, 1996), and antagonistic pleiotropy between genes that influence both male and female reproductive success (Rice and Chippindale, 2001). Our results provide another alternative: male ornament genes may be linked or exhibit pleiotropy with genes having nonadditive effects on female traits under selection. This possibility is suggested by the presence of nonadditive X-linked effects for female reproductive traits while the same chromosomal regions exhibit additive effects on male eye span. In effect, under or over-dominance in females at loci that exhibit additive allelic effects in males is a form of sexual antagonism because males and females have different genetic optima. Thus, sexual antagonism is possible even though we observed a positive, rather than a negative (Chippindale et al., 2001), correlation between the expression of a male ornament and a female reproductive trait. Whether such sex-biased nonadditive genetic effects occur in other sexually selected species merits additional study.

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