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SHORT COMMUNICATION

Sex chromosome evolution in the clam shrimp, *Eulimnadia texana*

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Abstract

Chromosomes that determine sex are predicted to evolve differently than autosomes: a lack of recombination on one of the two sex chromosomes is predicted to allow an accumulation of deleterious alleles that eventually leads to reduced functionality and potential physical degradation of the nonrecombining chromosome. Because these changes should occur at an elevated evolutionary rate, it is difficult to find appropriate species in which to test these evolutionary predictions. The unique genetic sex-determining mechanism of the crustacean *Eulimnadia texana* prevents major chromosome degeneration because of expression of both 'proto-sex' (i.e. early stage of development) chromosomes in homozygous form (ZZ and WW). Herein, we exploit this unique genetic system to examine the predicted accumulation of deleterious alleles by comparing both homogametic sexual types to their heterogametic counterpart. We report differences in crossing over in a sex-linked region in the ZW hermaphrodites (~ 3%) relative to the ZZ males (~ 21%), indicative of cross-over suppression in the ZW hermaphrodites. Additionally, we report that both ZZ and WW genotypes have reduced fitness relative to ZW hermaphrodites, which is consistent with the prediction of harboured recessive mutations embedded on both the Z and the W chromosomes. These results suggest that the proto-sex chromosomes in *E. texana* accumulate recessive deleterious alleles. We hypothesize that recessive deleterious alleles of large effect cannot accumulate because of expression in both ZZ and WW individuals, keeping both chromosomes from losing significant function.

Introduction

Among chromosomes, those that contain the genes determining sexuality (termed 'sex chromosomes') are often unique: one of the two chromosomes is commonly degenerate (Bull, 1983; Charlesworth & Charlesworth, 2000). When this chromosome determines maleness, it is termed the 'Y' chromosome and when it determines femaleness, it is termed the 'W' chromosome (Bull, 1983). The degeneration of the Y(W) chromosome is thought to be a direct result of restricted recombination between the sex chromosomes in the heterogametic sex (Bull, 1983; Rice, 1987; Charlesworth, 1991; Charlesworth & Charlesworth, 2000). Beginning with the initial stages of the evolution of sexual dimorphism, the genes causing sexual specialization must have restricted recombination: 'If sex is determined by two or more genes, it is essential for these genes to be inherited together as a unit. This requires inhibition of recombination between the differentiated segments of the X (or Z) and Y (or W) chromosomes' (Nei, 1969). The initial selective advantage of reduced recombination of the sex-determining region on the sex chromosomes is thought to be a necessary outcome of the negative effects of the recombining of multiple sex-determining alleles into infertile 'intersexes' (Nei, 1969; Charlesworth, 1991, 2004). This region of reduced recombination on the developing sex

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chromosome is predicted to grow as additional sex-specific genes are selected to transpose to areas near the sex-determining region of the early-stage (or 'proto'-) sex chromosomes (Rice, 1987; Charlesworth *et al.*, 2005). The permanent heterozygosity of Y(W)-linked genes, as well as their smaller effective population sizes, causes these genetic regions to have a much higher probability of accumulating deleterious recessive mutations (Muller & Painter, 1932; Charlesworth & Charlesworth, 2000) and/or transposable elements (Steinemann *et al.*, 1993) than other regions of the genome. This is because the permanently heterozygous sex chromosome cannot recombine with a homolog to allow natural selection to effectively purge these deleterious elements. The accumulation of these DNA abnormalities can then lead to the eventual degradation of the Y(W) chromosome's content (Bull, 1983; Rice, 1996).

A great difficulty in testing these ideas lies in the fact that most species have progressed beyond the point where the predicted processes are easily detectable (Charlesworth & Charlesworth, 2000). The standard way to overcome this problem is to examine species in the earliest stages of sex chromosome development (Charlesworth *et al.*, 2005). These can be dioecious species recently derived from hermaphrodites (wherein fully differentiated sex chromosomes have not yet evolved) or in more established dioecious species, where an autosome (or autosomal segment) has recently fused with existing sex chromosomes causing the process of chromosomal degradation to begin anew in this newly fused segment (Charlesworth & Charlesworth, 2000). Because of the limited number of well-understood systems of this type, studies of sex chromosome evolution in animal species other than *Drosophila* are uncommon (Charlesworth & Charlesworth, 2000).

If a species was found in which the progression of chromosomal degradation was held at some intermediate level for an extended period, testing of several of these predictions would be facilitated. Herein, we report evidence that sex chromosomal evolution in the clam shrimp *Eulimnadia texana* is at the 'proto-sex' chromosomal stage of development. We propose that the unique sex-determining mechanism found in these shrimp slows the evolution of the sex chromosomes such that chromosomal degradation should be limited to the earliest stages of degradation for an extended period. *Eulimnadia texana* are androdioecious, with males coexisting with hermaphrodites (Sassaman & Weeks, 1993). Hermaphroditism is the dominant condition (i.e. a ZW chromosomal system), and males are homogametic (ZZ). Hermaphrodites are self-compatible, and thus a selfing ZW hermaphrodite can produce three types of offspring: ZZ males, ZW hermaphrodites (termed 'amphigenics') and WW hermaphrodites (termed 'monogenics,' Sassaman & Weeks, 1993). Because both sex chromosomes are expressed in homozygous individuals, we suggest that recombination and selection can eliminate any large-effect, deleterious mutations or insertions of transposable elements that lead to the silencing of one or more genes because such mutants would be selected against when expressed in ZZ and WW offspring. Given the expression of both chromosomes in homozygous states, the progression of major structural differences between the Z and W chromosomes is limited. Herein, we report data from *E. texana* that tests the prediction that deleterious recessive alleles have accumulated on the Z and W chromosomes by comparing crossing over rates in the heterogametic and homogametic sexes and noting whether there is evidence of an accumulation of deleterious recessive alleles on one or both of the proto-sex chromosomes.

Methods and materials

Rearing and genetic assays of parental generation

All offspring used in the current study were derived from amphigenic (ZW) hermaphrodites. When hermaphrodites are paired with males, they continue to self some proportion of their eggs (Weeks *et al.*, 2004), so that each clutch of offspring from such matings contains five identifiable categories of offspring (selfed males, selfed amphigenics, selfed monogenics, outcrossed males and outcrossed amphigenics) in any clutch produced by pairing a male with an amphigenic hermaphrodite. These five categories were then used to assess rates of crossing over and to test the prediction that the proto-sex chromosomes are accumulating deleterious recessive alleles.

Shrimp rearing procedures were as outlined in previous publications (e.g. Weeks *et al.*, 1999). The parental generation was reared from soil collected from Portal, Arizona, from a cattle tank previously defined as WAL. After the shrimp matured, they were separated by sex into holding tubs for 24 h to ensure that any eggs the hermaphrodites had in their brood chambers were expelled and thus that no eggs were produced via outcrossing before isolation (hermaphrodites cannot store sperm, Weeks *et al.*, 2000b). After this waiting period, male and hermaphrodite shrimp were paired in individually-labelled, 500-ml cups to mate. After 7 days, the shrimp were frozen (−80 °C) for allozyme surveys.

Cellulose acetate electrophoresis was run for five loci: Idh-1, Idh-2 (isocitrate dehydrogenase, EC 1.1.1.42), Mpi (mannose–phosphate isomerase, EC 5.3.1.8), Pgm, (phosphoglucomutase, EC 5.4.2.2) and Fum (fumarate hydratase, EC 4.2.1.2). Matings in which the parents were homozygous for alternate alleles at any locus and in which the hermaphroditic parent was heterozygous at one or more of the sex-linked loci (Fum, Idh-1, or Idh-2) were chosen for further analyses. The alternately homozygous loci allowed the detection of outcrossing, and any heterozygote at a sex-linked locus distinguished the

amphigenic hermaphrodites from the monogenics (Weeks *et al.*, 1999).

Rearing and genetic assays of offspring generation

The isolation cups from mated parents with the above allozymic characteristics were then hydrated. The resulting nauplii were transferred to larger isolation tubs for 24 h. The offspring were left in these tubs until they matured and were then moved into individual 500-ml isolation cups for the duration of their life. Immediately after the offspring died, their carapace length was measured (using SCION imaging software, Scion Corp, Frederick, MD, USA) and their lifespan was recorded. They were then frozen for electrophoretic scoring.

Cellulose acetate electrophoresis was run on the offspring as described above for each locus previously identified from the parents. This information, combined with sexual identification (which is easily scored morphologically, Mattox, 1959), allowed identification of the five mating types noted above.

Estimates of crossing over frequency

Crossing over was estimated in 69 male and 132 hermaphroditic offspring raised from the nine families with the highest levels of outcrossing noted in the cellulose acetate analyses above. Outcrossed and selfed offspring were run for Fum (known to be linked to the sex-determining region; Sassaman & Weeks, 1993) and for seven microsatellite loci. For the microsatellite analyses, DNA was extracted from frozen offspring using the DNeasy tissue kit (Qiagen, Valencia, CA, USA). Following DNA extractions, six microsatellite loci (CS3, CS7, CS8, CS11, CS15 and CS16) previously characterized for *E. texana* (Duff *et al.*, 2004) and a newly identified locus (CS9: CS9F 5′-CTTGTTATCGGAAATCGATTGA and CS9R 5′-CAAACGCACTCTGGTTTACCTGT) were amplified with forward fluorescent-labelled primers and reverse primers modified with a GTTT tail to enhance the 3′-adenylation. Each 10-μl reaction included 10 pmol of each primer; 2 mM of each dNTP; 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.4 mM $MgCl_2$, Qiagen); and template DNA (1–20 ng). The LIZ-600 size standard was added directly to the amplified products and both were directly visualized in individual capillaries on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA, USA). Scoring of genotypes was aided by GeneMapper Software v4.0 (Applied Biosytems). Of the 201 offspring run, 197 produced scoreable results. Three of the eight microsatellite loci (CS8, CS11 and CS15) were linked to one another and to Fum (and thus to the sex-determining region; Weeks *et al.*, 1999). Initial estimates of crossing over were made among these four loci by comparing parental to crossed inheritance patterns in male- and amphigenic hermaphrodite-originating chromosomes, inferring preliminary marker order. Our dataset was not the standard set of intercrosses or backcrosses used in mapping studies: we were working with data derived from offspring of selected couples, in various combinations of homozygote and heterozygote parents, and in these 'natural' crosses, we had occurrence of both selfing and outcrossing. This made our dataset somewhat difficult to treat using 'traditional' software. We analysed data with the software CarthaGène (De Givry *et al.*, 2005), to map our markers. CarthaGène's underlying mathematical model assumes a linear ordering of markers with no interference. We coded heterozygous (H) and homozygous (A) markers in two datasets, one for the maternal (hermaphrodite) and one for the paternal (male) parents. We ran the hermaphrodite scores first in the 'selfing' mode and then in the 'backcross' mode: part of the offspring of hermaphrodites can be the result of selfing even during a single outcrossing event, as noted. We had a reduced dataset for males, which we analysed in 'backcross' mode. Backcross data can be considered analogous to haploid data (two genotypes, with comparisons of homozygotes vs. heterozygotes). We used the SEM command (single EM algorithm) to analyse the maximum likelihood of the genetic map using the marker order initially determined. Attempts to estimate better maps (maximum likelihood estimations with 2-point distances) resulted in only minimal improvement of the likelihood, and similar values of genetic distance among markers and linkage scores. We obtained maps with linear distances between markers and we evaluated the logarithm of odds (LOD) scores. LOD scores vary as a function of the recombination rate, and LOD scores > 3.0 are considered evidence of linkage (Risch, 1992). CarthaGène associates with each map a log-likelihood value. Such values are reported in the results to give a statistical value of how well our map fits the dataset, with smaller negative log-likelihood values indicating a better fit of the map to the data.

Fitness estimates among offspring types

Size (length at death) and lifespan were used as surrogates of fitness and were analysed using the statistical package JMP (SAS Inc., Cary, NC, USA). Size has previously been found to be strongly positively correlated with egg production in hermaphrodites (Weeks *et al.*, 1997), and larger males are better able to guard females before copulation (Benvenuto *et al.*, 2009), both of which suggest that size is a good surrogate of reproductive success in clam shrimp. Lifespan needed square-root transformation to normalize residuals. Both dependent variables were analysed separately in blocked, one-way ANOVA's and simultaneously in a blocked, one-way MANOVA. Offspring family was considered the blocked effect. The five offspring types (outcrossed amphigenics, outcrossed males, selfed amphigenics, selfed males and selfed monogenics) constituted the main effect. Three *a priori* contrasts were performed in the MANOVA: a

comparison of males to amphigenic hermaphrodites in the outcrossed group, males to monogenic hermaphrodites in the selfed group, and the relative difference between males and amphigenic hermaphrodites in outcrossed and selfed offspring.

To visualize the combined fitness effects of both size and lifespan, we used the first canonical scores output from the MANOVA analysis to combine both measures into a single dimension (Chatfield & Collins, 1980). The first canonical score accounted for 88% of the total variance in the two dependent variables across the five offspring types, and thus this score was used to compare relative performance among these five offspring types.

Results

Estimates of crossing over frequency

Linkage was evident among the four genetic markers: one allozyme (Fum) and three microsatellite loci (CS8, CS11 and CS15; Table 1). Rates of crossing over were consistently lower on sex-chromosomal regions derived from amphigenic hermaphrodites (ZW) relative to males (ZZ) for all six pairwise comparisons among these four loci: males had ~ 21% crossing over whereas hermaphrodites had ~ 3% crossing over. We ran the hermaphrodite dataset in CarthaGène twice and obtained similar results, regardless of whether we analysed the cross as a selfing event or a backcross event. The four markers were relatively close, and the LOD scores were very high (all greater than 3.0, see Table 1; the genetic map was associated with a log-likelihood = −95.48). We had a smaller dataset for males, which displayed a longer map and lower LOD values (see Table 1; the genetic map was associated with a log-likelihood = −24.65). Male alleles presented an LOD of 0.1 whereas hermaphroditic alleles had LOD scores between 12 and 30, implying high linkage in the latter. This pattern of linkage clearly indicates that recombination rates were consistently lower on sex-chromosomal regions derived from amphigenic hermaphrodites (ZW) relative to males (ZZ) among these four loci. Thus, heterogametic amphigenic hermaphrodites revealed a consistent pattern of crossing over suppression between the Z and W chromosomes relative to their homogametic male (ZZ) counterparts. We did not have sufficient variable markers throughout the genome to assess rates of recombination in other chromosomes, so we can only assess recombination between the 'proto-sex' chromosomes at this stage. Thus, at this point, we cannot specify whether these crossing over differences are because of specific crossover suppression between the sex chromosomes or a genome-wide crossover suppression in ZW relative to ZZ individuals.

Table 1 Linear map of four markers obtained from offspring (164 hermaphrodites and 33 males) derived from nine males mated to nine hermaphrodites. Data were analysed for hermaphrodites (in backcross and self mode) and males (backcross) using CarthaGène software. For each marker, the position, the distance (using Kosambi and Haldane mapping functions), the recombination ratio theta and two-point logarithm of odds (LOD) scores are reported. A LOD score > 3.0 is considered evidence of linkage. Four loci are linked to the sex-determining region: Fum (Fumarase) and three microsatellite loci (CS8, CS11 and CS15).

Marker	Position	Distance Haldane (cM)	Cumulative Haldane (cM)	Distance Kosambi (cM)	Theta (%)	2pt LOD
Hermaphrodites						
Self						
Fum	1	9.1	9.1	8.4	8.3	14.7
CS11	2	2.4	11.5	2.4	2.4	27.50
CS15	3	1.3	12.8	1.3	1.3	30.9
CS8	4					
		12.8		12.0		
Backcross						
Fum	1	16.8	16.8	14.7	14.3	14.7
CS11	2	4.8	21.5	4.5	4.5	27.5
CS15	3	2.5	24.1	2.4	2.4	30.9
CS8	4					
		24.1		21.7		
Males						
Backcross						
Fum	1	78.4	78.4	53.8	39.6	0.1
CS11	2	93.9	172.3	62.3	42.4	0.0
CS15	3	72.8	245.1	50.6	38.3	0.1
CS8	4					
		245.1		166.7		

In the assays for crossing over, one of nine families studied (A8) showed evidence suggesting a recessive lethal associated with the sex-determining region. Among the 24 selfed offspring in this family, 9 were male and 15 were amphigenic hermaphrodites. No monogenics were found among the offspring. Within this one family, this represents a significant deviation ($\chi^2 = 8.25$; $P < 0.02$) from the 1 : 2 : 1 ratio of males to amphigenics to monogenics expected among the offspring of a selfing amphigenic (Sassaman & Weeks, 1993). However, when considering all nine families, the deviation in this one family is no longer significant. Nonetheless, the lack of monogenic hermaphrodites in this family is suggestive of a recessive lethal associated with the hermaphrodite-determining region of the W sex chromosome that kills monogenics at some early stage of their development.

Fitness estimates among offspring types

Significant differences in both growth and lifespan were found among the five offspring types when the effects of family were removed (Table 2). The relative differences for these two surrogates of fitness were similar among offspring types, which are reflected in the similar results of the MANOVA analyses to the univariate analyses (Table 2). Thus, we reported the overall effects of

Table 2 Analysis of variance results for length and lifespan.

Effect	d.f.	Sum of squares	*F*-ratio	*P*-value
Family				
Length	14	254.12	32.96	< 0.0001
Lifespan*	14	72.97	22.70	< 0.0001
MANOVA†	28	0.302	29.42	< 0.0001
Offspring type				
Length	4	31.94	14.50	< 0.0001
Lifespan*	4	6.21	6.76	< 0.0001
MANOVA†	8	0.880	8.31	< 0.0001
Error				
Length	504	277.58		
Lifespan*	504	115.70		
MANOVA†	1006			
Contrasts‡				
1. Amph-O vs. male-O	2	0.016	4.02	0.0186
2. Mono-S vs. male-S	2	0.001	0.36	0.6993
3. (AO – MaO) vs. (AS – MaS)	2	0.017	4.20	0.0155

*Square-root transformed.
†Wilks' Lambda reported instead of sum of squares values.
‡Contrasts on MANOVA results.
Amph, amphigenic hermaphrodites; mono, monogenic hermaphrodites; O, outcrossed; S, selfed; AO, amphigenic, outcrossed; MaO, male, outcrossed; AS, amphigenic, selfed; MaS, male, selfed.

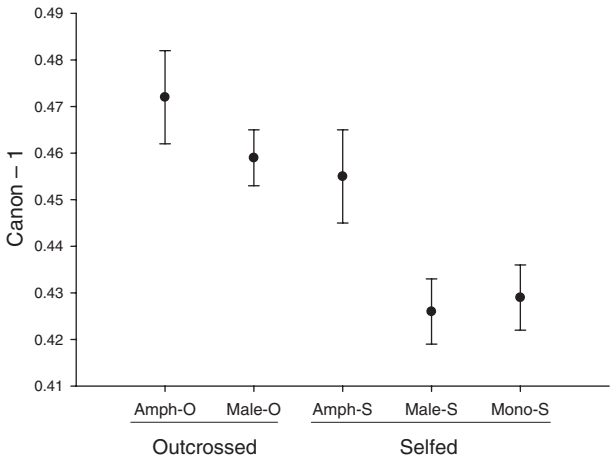

Fig. 1 Fitness comparison among the five offspring types produced by mated amphigenic hermaphrodites. The *y*-axis depicts the first canonical scores generated in the MANOVA analysis, which combines survival (canonical coefficient = 0.021) and growth (canonical coefficient = 0.053) measures for the five offspring types. Amph, amphigenic hermaphrodites; mono, monogenic hermaphrodites; O, outcrossed; S, selfed. Error bars depict one standard error of the mean.

treatment on our combined estimate of performance using canonical scores generated in the MANOVA analysis, with the first canonical score portraying differences in the estimate of fitness (Fig. 1).

The MANOVA contrast of outcrossed males to outcrossed amphigenics (Table 2, contrast 1) revealed a significant reduction in fitness of the former (Fig. 1), which primarily reflected a shorter lifespan for males than for hermaphrodites. Among the selfed offspring, both homogametic offspring types (selfed males and monogenics) had significantly reduced fitness relative to their heterogametic counterparts (selfed amphigenics). Although the selfed males were slightly lower in fitness than the selfed monogenics (Fig. 1), this difference was not significant (Table 2, contrast 2). The difference between males and amphigenic hermaphrodites was significantly greater in the selfed than in the outcrossed offspring (Table 2, contrast 3).

Discussion

In the evolution of the sex-determining chromosomes, a lack of crossing over between the male and female chromosomes (X and Y or Z and W) is thought to begin a process of degeneration of the sex chromosome that is only found in the heterogametic sex (the Y or W; Rice, 1987; Charlesworth, 1991; Rice, 1996; Charlesworth & Charlesworth, 2000; Charlesworth *et al.*, 2005). The unique qualities of the clam shrimp *E. texana* should prevent major degeneration of proto-W chromosome because this chromosome is expressed in the WW hermaphrodites. Integral to this argument is the assumption that there is cross-over suppression in the heterogametic sex and free (or high) recombination in the homogametic sex. From previous studies on clam shrimp, we have estimated crossing over between allozyme loci linked to the sex-determining genes to be ~ 1–2% in the heterogametic sex (Weeks *et al.*, 1999). Herein, we found a similar rate of crossing over of ~ 3% among linked microsatellite loci and one allozyme locus. These rates are similar to those found in the brine shrimp *Artemia* (Bowen, 1965). Crossing over in the homogametic sex (males) is 7-fold higher (~ 21%) than in the heterogametic hermaphrodites (amphigenics). Because all monogenic (WW) hermaphrodites are produced via self-fertilization, finding a heterozygous monogenic is exceptionally rare. Thus, we were not able to test for crossing over in monogenics at this point. Nonetheless, we do have solid evidence of suppression of crossovers in the ZW hermaphrodites and much higher crossing over in the ZZ males. Sexual differences in crossing over have been demonstrated in other arthropods (Sturtevant & Beadle, 1936) including the branchiopod *Artemia* (Bowen, 1965). The current data, as well as these earlier observations, are all consistent with the prerequisite for sex chromosome evolution: reduced crossing over between the sex chromosomes in the heterogametic relative to the homogametic sexes (Bull, 1983; Rice, 1987; Charlesworth, 1991; Charlesworth & Charlesworth, 2000).

Once we determined the necessary conditions for the potential for accumulating recessive deleterious alleles in

the sex-determining system, we then tested for the presence of these recessive alleles by comparing the fitness of the heterogametic (ZW) hermaphrodites to both homogametic genotypes (ZZ & WW). A previous study (Weeks *et al.*, 2001) noted a significant fitness difference between monogenic (WW) and amphigenic (ZW) hermaphrodites, which was attributed to an accumulation of deleterious recessive alleles on the W chromosome being expressed in the WW hermaphrodites. In the current study, we found a similar reduction of fitness for WW compared to ZW hermaphrodites (Fig. 1) but also noted an equally high fitness reduction for ZZ compared to ZW hermaphrodites (Table 2, contrast 2). The latter is confounded by a known reduction of survival for males relative to hermaphrodites (Zucker *et al.*, 2001). Our current experimental design allows us to assess the potential contribution of reduced male fitness by noting the fitness of the two outcrossed categories (males and amphigenic hermaphrodites) which should have no inbreeding effect and thus any difference between them should be because of a 'sex' effect. Indeed, there was a general sex effect in that outcrossed males had lower fitness than outcrossed ZW hermaphrodites (Table 2, contrast 1). Contrast 3 was a test of the relative difference between males and amphigenic hermaphrodites when both are the result of an outcrossing or a selfing event. If the difference between these two sexes among selfed offspring is only because of some inherent 'sex-difference', then the fitness difference (as assessed via growth and survival) would not differ in the selfed vs. outcrossed groups. On the other hand, if selfing differentially affects males relative to amphigenic hermaphrodites (e.g. because selfing exposes the embedded deleterious alleles on the Z chromosome in ZZ males whereas these deleterious alleles are masked in heterozygous form in the ZW amphigenic hermaphrodites), then the difference in fitness between males and amphigenic hermaphrodites would be greater in the selfed relative to the outcrossed comparison. Contrast 3 clearly revealed the latter situation: the 'sex effect' did not completely explain the magnitude of fitness reduction noted between selfed ZW hermaphrodites and males (Fig. 1), and thus we conclude that the Z chromosome also contains embedded deleterious recessive alleles that reduce fitness in selfed ZZ males. Therefore, evidence presented in this study as well as in previous work (Weeks *et al.*, 2000a, 2001) suggests that the Z and W chromosomes have embedded deleterious recessive alleles, most likely in the nonrecombining region surrounding the sex-determining loci (Weeks *et al.*, 2001).

The current finding of detectable levels of deleterious recessive alleles in the Z chromosome was unexpected. Because males have high levels of recombination among sex-linked loci (Table 1), we expected to find evidence of many fewer embedded deleterious recessives on the Z chromosome than on the W chromosome, as is predicted in models of sex chromosome evolution when the sex chromosomes substantially differ in recombination rates (Charlesworth, 1978; Charlesworth & Charlesworth, 2000). However, the effectiveness of recombination producing new genotypes with few deleterious alleles is dependent on the rates of outcrossing; recombination between homologous chromosomes (ZZ or WW) derived from a selfing ZW hermaphrodite will produce very little variation from which to select the 'least loaded' lines because both chromosomes will be inherited essentially as duplicates of one another because of the very low rates of recombination in the ZW parent. Thus, the only way to retard the accumulation of deleterious recessives on these chromosomes is to have crossing over between different pairs of sex chromosomes derived from separate parents (Charlesworth, 1978). This can only occur in ZZ males that are the product of an outcrossing rather than a selfing event. Because outcrossing is known to be quite low in *E. texana* (25–40%; Weeks & Zucker, 1999; Weeks *et al.*, 2004), it is likely that the Z chromosome can also accumulate deleterious recessive alleles even though males have high levels of crossing over on the Z sex chromosome.

Although we now have ample evidence that deleterious recessive alleles have accumulated to some degree in this species (Fig. 1 and see also Weeks *et al.*, 2000a, 2001), we do not anticipate that the predicted longer-term degeneration of the W sex chromosome (Rice, 1987, 1996; Charlesworth, 1991; Charlesworth & Charlesworth, 2000; Charlesworth *et al.*, 2005) can occur in this species because the proto-W sex chromosome is expressed in homogametic (WW) form. Exposing the otherwise recessive alleles to selection in WW hermaphrodites greatly reduces the likelihood of fixing lethal mutations within the linkage group. In this study, we found one nonsignificant but suggestive case in which this 'recessive exposure' may have occurred: one family of 24 offspring (family A8) had no monogenic hermaphrodites (WW), even though nine males (ZZ) were found and six WW hermaphrodites were expected (Sassaman & Weeks, 1993). This lack of monogenics is suggestive of an expression of a recessive lethal on the W chromosome that the amphigenic parent (ZW) was carrying. If our speculation is correct, then this may be an example of the purging of deleterious recessives of large effect in WW hermaphrodites that would otherwise be hidden in permanently heterozygous ZW individuals that occur in other sex chromosomal systems.

In conclusion, the evidence of the sex-determining system in this species suggests that a large linkage group (or possibly a whole sex chromosome) controls sex and sexual dimorphism (Weeks *et al.*, 2000a, 2001, 2006). Evidence presented herein and previously (Weeks *et al.*, 2000a, 2001) suggests that this linkage group has accumulated recessive deleterious alleles, which is predicted by models of sex chromosome evolution (Bull, 1983; Rice, 1987; Charlesworth, 1991; Charlesworth & Charlesworth, 2000). We hypothesize, however, that the

longer-term predictions of continued degradation of the W sex chromosome do not occur in this species because of its unique exposure of the homogametic WW sexual type, which effectively removes the larger-scale mutations and chromosomal rearrangements that would cause greatly reduced fitness in WW individuals. A useful comparison would be to contrast the level of deleterious alleles in these androdioecious clam shrimp to their dioecious close relatives to test the notion that the predicted chromosome degradation has progressed further in the latter relative to the former.

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References

Benvenuto, C., Knott, B. & Weeks, S.C. 2009. Mate-guarding behavior in clam shrimp: a field approach. *Behavioral Ecology* **20**: 1125–1132.

Bowen, S.T. 1965. The genetics of *Artemia salina.* V. Crossing over between the X and Y chromosomes. *Genetics* **52**: 695–710.

Bull, J.J. 1983. *Evolution of Sex Determining Mechanisms*. The Benjamin/Cummings Publ. Co., Inc., Menlo Park, CA.

Charlesworth, B. 1978. Model for evolution of Y chromosomes and dosage compensation. *Proc. Natl Acad. Sci. USA* **75**: 5618–5622.

Charlesworth, B. 1991. The evolution of sex-chromosomes. *Science* **251**: 1030–1033.

Charlesworth, B. 2004. Sex determination: primitive Y chromosomes in fish. *Curr. Biol.* **14**: R745–R747.

Charlesworth, B. & Charlesworth, D. 2000. The degeneration of Y chromosomes. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **355**: 1563–1572.

Charlesworth, D., Charlesworth, B. & Marais, G. 2005. Steps in the evolution of heteromorphic sex chromosomes. *Heredity* **95**: 118–128.

Chatfield, C. & Collins, A.J. 1980. *Introduction to Multivariate Analysis*. Chapman and Hall, Ltd., London.

De Givry, S., Bouchez, M., Chabrier, P., Milan, D. & Schiex, T. 2005. CARTHAGÈNE: multipopulation integrated genetic and radiation hybrid mapping. *Bioinformatics* **21**: 1703–1704.

Mattox, N.T. (1959) Conchostraca. In: *Freshwater Biology* (W.T. Edmundson ed.). pp. 577–586. Wiley, New York, NY.

Muller, H.J. & Painter, T.S. 1932. The differentiation of the sex chromosomes of *Drosophila* into genetically active and inert regions. *Zietschrift fur Induktive Abstammungs und Vererbungslehre* **62**: 316–365.

Nei, M. 1969. Linkage modification and sex differences in recombination. *Genetics* **63**: 681–699.

Rice, W.R. 1987. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex-chromosomes. *Evolution* **41**: 911–914.

Rice, W.R. 1996. Evolution of the Y sex chromosome in animals. *Bioscience* **46**: 331–343.

Risch, N. 1992. Genetic linkage: interpreting Lod scores. *Science* **255**: 803–804.

Sassaman, C. & Weeks, S.C. 1993. The genetic mechanism of sex determination in the conchostracan shrimp *Eulimnadia texana*. *Am. Nat.* **141**: 314–328.

Steinemann, M., Steinemann, S. & Lottspeich, F. 1993. How Y chromosomes become genetically inert. *Proc. Natl Acad. Sci. USA* **90**: 5737–5741.

Sturtevant, A.H. & Beadle, G.W. 1936. The relations of inversions in the X-chromosomes of *Drosophila melanogaster* to crossing over and disjunction. *Genetics* **21**: 554–604.

Weeks, S.C. & Zucker, N. 1999. Rates of inbreeding in the androdioecious clam shrimp *Eulimnadia texana*. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **77**: 1402–1408.

Weeks, S.C., Marcus, V. & Alvarez, S. 1997. Notes on the life history of the clam shrimp, *Eulimnadia texana*. *Hydrobiologia* **359**: 191–197.

Weeks, S.C., Marcus, V. & Crosser, B.R. 1999. Inbreeding depression in a self-compatible, androdioecious crustacean, *Eulimnadia texana*. *Evolution* **53**: 472–483.

Weeks, S.C., Crosser, B.R., Bennett, R., Gray, M. & Zucker, N. 2000a. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: estimates of inbreeding depression in two populations. *Evolution* **54**: 878–887.

Weeks, S.C., Crosser, B.R., Gray, M.M., Matweyou, J.A. & Zucker, N. 2000b. Is there sperm storage in the clam shrimp *Eulimnadia texana*? *Invertebrate Biology* **119**: 215–221.

Weeks, S.C., Crosser, B.R. & Gray, M.M. 2001. Relative fitness of two hermaphroditic mating types in the androdioecious clam shrimp, *Eulimnadia texana*. *J. Evol. Biol.* **14**: 83–94.

Weeks, S.C., Marquette, C.L. & Latsch, E. 2004. Barriers to outcrossing success in the primarily self fertilizing clam shrimp, *Eulimnadia texana* (Crustacea, Branchiopoda). *Invertebrate Biology* **123**: 146–155.

Weeks, S.C., Reed, S.K., Cesari, M. & Scanabissi, F. 2006. Production of intersexes and the evolution of androdioecy in the clam shrimp *Eulimnadia texana* (Crustacea, Branchiopoda, Spinicaudata). *Invertebrate Reproduction & Development* **49**: 113–119.

Zucker, N., Stafki, B. & Weeks, S.C. 2001. Maintenance of androdioecy in the freshwater clam shrimp *Eulimnadia texana*: longevity of males relative to hermaphrodites. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **79**: 393–401.

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