Chromosome-Wide Impacts on the Expression of Incompatibilities in Hybrids of *Tigriopus californicus*

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**ABSTRACT**

Chromosome rearrangements such as inversions have been recognized previously as contributing to reproductive isolation by maintaining alleles together that jointly contribute to deleterious genetic interactions and postzygotic reproductive isolation. In this study, an impact of potential incompatibilities merely residing on the same chromosome was found in crosses of populations of the copepod *Tigriopus californicus*. When genetically divergent populations of this copepod are crossed, hybrids show reduced fitness, and deviations from expected genotypic ratios can be used to determine regions of the genome involved in deleterious interactions. In this study, a set of markers was genotyped for a cross of two populations of *T. californicus*, and these markers show widespread deviations from Mendelian expectations, with entire chromosomes showing marked skew. Despite the importance of mtDNA/nuclear interactions in incompatibilities in this system in previous studies, in these crosses the expected patterns stemming from these interactions are not widely apparent. Females lack recombination in this species, and a striking difference is observed between male and female backcrosses. This suggests that the maintenance of multiple loci on individual chromosomes can enable some incompatibilities, perhaps playing a similar role in the initial rounds of hybridization to chromosomal rearrangements in preserving sets of alleles together that contribute to incompatibilities. Finally, it was observed that candidate pairs of incompatibility regions are not consistently interacting across replicates or subsets of these crosses, despite the repeatability of the deviations at many of the single loci themselves, suggesting that more complicated models of Dobzhansky-Muller incompatibilities may need to be considered.

**KEYWORDS**

postzygotic reproductive isolation
Dobzhansky-Muller incompatibilities
copepod
Mendelian ratio deviations

The nature and structure of chromosomes can play an important role in the genetic patterns that underlie postzygotic reproductive isolation. One broadly observed example of this is the impact of sex chromosomes in hybrids, which leads to two important patterns: Haldane’s rule and the large X-effect. The large X-effect is the pattern of a disproportionately high number of Dobzhansky-Muller (DM) incompatibilities occurring on the X (or Z) chromosome relative to autosomes (Masly and Presgraves 2007; Good et al. 2008). Haldane’s rule is the observation that if one sex is inviable or sterile, it is most often the heterogametic sex, and relies on the recessive nature of most incompatibility interactions (Haldane 1922; Turelli and Orr 2000). These DM incompatibilities are deleterious interactions between alleles in the hybrid genome, and are thought to cause the lowered hybrid viability or fertility observed in crosses of genetically divergent taxa (Coyne and Orr 2004; Masly and Pregraves 2007). Taxa that lack sex chromosomes or have lower levels of sex chromosome differentiation (lower heteromorphism) show slower rates of build-up of postzygotic reproductive isolation relative to taxa that have highly differentiated sex chromosomes (Lima 2014). It is clear then that differentiated sex chromosomes have an impact on postzygotic reproductive isolation. However, it is less clear in taxa that lack sex chromosomes whether, when multiple DM incompatibility loci occur on the same chromosome, this distribution will influence the nature or accumulation of DM incompatibilities.
One way that chromosomal differences could contribute to postzygotic reproductive isolation is by chromosomal rearrangements, and these rearrangements could act in either a direct or indirect way. Chromosomal inversions can contribute directly to hybrid sterility via the disruption of meiosis, which is then associated with lowered hybrid fertility; however, it is unclear how often this occurs, particularly among animal species (Dehleri et al. 2003; Faria and Navarro 2010). Indirectly, chromosomal rearrangements can limit recombination between divergent chromosomal regions in hybridizing taxa, holding together sets of alleles that can contribute to reproductive isolation (Noor et al. 2001; Navarro and Barton 2003; Strasburg et al. 2009).

In second-generation hybrid offspring (F2), the lack or lower levels of recombination in one sex could also influence the impacts of incompatibilities in these taxa. A wide range of taxa show either large differences in recombination rates between the sexes, or even the complete absence of recombination in one sex (Morgan 1914; Lenormand and Duthiel 2005). For heterogametic taxa, when recombination is absent in one sex it is almost always the heterogametic sex (Huxley 1928; Lenormand and Duthiel 2005), and this has been suggested as a mechanism to ensure continued opportunities for recombination across the entire X (or Z) chromosome in the homogametic sex (Lenormand and Duthiel 2005). In Tigriopus californicus, a species that lacks sex chromosomes, females lack recombination (Ar-rushdi 1962). Edmands (2008) found that recombinant backcrosses (hybrid male backcrosses) result in higher hybrid fitness in comparison to nonrecombinant backcrosses (hybrid female backcrosses). However, it is unclear if this difference could impact the expression of potential DM incompatibilities in this system.

Tigriopus californicus is a copepod that inhabits splash pools in the rocky intertidal pools along the Pacific coast of North America, and has been used extensively as a system to study the genetics of postzygotic reproductive isolation (Burton et al. 2006; Willett 2011a; Foley et al. 2013). Populations of this species can have high levels of genetic divergence, particularly for mtDNA (Burton 1998; Edmands 2001; Willett and Ladner 2009; Willett 2012). These genetically divergent populations, when crossed in the laboratory, show some level of hybrid inviability, particularly for F2 hybrids (Burton 1987, 1990; Edmands 1999). Interactions between the mtDNA genome and nuclear genomes appear to play an important role in DM incompatibilities in this system (Ellison and Burton 2008a; Burton and Barreto 2012), but there is also evidence for autosome-autosome interactions (Willett 2006; Foley et al. 2013).

In F2 hybrids of *T. californicus*, significant deviations from expected Mendelian inheritance are seen at many loci for adult hybrids, but not generally for the first larval stage (nauplii), indicating potential regions of the genome involved in DM incompatibilities (Willett and Burton 2001; Willett 2006; Harrison and Edmands 2006; Pritchard et al. 2011; Foley et al. 2013). This paper will focus on one particular cross in which previous studies have uncovered a marker, malic enzyme (ME2), with a dramatically high level of distortion in F2 hybrids and at least one other marker (*GOT2*) that showed a significant interaction with this ME2 genomic region (Burton 1987; Willett and Berkowitz 2007; Willett 2011b). Previous studies of this cross have not examined this ME2 marker in conjunction with other markers on the same chromosome, or markers on other chromosomes.

Here, we conducted a series of crosses with targeted genotyping of F2 hybrids from a cross of two populations of *T. californicus* to examine the wider chromosomal context of potential DM incompatibilities. First, a set of markers spanning the chromosome with the ME2 marker and each of the other 11 chromosomes was examined to determine the extent to which the distortion detected at ME2 extends into the rest of its chromosome, and to determine other potentially interacting genomic regions. Next, we looked at a full set of backcrosses of these two populations to determine the impact of sex-limited intrachromosomal recombination in the expression of this potential incompatibility associated with ME2, to explore potential interacting chromosomal regions. The results of this study provide a more complete view of the wider genomic impacts of hybridization, and the potential impacts of differing patterns of sex-limited intrachromosomal recombination on DM incompatibilities.

**MATERIALS AND METHODS**

**Copepod culturing and crossing**

*T. californicus* copepods used for crosses were collected from intertidal rock pools at two southern California locations, San Diego (SD, 32.7457°N, 117.2550°W, San Diego County, CA) and Abalone Cove (AB, 33.7377°N, 118.3753°W, Los Angeles County, CA). The copepods were maintained in the laboratory in mass culture in artificial seawater (Instant Ocean, Aquarium Systems Inc.) in 400-ml beakers at 20° with a 12:12 light-dark photoperiod (L:D). Copepods were maintained at a saltwater concentration of 35 parts per 1000 (similar to normal coastal ocean salinities), and fed with commercial flake fish food. Copepods also consumed natural algal growth and detritus in these cultures.

A series of different crosses were set up between copepods from the AB and SD populations. The general practice used for these crosses was to first collect virgin females by separating clasped pairs to obtain putatively unmated females. These females were individually monitored for approximately a week after maturity to verify that they produced no offspring and were indeed virgin females. Females were then mated to males from the other population. After first generation hybrid nauplii were observed in a cross, males were removed. Females were removed when offspring reached the copepodid stage. With the exception of one cross (discussed below), the crosses were done at 20° with a 12:12 L:D cycle.

**F1 x F1 cross and iPlex gold SNP assays (DA1 and AD1)**

One set of crosses was set up to generate F2 adult and nauplii hybrids to genotype for a set of SNPs covering all 12 chromosomes (Figure 1A). To generate F1 hybrids for these crosses, two Petri dishes, each with 20 females and 20 males, were set up for the two reciprocal crosses between copepods from the AB and SD populations. AB females crossed with SD males will be called AD, while SD females crossed with AB males will be DA. F1 hybrids were mixed across replicate dishes of the same cross to help minimize any chance of inbreeding. Collection dishes for F2 progeny were then set up in new Petri dishes starting with 20–25 mated F1 females. Replicated sets of these F2 collection Petri dishes were then placed at each of two temperatures (16° and 20°), and the female copepods left to produce progeny. Previous studies had shown some impacts of temperatures on the expression of incompatibilities and interactions (Willett and Burton 2003; Willett 2008), and we used these two temperatures to help determine the stability of patterns of interactions and marker distortion in the current crosses. F2 hybrid, first-stage nauplii were collected from the SD × ABm cross at 20°. Adult male and female F2 hybrids were collected from each of the four crosses (two reciprocal crosses each with two temperatures) in the numbers recorded in Table 1. Note that males and females were collected as they matured and reflect the biased sex ratios produced for these crosses. Copepods were collected and placed into 20-μl of lysis buffer in 96-well plates for genotyping (Willett 2011b).

A set of 32 SNP markers was selected that both spanned the 12 chromosomes of *T. californicus* and also included a number of other
To further explore the impact of chromosome 3 and any potentially interacting chromosomes, a multigenerational backcross was done, and followed by a controlled genotype intercross with backcross genotypes (Figure 1C). For these crosses DA F1 x ABm backcross hybrid progeny were backcrossed for two generations to AB males. The hybrid female parent was scored in each cross, and only the crosses where the female was heterozygous for chromosome 3 were retained as scored by genotyping progeny for each of these eight possible backcrosses. These additional backcross progeny were initially genotyped for the ME2 locus (both closely associated with marker 3d); primers and more details are given in Table S2.

**Backcrosses to test impact of chromosome 3**

To explore the impacts of females lacking recombination on any potential DM inviability loci on chromosome 3, a set of backcrosses were conducted using F1 hybrids from the AB and SD population crosses (Figure 1B). These crosses were done in the same manner as the DA2 cross using 24-well culture plates. All eight possible combinations of backcrosses were performed using F1 males and females from the two parental reciprocal crosses (Table 1 gives all combinations and numbers of progeny collected for each). An initial set of up to 48 progeny was genotyped for each of these eight possible backcrosses for the markers ME2 (3d) and G0T2 (8d). Further crosses were done to extend and confirm the results from the initial set of backcross progeny for four of these backcrosses. These additional backcross progeny were initially genotyped for the ME2 locus and/or the 3FBLR locus (both closely associated with marker 3d). A set of progeny from the AD F1 x SDm cross was further genotyped for a single marker on each of the 12 chromosomes (Table S2). This cross is a female backcross, so no recombination should occur between the two different populations’ chromosomes, and a single marker will be sufficient to characterize the genotype for each chromosome in each hybrid individual.

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both parents. Four heterozygote/heterozygote lineages were obtained from these crosses and these produced 64, 51, 14, and six adult progeny. The parents and a set of progeny of these crosses were scored for a single marker on each of the 12 chromosomes of *T. californicus*.

**Data availability**

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. In addition to the summary results given in the Supplemental Material, genotyping data and R source code has been archived in the Dryad data repository at [http://dx.doi.org/10.5061/dryad.gh2pb](http://dx.doi.org/10.5061/dryad.gh2pb).

**RESULTS**

**iPlex SNP genotyping**

To look for the potential impacts of DM incompatibilities and find potential interactions we scored F2 hybrid progeny from four different crosses between the AB and SD populations of *T. californicus* for a set of 25 markers spanning the 12 chromosomes of this species (consisting of two reciprocal crosses at two temperatures: Table 1 and Table S3). These 25 markers were pared down from an original set of 32 markers, seven of which were deemed unreliable based on control scoring or aberrant genotyping results. Genetic maps constructed from these crosses and these produced 64, 51, 14, and six adult progeny. The parents and a set of progeny of these crosses were scored for a single marker on each of the 12 chromosomes of *T. californicus*. Four heterozygote/heterozygote lineages were obtained from these crosses and these produced 64, 51, 14, and six adult progeny.

The genotypes of the F2 progeny at many of these 25 loci showed significant deviations from Mendelian ratios, as well as some differences across reciprocal crosses. The results for the DA1 and AD1 crosses, a subset of the total progeny collected were genotyped. Total collected for each of these four cross/temperature combinations are given here to provide an estimate of the sex ratio of the progeny in each cross.

Table 1 AB × SD *T. californicus* hybrid cross sample sizes

| Cross                  | Pool | Genotyped   | Total Collecteda |
|------------------------|------|-------------|------------------|
|                        |      | Female | Male | Adult | Nauplii | Male |                     |
| SDF × ABm (DA1)        | 16°  | 214    | 25   | 235   | 302    | 25   |                     |
|                        | 20°  | 332    | 53   | 388   | 96     | 640  | 124                 |
| SDm × ABf (AD1)        | 16°  | 142    | 134  | 276   | 237    | 229  |                     |
|                        | 20°  | 184    | 114  | 298   | 186    | 117  |                     |
| SDF × ABm (DA2)        | 1 d  | 142    | 134  | 276   | 237    | 229  |                     |
|                        | 2 d  | 184    | 114  | 298   | 186    | 117  |                     |
| F1f (DA) × SDm         | Adult| 137    | 87   | 224   |                     |      |                     |
| F1f (DA) × SDf         |      | 16     | 16   | 54    |                     |      |                     |
| F1m (DA) × SDf         |      | 17     | 7    | 22    |                     |      |                     |
| F1f (AD) × SDm         |      | 34     | 53   | 206   |                     |      |                     |
| F1m(AD) × SDf          |      |        |      | 43    |                     |      |                     |
| F1f (DA) × ABm         |      | 34     | 53   | 206   |                     |      |                     |
| F1m(AD) × ABf          |      | 119    |      | 45    |                     |      |                     |
| F1f (AD) × ABm         |      | 48     |      | 43    |                     |      |                     |
| F1m (AD) × ABf         |      | 48     |      | 43    |                     |      |                     |

For the DA1 and AD1 crosses, a subset of the total progeny collected were genotyped. Total collected for each of these four cross/temperature combinations are given here to provide an estimate of the sex ratio of the progeny in each cross. For backcrosses, progeny were collected across different independent crosses and sex was not recorded for several of these crosses.

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| F1f (DA) × SDm         | Adult| 137    | 87   | 224   |         |       |       |                     |
| F1f (DA) × SDf         |      | 16     | 16   | 54    |         |       |       |                     |
| F1m (DA) × SDf         |      | 17     | 7    | 22    |         |       |       |                     |
| F1f (AD) × SDm         |      | 34     | 53   | 206   |         |       |       |                     |
| F1m(AD) × SDf          |      |        |      | 43    |         |       |       |                     |
| F1f (DA) × ABm         |      | 34     | 53   | 206   |         |       |       |                     |
| F1m(AD) × ABf          |      | 119    |      | 45    |         |       |       |                     |
| F1f (AD) × ABm         |      | 48     |      | 43    |         |       |       |                     |
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in limited subsets of the crosses. Five other pairs of chromosomes showed relatively strong deviations from independence, but these did not reach the Bonferroni correction level (Table 2), and numerous other pairs of loci were significant with lower levels of stringency (Table S4). For three-locus interactions no comparisons were significant with a Bonferroni correction, and only a fairly small number were significant at the $P < 0.001$ level (Table 2; full results in Table S5).

**Genotypes from first and second day hybrid nauplii**

We looked at the time course for the expression of incompatibilities by examining several markers in the earliest free-living life stage, F2 nauplii. Targeted genotyping of three different SNP markers showed that markers on chromosome 3 change quickly in their genotypic ratios over the course of development (Figure 4). The F2 hybrids for this targeted genotyping were from another replicate Sdf × ABm cross (DA2). The three markers included two on chromosome 3 (3b and 3d) and a third on chromosome 11, and were scored using a PCR-based genotyping method (these did not score the exact same SNP as the nearby iPlex markers). Both markers on chromosome 3 were shifted in their genotypic ratios from the first to the second day (with one of the two markers showing a significant shift), and then another significant shift in genotypes from d2 to adults. For the chromosome 11 marker, there was little change for the first and second day nauplii, but the genotypic ratios shifted significantly for adults. Another marker on chromosome 8 (c8_3336) showed no shifts in genotypic ratios across these three developmental points (included in Table S6).
Backcross genotyping
We took advantage of the lack of crossing over in females to determine whether markers that reside on the same chromosome could impact the expression of hybrid incompatibilities differently in male vs. female backcrosses. The patterns of segregation for two chromosomal markers were examined across the eight possible backcrosses of the AB and SD populations of *T. californicus*. The first marker, 3d, was highly skewed in two nonrecombinant backcrosses when F1 hybrid females were backcrossed to the SD population (Figure 5A). The second marker, on chromosome 8 (*GOT2*, 8d), showed no significant differences from expected patterns of inheritance across these eight crosses (Table S7).

We then focused on a subset of individuals for the nonrecombinant backcross AD F1f · SDm, and genotyped a single marker per chromosome to determine if any associations between chromosomes could be found (particularly for the individuals that were SD/SD at marker 3d). While two additional chromosomes (1 and 9) were skewed in these backcross hybrids (Figure 5B), no significant associations were found between chromosomes (Table S8).

A final set of backcrosses was done to help determine whether other chromosomes had to cosegregate with chromosome 3 to maintain viability. These backcrosses were done for two generations for the nonrecombinant backcross, DA F1f × ABm, and genotyped a single marker per chromosome to determine if any associations between chromosomes could be found (particularly for the individuals that were SD/SD at marker 3d). While two additional chromosomes (1 and 9) were skewed in these backcross hybrids (Figure 5B), no significant associations were found between chromosomes (Table S8).

DISCUSSION

**Chromosome-wide deviations for chromosome 3**
One striking pattern to emerge from the analyses of the genetic markers in the DA1 and AD1 crosses of the AB and SD populations of *T. californicus* is the strong and potentially chromosome-wide deviations observed for chromosome 3 (Figure 2). Previous studies had consistently found that the SD/SD genotype at the ME2 locus was nearly completely inviable in F2 adult hybrids from these two populations...
for both directions of the cross (Willett and Berkowitz 2007; Willett 2011b), and this study shows that this pattern extends to the entire surveyed portion of the 3rd chromosome. Results from complete genome sequencing of pools of F2 hybrids also confirm a strong AB allele bias across the entire 3rd chromosome for this cross (T. G. Lima and C. S. Willett, unpublished data). Based on the genetic map of Foley et al. (2011) for a different pair of populations (SD and SC), the markers used in this study extend close to both ends of the chromosome 3 linkage group. The linkage map distances for chromosome 3 markers are comparable to those obtained by Foley et al. (2011); also, chromosome 3 showed little evidence of deviations from expected Mendelian inheritance for this SD × SC cross (Foley et al. 2013). The comparison of these results suggests that recombinant DNA is similar for chromosome 3 for these two different F1 × F1 crosses (with recombination occurring presumably in the male F1 parent) that are nonetheless showing dramatically different patterns of genotypic viability for this chromosome.

Given the importance of sex chromosomes for the expression of DM incompatibilities in other systems (Coyne and Orr 2004; Masly and Presgraves 2007; Good et al. 2008; Lima 2014), it is interesting to ask whether the chromosomal regions that show strong deviations in these crosses are connected with sex determination in this T. californicus species. Clearly, a number of chromosomes show strongly biased genotypic ratios between males and females—in particular chromosomes 2, 4, 6, and 10 (Figure 3). Interestingly, these same chromosomes were shown to harbor sex determination QTL in a recent study by Alexander et al. (2015) that used a cross between SD and a population from British Columbia, Canada. Given that the SD population was used in both of these crosses (and chromosome 10 previously has been suggested to harbor a sex determination locus in a cross of SC and SD; Foley et al. 2013), our results lend further support to at least the SD population harboring sex ratio altering alleles on these chromosomes. However, these results do not suggest that the large impacts of markers on chromosomes 1 and 3 have any connection to sex, nor that chromosome 3 is likely to be a proto-sex chromosome.

In contrast to the results for F2 adults for chromosome 3, F2 nauplii show inconsistent deviations from Mendelian expectations in hybrids for markers on this chromosome. For the DA1 cross, four of five markers show an excess of AB/AB homozygotes (Figure 2), while in the DA2 cross, nauplii tend to show an excess of SD/SD homozygotes at ME2 (marker 3d; Figure 4). Interestingly, the genotypic frequencies for a marker on chromosome 3 shift rapidly in the first 2 d after naupliar eclosion from the egg sac, suggesting the deleterious impacts of the 3rd chromosome in a hybrid genetic background might be particularly strong during this early free-living phase of the life cycle. Nauplii have undergone significant development in the egg sac before emerging as free-swimming organisms, and it is possible that maternal effects could lead to differences across clutches at hatching (perhaps mediated by different amounts of resources provided by different females). Previous studies had found either no or inconsistent deviations for first-day nauplii for the ME2 marker in crosses of these and other related populations (Willett and Berkowitz 2007; Willett 2011b), while markers on other chromosomes have shown little evidence for deviations for markers across the genome in first-stage nauplii in this and other crosses of this copepod species (Pritchard et al. 2011; Foley et al. 2013; T. G. Lima and C. S. Willett, unpublished data).

The results from the progeny of first-generation backcrosses highlight the importance of females lacking recombination in T. californicus for the expression of potential DM incompatibilities on chromosome 3 (Figure 5A). It is only for crosses in which there is a hybrid female parent crossed to an SD male that there is a significant deviation from the expected 1:1 ratio for the progeny. For F1 hybrid male parents crossed to SD females, there is no evidence for a deviation from
expected ratios. This observation implies that an intact chromosome 3 is important for the expression of this incompatibility in this context. In hybrid male parents there will be recombination between the AB and SD alleles for this chromosome, while in females the chromosome will be passed on to the offspring intact. These results could suggest that two or more factors on this chromosome (and potentially chromosome 1 as well) contribute jointly to hybrid breakdown in these backcross hybrids, and that the lack of intrachromosomal recombination in one sex can be an important factor for the expression of DM incompatibilities.

The importance of an intact chromosome for expressing a subset of DM incompatibilities parallels the impact that chromosomal rearrangements can have in maintaining a set of coadapted alleles that together can produce incompatibilities in hybrids (Noor et al. 2001; Navarro and Barton 2003; Strasburg et al. 2009). However, there is no evidence in these crosses of T. californicus that the chromosome-wide skew patterns are associated with chromosomal rearrangements or reductions in recombination in hybrid males. The genetic maps in crosses of three different highly divergent populations show the same relative ordering of markers, and no regions of highly clumped markers that could indicate rearrangements (Foley et al. 2011; Table S1). The contrast between the male backcrosses and female backcrosses (Figure 5A) also indicate that it is the lack of recombination in females, and not chromosomal rearrangements, that are responsible. A study by Edmands (2008) examined the fitness consequences of nonrecombinant backcrosses in T. californicus using a population from Laguna Beach, CA (genetically most similar to SD; Peterson et al. 2013), and the Royal Palms, CA population (very closely related to AB). She found that recombinant crosses had faster development rates than nonrecombinant backcrosses in the two temperature regimes did not have a large impact on the size of hybridity makes a difference in the expression of incompatibilities (Maheshwari and Barbash 2011), it could be that single interactions that are lethal in a genome with a higher level of hybridity (i.e., the F1 × F1 hybrids) may not be lethal in the backcrosses, and additional DM incompatibilities would be needed to cause lethality (where 75% of the genome is from one parental population). If these additional loci reside on the same chromosome, limiting recombination would increase the percentage of times they segregate together in hybrids.

Interactions between markers

There is little evidence for strong, consistent interactions between markers in this dataset that could indicate regions of the genome that are interacting to cause DM incompatibilities across all conditions and crosses (Table 2). The two-way interactions that were significant with a Bonferroni correction (between chromosomes 2/3 and 3/4) were not observed for all markers on these chromosomes, nor were they found in many subsets of these crosses. The second-generation backcross results do lend some further support to the possibility of an interaction between chromosomes 3 and 4, but more lines are needed to strengthen this conclusion (Table 3). Previous studies using hybrids from this pair of populations had shown significant interactions between ME2 (3d) and GOT2 (8d) in one dataset, and in another, between CYC (6a), CYC1 (4a), RISP (8a), which are associated with the mitochondrial ETS complex III (Willett 2006, 2011b). Of these interactions, only the CYC1 (4a) and RISP (8a) markers show a strong interaction in the present dataset, but only for the combined DA crosses. Deviations at single loci for crosses of T. californicus have sometimes shown a large degree of variation across repeated crosses (Willett 2008)—a pattern that contrasts with the consistently strong deviations associated with ME2 in this AB × SD cross (Burton 1987; Willett and Berkowitz 2007; Willett 2011b). In this study, the repeated crosses across the two temperature regimes did not have a large impact on the size or direction of single-locus deviations (Figure S1), but they did appear to impact which sets of two- and three-way interactions between loci were significant (Table 2). Variation from cross to cross could result from uncontrolled environmental factors such as the...
Previous work looking at crosses involving populations of *T. californicus* has found both a number of nuclear/nuclear interactions as well as nuclear/mitochondrial interactions (Foley et al. 2013). Foley et al. 2013 used crosses between the SD population and the SC population from central California, and found significant two-way interactions between chromosomes 4 and 7 across both reciprocal crosses, as well as several significant differences between the reciprocal crosses that could be due to mitochondrial interactions. In most cases, markers from across each of these chromosomes showed similar patterns of interactions. They found 15 different chromosome three-way interactions that were significant with a Bonferroni correction, suggesting an increased number of more complex interactions were occurring in comparison to two-way interactions. These results suggest that the stability and strength of epistatic interactions could differ markedly across different crosses of populations of this copepod.

Table 3 Parental genotypes in second generation backcross hybrids between the AB and SD populations of *T. californicus* (DA F1f x ABm)

| Chromosome | Marker | Line 1 | Line 2 | Line 3 | Line 4 |
|------------|--------|--------|--------|--------|--------|
| 1          | c1_1718| AA     | AA     | HA     |        |
| 2          | c2_5   | AA     | AA     | HH     | AH     |
| 3          | 3d     | HH<sup>b</sup> | HH<sup>b</sup> | HH<sup>d</sup> | HH     |
|            | AB rel. | 2.17   | 2.26   | 1.75   | 2.0    |
|            | SD rel. | 0.35   | 1.39   | 0.67   |        |
| 4          | 4a     | HH<sup>b</sup> | AA     |        |        |
| 5          | P5CS   | AH<sup>c</sup> | HA     |        |        |
| 6          | 6a     | HA     | AA     | HH     |        |
| 7          | c7_2276| AA     | AA     | HA     |        |
| 8          | 8d     | AH     | AH     | HH     |        |
| 9          | c9_2203| AH<sup>b</sup> | AA     |        |        |
| 10         | c10_1464| AH     | AA     | AH     |        |
| 11         | 11     | AA     | AA     | AA     | HH     |
| 12         | 12     | HA<sup>a</sup> | AH<sup>b</sup> | AA     |        |
| Total      |        | 51     | 73     | 14     | 6      |

Table 3. Parental genotypes in second generation backcross hybrids between the AB and SD populations of *T. californicus* (DA F1f x ABm).

<sup>a</sup> The genotypes of the two parents for scored chromosome: AH = AB/AB female and AB/SD male parent; HA = AB/SD female and AB/AB male parent; HH = both parents AB/SD; AA = both parents AB/AB.

<sup>b</sup> The progeny in these lines showed a significant deviation from expected Mendelian ratios when correcting for multiple tests with a Bonferroni correction (19 tests, adjusted P = 0.0026), full results in Table S9.

<sup>c</sup> The progeny of these lineages showed no evidence for deviations from expected Mendelian patterns of inheritance (P > 0.05 with more than 20 genotyped progeny).

<sup>d</sup> No SD/SD homozygotes were found for this lineage at marker 3d, and P value was 0.0092 for departure from 1:2:1 ratio.
Conclusions

This study has shown that the linkage of potential DM incompatibilities in a chromosome could have impacts on their expression in hybrids of the copepod *T. californicus*. Strong deviations were found for the entire 3rd chromosome, and, in backcross hybrids, these deviations were observed only when individuals inherited recombinant chromosomes from both parents. As illustrated by difference between the backcrosses with males (with recombination) and females (no recombination), it is possible that a contribution to hybrid inviability is made by the chromosome holding together a set of loci that can jointly cause incompatibilities in a manner analogous to the role played by inversions in crosses of other species (Noor et al. 2001; Navarro and Barton 2003; Strasburg et al. 2009). However, it is unlikely that the sex-limited lack of recombination completely determines the patterns of viability at chromosome 3 in F1 × F1 crosses. Given the magnitude of the deviations in F2 hybrids for markers across chromosome 3, it is most likely that there are other factors that do not depend on having a complete 3rd chromosome, and these factors could interact with novel homozygous genotypes in these crosses (that cannot be produced in a backcross).

The regions of the genome that are interacting to cause these DM incompatibilities in this cross of *T. californicus* are less clear. In this study, a number of different regions of the genome show some evidence for interactions, but these are not consistently displayed across different subsets of the data. Additionally, strong two-way interactions that were observed in previous studies in this same cross were no longer detected in this dataset. This apparently sporadic expression of interactions stands in contrast to the consistently strong deviations for the single-locus effects stemming from the region of chromosome 3 marked by the ME2 (3d) locus across a number of different replicates of this cross for these and closely related populations (Burton 1987; Willett and Berkowitz 2007; Willett 2011b; this study). Inconsistency could result from differences in density or culture conditions, as discussed previously. Alternatively, these results suggest that it is worth considering other DM incompatibilities model variants, including interactions with widespread genetic elements, novel responses that occur due to the unique nature of the hybrid genome overall (Maheshwari and Barbash 2011; Satyaki et al. 2014), or potentially a unique metabolic syndrome in hybrid individuals (Barreto et al. 2014).

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LITERATURE CITED

Alexander, H. I., J. M. L. Richardson, S. Edmands, and B. R. Anholt, 2015 Sex without sex chromosomes: multiple independently segregating loci across the genome determines sex ratios in the copepod *Tigriopus californicus*. J. Evol. Biol. 28(12): 2196–2207.

Ar-rushdi, A. H., 1962 The cytology of achiasmate meiosis in the female *Tigriopus* (copepoda). Chromosoma 13(5): 526–539.

Barreto, F. S., and R. S. Burton, 2013 Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. Proc. Biol. Sci. 280(1767): 20131521.

Barreto, F. S., R. J. Pereira, and R. S. Burton, 2014 Hybrid dysfunction and physiological compensation in gene expression. Mol. Biol. Evol. 32(3): 613–622.

Burton, R. S., 1987 Differentiation and integration of the genome in populations of the marine copepod *Tigriopus californicus*. Evolution 41(3): 504–513.

Burton, R. S., 1990 Hybrid breakdown in physiological response: a mechanistic approach. Evolution 44(7): 1806–1813.

Burton, R. S., 1998 Intraspecific phylogeography across the Point Concept biogeographical boundary. Evolution 52(3): 734–745.

Burton, R. S., and F. S. Barreto, 2012 A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? Mol. Ecol. 21(20): 4942–4957.

Burton, R. S., C. K. Ellison, and J. S. Harrison, 2006 The sorry state of F2 hybrids: consequences of rapid mitochondrial DNA evolution in allopatric populations. Am. Nat. 168(December, Suppl): S14–S24.

Coyne, J. A., and H. A. Orr, 2004 Speciation, Sinauer, Sunderland, MA.

Deleri, D., I. Colson, S. Grammenoudi, I. N. Roberts, E. J. Louis et al., 2003 Engineering evolution to study speciation in yeasts. Nature 422(6927): 68–72.

Edmands, S., 1999 Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53(6): 1757–1768.

Edmands, S., 2001 Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Mol. Ecol. 10(7): 1743–1750.

Edmands, S., 2008 Recombination in interpopulation hybrids of the copepod *Tigriopus californicus* release of beneficial variation despite hybrid breakdown. J. Hered. 99(3): 316–318.

Ellison, C. K., and R. S. Burton, 2006 Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. Evolution 60(7): 1382–1391.

Ellison, C. K., and R. S. Burton, 2008a Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. Proc. Natl. Acad. Sci. USA 105(11): 15831–15836.

Ellison, C. K., and R. S. Burton, 2008b Interpopulation hybrid breakdown maps to the mitochondrial genome. Evolution 62(3): 631–638.

Faria, R., and A. Navarro, 2010 Chromosomal speciation revisited: rearranging theory with pieces of evidence. Trends Ecol. Evol. 25(11): 660–669.

Foley, B. R., C. G. Rose, D. E. Rundle, W. Leong, G. W. Moy et al., 2011 A gene-based SNP resource and linkage map for the copepod *Tigriopus californicus*. BMC Genomics 12(1): 568.

Foley, B. R., C. G. Rose, D. E. Rundle, W. Leong, and S. Edmands, 2013 Postzygotic isolation involves strong mitochondrial and sex-specific effects in *Tigriopus californicus*, a species lacking heteromorphic sex chromosomes. Heredity 111(5): 391–401.

Good, J. M., M. D. Dean, and M. W. Nachman, 2008 A complex genetic basis to X-linked hybrid male sterility between two species of house mice. Genetics 179(4): 2213–2228.

Haldane, J. B. S., 1922 Sex ratio and unisexual sterility in hybrid animals. J. Genet. XII(2): 101–109.

Haldane, J. B. S., 1956 The estimation of viabilities. J. Genet. 59: 29–36.

Harrison, J. S., and S. Edmands, 2006 Chromosomal basis of viability differences in *Tigriopus californicus* interpopulation hybrids. J. Evol. Biol. 19(6): 2040–2051.

Huxley, J. S., 1928 Sexual difference of linkage in *Gammarus chevreuxi*. J. Genet. XIII(2): 145–156.

Lenormand, T., and J. Dutheil, 2005 Recombination difference between sexes: a role for haploid selection. PLoS Biol. 3(3): 0396–0403.

Lima, T. G., 2014 Higher levels of sex chromosome heteromorphism are associated with markedly stronger reproductive isolation. Nat. Commun. 5: 4743.
Lorieux, M., 2012 MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breed. 30(2): 1231–1235.

Maheshwari, S., and D. A. Barbash, 2011 The genetics of hybrid incompatibilities. Annu. Rev. Genet. 45(1): 331–355.

Masly, J. P., and D. C. Presgraves, 2007 High-resolution genome-wide dissection of the two rules of speciation in Drosophila. PLoS Biol. 5(9): 1890–1898.

Morgan, T. H., 1914 No crossing over in the male of Drosophila of genes in the second and third pairs of chromosomes. Biol. Bull. 26: 195–204.

Navarro, A., and N. H. Barton, 2003 Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. Evolution 57(3): 447–459.

Noor, M.A, K. L. Grams, L. A. Bertucci, and J. Reiland, 2001 Chromosomal inversions and the reproductive isolation of species. Proc. Natl. Acad. Sci. USA 98(21): 12084–12088.

Peterson, D. L., K. B. Kubow, M. J. Connolly, L. R. Kaplan, M. M. Wetkowski et al., 2013 Reproductive and phylogenetic divergence of tidepool copepod populations across a narrow geographical boundary in Baja California. J. Biogeogr. 40(9): 1664–1675.

Pritchard, V. L., L. Dimond, J. S. S. Harrison, C. C. Velázquez, J. T. Zieba et al., 2011 Interpopulation hybridization results in widespread viability selection across the genome in Tigriopus californicus. BMC Genet. 12(1): 54.

R Core Development Team. 2013 R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.r-project.org.

Rawson, P. D., and R. S. Burton, 2002 Functional coadaptation between cytochrome c and cytochrome c oxidase within allopatric populations of a marine copepod. Proc. Natl. Acad. Sci. USA 99(20): 12955–12958.

Satyaki, P. R. V., T. N. Cuykendall, K. H. C. Wei, N. J. Brideau, H. Kwak et al., 2014 The Hmr and Lhr hybrid incompatibility genes suppress a broad range of heterochromatic repeats. PLoS Genet. 10(3): e1004240.

Strasburg, J. L., C. Scotti-Saintagne, I. Scotti, Z. Lai, and L. H. Rieseberg, 2009 Genomic patterns of adaptive divergence between chromosomally differentiated sunflower species. Mol. Biol. Evol. 26(6): 1341–1355.

Turelli, M., and H. A. Orr, 2000 Dominance, epistasis and the genetics of postzygotic isolation. Genetics 154(4): 1663–1679.

Willett, C. S., 2006 Deleterious epistatic interactions between electron transport system protein-coding loci in the copepod Tigriopus californicus. Genetics 173(3): 1465–1477.

Willett, C. S., 2008 Significant variation for fitness impacts of ETS loci in hybrids between populations of Tigriopus californicus. J. Hered. 99(1): 56–65.

Willett, C. S., 2011a The nature of interactions that contribute to postzygotic reproductive isolation in hybrid copepods. Genetics 190(5): 575–588.

Willett, C. S., 2011b Complex deleterious interactions associated with malic enzyme may contribute to reproductive isolation in the copepod Tigriopus californicus. PLoS One 6(6): e21177.

Willett, C. S., and J. N. Berkowitz, 2007 Viability effects and not meiotic drive cause dramatic departures from Mendelian inheritance for malic enzyme in hybrids of Tigriopus californicus populations. J. Evol. Biol. 20(3): 1196–1205.

Willett, C. S., and J. T. Ladner, 2009 Investigations of fine-scale phylogeography in Tigriopus californicus reveal historical patterns of population divergence. BMC Evol. Biol. 9: 139.

Willett, C. S., and R. S. Burton, 2001 Viability of cytochrome c genotypes depends on cytoplasmic backgrounds in Tigriopus californicus. Evolution 55(8): 1592–1599.

Willett, C. S., and R. S. Burton, 2003 Environmental influences on epistatic interactions: viabilities of cytochrome c genotypes in interpopulation crosses. Evolution 57(10): 2286–2292.

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