Abstract

The outcome of hybridization can be impacted by environmental conditions, which themselves can contribute to reproductive isolation between taxa. In crosses of genetically divergent populations, hybridization can have both negative and positive impacts on fitness, the balance between which might be tipped by changes in the environment. Genetically divergent populations of the intertidal copepod *Tigriopus californicus* have been shown to differ in thermal tolerance at high temperatures along a latitudinal gradient. In this study, a series of crosses were made between pairs of genetically divergent populations of *T. californicus*, and the thermal tolerance of these hybrids was tested. In most cases, the first-generation hybrids had relatively high thermal tolerance and the second-generation hybrids were not generally reduced below the less tolerant parental population for high temperature tolerance. This pattern contrasts with previous studies from crosses of genetically divergent populations of this copepod, which often shows hybrid breakdown in these second-generation hybrids for other measures of fitness. These results suggest that high temperature stress could either increase the positive impacts of hybridization or decrease the negative impacts of hybridization resulting in lowered hybrid breakdown in these population crosses.

Key words: heterosis, hybridization, outbreeding depression, postzygotic reproductive isolation, thermal adaptation

The hybridization of genetically divergent lineages can result in hybrids that have increased fitness or decreased fitness depending on the nature of genetic interactions and the influence of the environment on these interactions. If there is a substantial amount of genetic load within populations that is masked by hybridization (i.e., the relief of inbreeding depression) or if there is overdominance at multiple loci, then the fitness of the hybrids can be higher than that of the parental populations (Charlesworth D and Charlesworth B 1987). Alternatively, hybridization can result in reduced hybrid fitness if deleterious gene interactions have accumulated between these lineages (e.g., Dobzhansky–Muller (DM) incompatibilities; Dobzhansky 1936; Muller 1942) or if hybridization results in a loss of local adaptation of each lineage to its specific environment (e.g., extrinsic reproductive isolation; Rundle and Whitlock 2001). Beyond even the case of extrinsic reproductive isolation, the environment can influence the results of both inbreeding and outbreeding making the potential fitness outcomes of hybridization complex and dependent on environmental conditions.

Outbreeding depression can be completely dependent on environmental differences if the fitness loss is a result of extrinsic reproductive isolation or largely independent of the environment for fitness losses stemming from intrinsic DM incompatibilities. There have been a number of examples described of reproductive isolation resulting from a loss of lineage-specific adaptation in hybrids (Feder et al. 1994; Hatfield and Schluter 1999; Hawthorne and Via 2001; Nosil et al. 2002; Fuller 2008; Egan and Funk 2009; Matute et al. 2009; McBride and Singer 2010). Matute et al. (2009) found evidence for one-way isolation as a result of a loss in temperature adaptation in 2 species of *Drosophila* that hybridize in nature. Although many DM incompatibilities may be largely intrinsic in nature, environmentally dependent genetic incompatibilities represent a type of DM incompatibility that is responsive to the environment (Bordenstein and Drapeau 2001; Fuller 2008). Temperature has often been found to be an important environmental factor moderating the expression of DM incompatibilities (Wade et al. 1999; Bordenstein and Drapeau 2001; Edmands and Deimler 2004;
Environmental stress can influence the expression of both inbreeding depression and outbreeding depression. The impacts of environmental stress on inbreeding depression have received more study and have shown a general pattern of increased inbreeding depression as environmental conditions become more stressful (Armbruster and Reed 2005; Fox and Reed 2011). Heat tolerance as a specific stress response has been found to be reduced in at least one case (Pedersen, et al. 2005) but in other cases not altered by inbreeding (Dahlgaard and Hoffmann 2000; Mikkelsen et al. 2010). In contrast to inbreeding depression, the expression of outbreeding depression may be lowered under more stressful conditions although data addressing this question are limited (Edmands and Deimler 2004; Edmands 2007). One proposed reason that outbreeding depression could be lowered under more stressful conditions is that stress could enhance the positive effects of outbreeding via increased heterosis rather than increasing the deleterious epistatic interactions that can cause outbreeding depression (Armbruster et al. 1997).

The intertidal copepod *Tigriopus californicus* is an excellent system in which to study the combined impacts on fitness of outbreeding depression, inbreeding depression, and the environmental interactions of each of these. This species occurs along the Pacific coast of North America in rocky intertidal pools and shows a great deal of genetic divergence between populations on different rocky outcrops. Gene flow appears to be limited on multiple scales from the local level where there are often stable differences in allele frequencies among populations (Burton 1997) to longer distances where pairwise divergence in mitochondrial-encoded (mtDNA) genes can exceed 20%, and nuclear-encoded genes can show lower but still substantial divergence (Burton 1998; Edmands 2001; Willett 2004; Willett and Ladner 2009). This divergence in mtDNA appears to contribute to DM incompatibilities that cause hybrid breakdown in population crosses of this species (Burton, et al. 2006; Ellison and Burton 2008; Willett 2011). Populations of this species can be reared in the laboratory and hybrids produced by controlled crosses. In crosses of genetically divergent populations from California, the F1 hybrids are generally equal in fitness to (or sometimes higher than) the parental copepods but have lowered fitness in the F2 generation for fitness measures, including developmental time, survival, hatching, fertility, and salinity tolerance (Burton 1986, 1987, 1990; Edmands 1999; Edmands and Deimler 2004; Ellison and Burton 2008; Willett 2008). Inbreeding depression is not nearly as well studied as outbreeding depression in *T. californicus* with a single study by Palmer and Edmands (2000) that found evidence for significant reductions in at least one measure of fitness with inbreeding.

Previously, I have shown that populations of *T. californicus* have a pattern of resistance to high temperature stress that is consistent with thermal adaptation across a latitudinal gradient for a series of populations from southern California to Vancouver in British Columbia, Canada (Willett 2010). The populations in the northern portion of this range have a significantly reduced ability to handle both acute short-term exposures to high temperatures and chronic exposure to a constant high temperature. Interestingly, in this study, I also found that for several sets of populations there appears to be fitness trade-offs associated with higher thermal resistance; the southern California populations that have higher resistance have lower competitive fitness at moderate temperatures (and the less-tolerant northern California populations have lower competitive fitness at high but nonlethal temperatures). A recent study by Kelly et al. (2011) also found a similar pattern in thermal adaptation across populations of *T. californicus* that extended further down into central Baja California, Mexico with a continued increase in high temperature thermal tolerance with decreasing latitude.

The impacts of temperature and thermal stress on outbreeding depression and the expression of incompatibilities have received only limited study in crosses of *T. californicus* populations to date. Edmands and Deimler (2004) found that survival and metamorphosis for F2 hybrids from one cross of populations showed less hybrid breakdown at an elevated temperature in comparison to a more moderate temperature. Temperature does appear to alter the nature of gene and protein interactions that may contribute to reproductive isolation in hybrids of crosses of *T. californicus*. Willett and Burton (2003) found that an incompatibility in hybrid copepods involving the cytochrome c (CYC) region of the genome appears to be conditional on the temperature at which copepods were reared. Also, the degree of functional coadaptation between CYC- and mtDNA-encoded subunits of the electron transport system has been shown to be dependent on temperature (Rawson and Burton 2002; Harrison and Burton 2006).

In the present study, I measure the high temperature thermal tolerance of hybrids of population crosses of *T. californicus* to determine the consequences of hybridization on this trait. I look at the ability of F1 and F2 hybrids to handle both chronic and acute thermal stress. These hybrids come from a set of crosses involving either 2 southern and more heat-tolerant populations or a southern population and a northern California, less heat-tolerant population. I find that hybrids are often higher in thermal tolerance than at least one of the parental populations suggesting that there is reduced outbreeding depression for this measure of fitness in the hybrid copepods. These results suggest that the ability of this trait to contribute to extrinsic reproductive isolation is likely to be somewhat limited at least in early hybrid generations.

### Materials and Methods

#### Copepod Collection and Crosses

*Tigriopus californicus* copepods were collected from the field from a set of outcrops from central Baja California, Mexico...
to central California, United States for use in crosses to generate hybrid individuals for temperature stress assays. These populations included 1 population from Baja California, Mexico, Punto Morro (PM) (31°31'51.690"N, 116°40'122"W), 3 populations from southern California, and 2 from northern California (Figure 1; sites other than PM are described previously in Willett and Ladner 2009). Copepods were collected from pools at these sites between 2000 and 2006 (for collection dates for each population used in each cross, see Table 1) and reared in the laboratory for at least one generation before initiating crosses. These same copepod collections were also used by Willett (2010) to examine high temperature heat tolerance in most of the parental populations. Many crosses were initiated with recently collected copepods; however, in some cases, copepod populations were maintained in the laboratory for longer periods of time before crossing. These populations were maintained in sets of both 400-ml beakers and multiple petri dishes with a starting size of 50 and 25 mated females, respectively. To help maintain genetic diversity within these populations, when new petri dishes or beakers were started, the founding females were collected from across multiple (4 or 5) petri dishes/beakers. All cultures and crosses were maintained at concentration of 35 parts per thousand in artificial seawater (Instant Ocean, Aquarium Solutions) at 20°C with a 12 h:12 h L:D daily light cycle. They were fed with commercial flake fish food, but copepods also consumed natural algal growth and detritus in the petri dishes and beakers.

Crosses were initiated by mating virgin female *T. californicus* from one population with males from the second population in the laboratory. Crosses were set up in the period between 2003 and 2006 for 7 different pairs of populations with reciprocal crosses done for 6 of these pairs (Table 1). In some

![Figure 1. Map of populations of *Tigriopus californicus* used in crosses to produce hybrids. Populations were sampled from tidepools ranging from northern California, United States to northern Baja California, Mexico. Further details of sampling sites can be found in Willett and Ladner (2009).](https://academic.oup.com/jhered/article-abstract/103/1/103/901259)

### Table 1

Number of assays performed for chronic and acute heat stress on hybrid copepods from crosses of populations of *Tigriopus californicus*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Years</th>
<th>32°C Assays</th>
<th>F₁ acute assays</th>
<th>F₂ acute assays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F₁ (f/m)⁵</td>
<td>37°C (f/m)</td>
<td>38°C (f/m)</td>
</tr>
<tr>
<td>PMf × SDm</td>
<td>2000, 2004</td>
<td>18/6</td>
<td>22/22</td>
<td>3/0</td>
</tr>
<tr>
<td>PMm × SDLf</td>
<td>2000, 2004</td>
<td>9/9</td>
<td>18/18</td>
<td>2/2</td>
</tr>
<tr>
<td>SDf × LJSm</td>
<td>2004, 2002</td>
<td>16/16</td>
<td>20/18</td>
<td>22/22</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>12/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDm × LJSf</td>
<td>2004, 2002</td>
<td>6/6</td>
<td>24/24</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>5/5</td>
<td>8/8</td>
<td></td>
</tr>
<tr>
<td>SDF × ABof</td>
<td>2004 ⁷</td>
<td>16/12</td>
<td>17/12</td>
<td>4/4</td>
</tr>
<tr>
<td>SDm × ABof</td>
<td>2002, 2000</td>
<td>4/4</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2004 ⁷</td>
<td>16/12</td>
<td>19/17</td>
<td>8/8</td>
</tr>
<tr>
<td>LJSf × ABof</td>
<td>2002, 2004</td>
<td>18/17</td>
<td>20/19</td>
<td>6/5</td>
</tr>
<tr>
<td>LJSf × ABof</td>
<td>2002, 2004</td>
<td>8/6</td>
<td>20/19</td>
<td>6/5</td>
</tr>
<tr>
<td>SDF × SCNf</td>
<td>2002, 2000</td>
<td>4/3</td>
<td>4/4</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>2004 ³</td>
<td>14/11</td>
<td>18/18</td>
<td>4/4</td>
</tr>
<tr>
<td>SDm × SCNf</td>
<td>2002, 2004</td>
<td>16/16</td>
<td>16/16</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td>2004 ³</td>
<td>16/16</td>
<td>16/16</td>
<td>15/15</td>
</tr>
<tr>
<td>ABf × SCNf</td>
<td>2000</td>
<td>4/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2004 ³</td>
<td>11/15</td>
<td>22/22</td>
<td>8/9</td>
</tr>
<tr>
<td>ABof × SCNf</td>
<td>2004 ³</td>
<td>19/18</td>
<td>23/21</td>
<td>12/12</td>
</tr>
<tr>
<td>SDm × BHBf</td>
<td>2006, 2004</td>
<td>12/13</td>
<td>3/3</td>
<td>8/8</td>
</tr>
</tbody>
</table>

⁤ Years denotes the years that copepods used in cross were collected from the field listed in the same order as the cross. Crosses were generally performed during the course of the year of the later collection date listed with the following exceptions: ABf × SCNf (2000) crosses were set up in 2004 and for years marked with a †, a subset of assays were from crosses done within 2 years of the last collection date.

⁣ Unequal numbers of males and females for acute assays indicate that unmatched females or males were set up as 20 same sex copepods rather than 10 female/10 male assays.
cases, populations were crossed that had been maintained for different periods of time in the laboratory. In Table 1 and for statistical analyses, “Years” refers to the year each of the parental populations in a cross were collected. Each cross was typically set up with 20 males and 20 virgin females per petri dish and generally 2 or more replicate petri dishes per cross. Hybrid F1 and F2 copepods were obtained as described in Willett (2006) and reared until they were adults to be used in temperature stress assays. For statistical analyses, the source was the individual petri dish from which the copepods in a single assay were collected.

Tests of Thermal Stress in Hybrids

Hybrid adult *T. californicus* individuals were exposed to both long-term chronic heat stress and short-term acute heat stress to look at their ability to handle extreme high temperatures. The number of assays done for each cross is given in Table 1. Most parental population thermal tolerance data come from Willett (2010) from assays that were undertaken at the same time as the hybrid assays described in this paper; the results from PM were not previously reported and they come from cultures that had been maintained in the laboratory for several years and may have lost some thermal tolerance due to laboratory adaptation. The PM parental and hybrid lineages were assayed at roughly the same time. Chronic and acute stress assays were done as previously described in Willett (2010), but briefly for the chronic temperature stress assays, daily survival of sets of 10 males or 10 females were monitored at the constant stressful temperature of 32 °C. The numbers of acute assays performed for each type of hybrid differed depending on the availability of those hybrids. Acute stress assays exposed groups of 10 males and 10 females that had been reared at 20 °C to a 1-h temperature of 37, 38, or 39 °C. After returning the copepods to 20 °C, survival was recorded after 3 days (preliminary work had shown that mortality from the heat shock declined dramatically after this length of time). For a few assays, 20 males or 20 females were used when there were not enough copepods from the opposite sex of a particular type of hybrid. A batch consisted of the set of assays that were conducted at the same time.

Statistical analyses for chronic stress were conducted with survival analyses using proportional hazards models in the program JMP (version 8.0.1, SAS Institute, Cary, NC). Chronic heat stress survival for each cross and reciprocal cross was analyzed with the data from the corresponding 2 parental populations. The minimum number of days survived was used in these models. Lineage, sex, and years
were included in each model (years was dropped for cases when all crosses were done in the same year). Willett (2010) showed some differences in thermal tolerance based on collection year for the parental populations used in these crosses. For the statistical analyses, an initial comparison was done between the parental populations and if there was not a significant effect of parental population (lineage) in the model, the parents were combined for subsequent comparisons to hybrids. In analyses of hybrid lineages, the reciprocal crosses for F1 and F2 hybrids were grouped together and then these were compared with parental populations. Subsequently, in the next set of models, the F1 and F2 hybrid lineages were compared with each other after dropping the parents out. Then, based on the results obtained from these sets of models, pairwise comparisons were made between parental lineages and single hybrid lineages (the F1 and F2 hybrids would be combined if they were shown not to be significantly different by the previous model). These last sets of comparisons were used to help determine the relative rankings of each hybrid lineage and parental population to one another.

Survival for the acute temperature stress experiments was modeled as binomial using generalized linear mixed models (function lmer, lme4 package; Bates et al. 2011) with R (version 2.8.1; R Development Core Team 2011). The best model for each set of experiments was chosen by starting with the simplest model and adding in effects and interactions sequentially. These nested models were compared with likelihood ratio tests to determine whether each additional factor improved the fit of the model significantly. In a number of cases, models in R would not converge for complex models involving crossed terms. The base model was set as the model that could not be improved on by adding additional factors. In these models, the factors source and batch were treated as random effects. A series of statistical models were undertaken as described above for chronic heat stress; first for parents, then for all lineages of hybrids and parents with hybrids grouped across reciprocal crosses, and then finally for hybrids. The impact of dropping lineage from each of these models was determined. Finally, pairwise comparisons were made based on these results to suggest the rankings of acute stress resistance among lineages analogous to that described above for chronic heat stress. These comparisons involved testing a single lineage (or set of nonsignificantly different lineages) with a second lineage to help order the acute stress resistance of each of the lineages. This process was repeated as many times as required to test how the lineages ranked against each other and whether they were different from one another.

### Results

#### Chronic Heat Stress

For the crosses involving 2 southern *T. californicus* populations where both parental populations have relatively high thermal resistance, the hybrids were generally either intermediate between the 2 parents or had higher chronic temperature stress resistance than one or both parents (Figure 2). In 3 of the 4 crosses, the F1 hybrids survived significantly longer at 32 °C than the F2 hybrids (Table 2).

### Table 2  Proportional hazards models for analysis of chronic heat stress of hybrids from crosses of the copepod *Tigriopus californicus*

<table>
<thead>
<tr>
<th>Cross</th>
<th>Factor</th>
<th>Parents only</th>
<th>All lineages</th>
<th>F1 and F2 hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L-R $\chi^2$</td>
<td>DF</td>
<td>P value</td>
</tr>
<tr>
<td>PM × SD</td>
<td>Lineage</td>
<td>2.24</td>
<td>1</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>14.5</td>
<td>1</td>
<td>0.0001</td>
</tr>
<tr>
<td>SD × LJS</td>
<td>Lineage</td>
<td>91.2</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>37.6</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Years</td>
<td>480.8</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD × AB</td>
<td>Lineage</td>
<td>3.56</td>
<td>1</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>32.9</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Years</td>
<td>216</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LJS × AB</td>
<td>Lineage</td>
<td>143.3</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>34.7</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD × SCN</td>
<td>Lineage</td>
<td>200.7</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>6.74</td>
<td>1</td>
<td>0.0094</td>
</tr>
<tr>
<td></td>
<td>Years</td>
<td>263.0</td>
<td>4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AB × SCN</td>
<td>Lineage</td>
<td>116.2</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>5.2</td>
<td>1</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Years</td>
<td>197.4</td>
<td>2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SD × BHB</td>
<td>Lineage</td>
<td>89.2</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>14.8</td>
<td>1</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

L-R $\chi^2$ is the likelihood ratio chi-square value for the indicated factor. For each cross, the tests are either down for parent only, all lineages, or a comparison of F1 and F2 hybrids only. For this table, the critical P value is 0.007 with a sequential Bonferroni correction for multiple tests.
the reciprocal cross had some impacts on chronic survival, but overall patterns were largely consistent with the results in Figure 2 (for a breakdown of year and reciprocal cross impacts, see Supplementary Figures S1 and S2). The largest difference between reciprocal crosses was for the SD/C2 AB crosses, where the difference between the F1 and F2 hybrids is driven by the ABf/C2 SDm reciprocal cross (Supplementary Figure S2).

CROSSES BETWEEN A NORTHERN AND A SOUTHERN CALIFORNIA POPULATION AGAIN SHOWED HYBRIDS THAT WERE INTERMEDIATE BETWEEN THE 2 PARENTS OR MORE HEAT RESISTANT THAN ONE OR BOTH PARENTAL POPULATIONS FOR CHRONIC TEMPERATURE STRESS TOLERANCE (Figure 3). A SERIES OF STATISTICAL TESTS USING PROPORTIONAL HAZARDS MODEL COMPARISONS BETWEEN LINEAGES (Supplementary Table S1) SUGGEST THAT THE NORTHERN CALIFORNIA POPULATION ALWAYS HAD THE LOWEST ABILITY TO TOLERATE CHRONIC HEAT STRESS IN EACH CROSS. ADDITIONALLY, F2 HYBRIDS HAD LOWER CHRONIC THERMAL STRESS TOLERANCE THAN THE SOUTHERN PARENTAL POPULATION IN 2 OF 3 CROSSES. IN THE THIRD CROSS, THE BHB × SD CROSS, THE SAMPLE SIZE OF THE F2 HYBRIDS WAS LIMITED (ONLY 6 ASSAYS). YEAR AND RECIPROCAL CROSS DID LEAD TO SOME VARIATION IN THESE ASSAYS, BUT RESULTS LARGELY DID NOT DEVIATE SUBSTANTIALLY FROM THE OVERALL PATTERN (see Supplementary Figures S3 and S4). THE RECIPROCAL CROSSES CONTAINING THE CYTOPLASM (AND mtDNA) FROM THE MORE HEAT-TOLERANT SOUTHERN CALIFORNIA POPULATION DID NOT SHOW A TEND TOWARD HIGHER THERMAL TOLERANCE THAN THOSE WITH A NORTHERN CALIFORNIA-TYPE CYTOPLASM.

Acute Heat Stress

Hybrids from crosses of 2 southern populations of *T. californicus* showed no evidence for a decline in high temperature acute stress tolerance in either the F1 or the F2 hybrid generation in comparison to the parental populations used in each cross (Figure 4 and Supplementary Table S2). In 3 of the 4 sets of crosses, at least one parent had lower heat resistance than both hybrid classes and in no case was a parental population consistently higher in acute thermal tolerance. Although the F2 hybrids tended to have lower survival than the F1 hybrids, this pattern is significant only for the SD × AB crosses. There was some variation across reciprocal crosses in these assays, and these results can be seen in Supplementary Figures S5 and S6. For the PM × SD cross, there was considerable variation across temperatures for the relative thermal resistance of F1 and F2 hybrids for the PMf × SDm reciprocal cross (Supplementary Figure S5). The factor years contributed significant variation in several crosses but showed the most variation for the crosses between the SD and LJS populations (Supplementary Figure S7).
Figure 4. Acute temperature stress survival for hybrids of crosses between 2 southern populations of *Tigriopus californicus*. Box plots show the proportion of copepods that survived an acute thermal stress for assays of hybrids combined across reciprocal crosses and parental assays from Willett (2010). Letters above each lineage are inferred differences among lineages based on a series of pairwise lineage statistical tests for each cross (Supplementary Table S2). Although letters are shown in the 38 °C panes of the figure, the statistical models incorporate the 2 or 3 temperatures used for each set of crosses. Dotted line is the midparent value (the average of the 2 parental populations median survival). Results broken down by reciprocal cross can be found in Supplementary Figure S5 for SD × PM crosses and Supplementary Figure S6 for the other 3 sets of crosses. *Note that the SD and PM populations are lumped due to the limited data available for the PM population.
In crosses between a northern and a southern California population of *Tigriopus californicus*, the F1 hybrids were roughly equal in acute stress resistance to the more thermally tolerant southern population, and both had significantly higher tolerance than the northern population (Figure 5 and Supplementary Table S2). In each cross, the northern...
parental population had the lowest thermal tolerance of any of the lineages. The F1 hybrids were higher in thermal tolerance than F2 hybrids for 1 of the 2 applicable comparisons with no significant difference in the second. The SCf × ABm reciprocal cross at 37 °C showed the largest deviation from the pattern of F1 hybrids having higher acute stress resistance than F2 hybrids among these crosses (Supplementary Figure S8). A comparison of the reciprocal crosses for these acute stress assays does not show a general trend for higher temperature resistance in hybrid lineages with the female parent of the cross (and the mtDNA) from the southern California population (Supplementary Figure S8). For acute and chronic stress assays, the females generally had higher thermal tolerance than the males both for parental populations and for hybrid lineages across all crosses.

Discussion

For acute and chronic thermal tolerance, hybrids of population crosses of *T. californicus* did not show dramatic reductions in thermal resistance in comparison to parental populations, a pattern that was particularly evident for F1 hybrids. In fact, for a number of the crosses in Figures 2–5, the F1 hybrids are better than the more thermally tolerant parental population (5 of 14). For the one case where an F1 lineage is less tolerant than one parental population, in the LJS × AB cross, there were only a fairly small number of chronic stress assays for only one reciprocal cross, and this pattern was not apparent for acute stress for this same cross (Figure 4). For crosses between northern and southern populations, these results suggest that the beneficial impacts of hybridization might outweigh the impacts of the genetic contribution from the less thermally tolerant northern population in first-generation hybrids. With additive inheritance, the heat tolerance of the F1 hybrids would be expected to fall midway between the 2 parental populations, but the F1 are clearly higher than this midpoint value.

The generally higher performance of F1 hybrids for these fitness characters is consistent with results found for several other fitness measures in crosses of *T. californicus* populations including developmental time, survivorship, and metamorphosis fraction (Burton 1990; Edmands 1999; Ellison and Burton 2008) and is commonly found as well in crosses of populations of other species for a variety of fitness measures (Rhode and Cruzan 2005). Edmands (1999) found that there was not a relationship between F1 and F2 fitness across a set of crosses nor was there a significant relationship between F1 fitness and geographic or genetic distance. These results suggest that there is heterosis occurring in these F1 hybrids that could result from either genetic load within populations that is being masked in hybrids or from overdominance that occurs at a number of loci. For thermal tolerance, there is variation among crosses in the extent of hybrid vigor seen for thermal stress variation that was also found by Edmands (1999) for other fitness measures. Variation among crosses in levels of hybrid vigor could be caused by different levels of genetic load among populations of *T. californicus* or differences in the allelic interactions that lead to overdominance. Variation in genetic load among populations could stem from the variable nature of the copepod’s habitat and fluctuations in population size that accompany this variability (Dethier 1980; Dybdahl 1994; Powluck 1998) that could result in the fixation of deleterious mutations during population bottlenecks.

The thermal tolerance of F2 hybrids was generally reduced in comparison to F1 hybrids. In part, this observation is consistent with the pattern seen for a number of other measures of fitness in crosses of populations of *T. californicus* where F2 hybrids have lowered fitness (Burton 1986, 1987, 1990; Edmands 1999; Edmands and Deimler 2004; Ellison and Burton 2008; Willett 2008). This pattern has been interpreted as a result of the expression of deleterious DM incompatibilities that act in a recessive fashion and are masked in the F1 generation but reduce fitness in the F2 hybrids (often referred to as hybrid breakdown). For crosses of substantially genetically divergent populations, these previous studies have also found that the fitness of the F2 hybrids is often significantly reduced below that of both of the parental populations used in the cross (including many of the same population crosses used in this study, particularly those among the SD, AB, and SCN populations, e.g., Ellison and Burton 2008). This hybrid breakdown is a key difference with the present study because for studies of acute and chronic heat stress, thermal tolerance of the F2 hybrids is never significantly lower than both parental populations (although it is sometimes lower than the more thermally tolerant parental population). This finding could suggest that either there is very little hybrid breakdown in these measures of thermal tolerance in F2 hybrids or that it is substantially reduced in magnitude. If there were no hybrid breakdown for high temperature tolerance, that would imply that the observed reduction in thermal tolerance of the F2 versus the F1 hybrids is a result of a reduction in heterosis in F2 hybrids. Such a reduction in heterosis could conceivably occur either by the decrease in heterozygosity in the F2 hybrids or by the reemergence of some of the deleterious effects of genetic load in these F2 hybrids.

Regardless of whether hybrid breakdown is eliminated or merely reduced, these results suggest that DM incompatibilities have a lower impact on thermal tolerance than they have on many other fitness traits in these F2 hybrids of *T. californicus*. There are at least 2 different reasons why the magnitude of the deleterious impacts of DM incompatibilities could be reduced for thermal stress in comparison to other measures of fitness. One possibility is that it could be important at what point during the lifespan the fitness measures are taken and a second explanation would be a general reduction in hybrid breakdown under stressful conditions. First, I will focus on the possibility that the time in which the fitness is measured is important. Timing could play a role in decreasing the impacts of hybrid breakdown later in the life cycle if F2 hybrid adults represent a greatly winnowed population from which the lowest fitness
individuals have been removed. In previous studies, most measures of F2 hybrid fitness in population crosses of *T. californicus* were taken during development to the adult stage, for example, survival, hatching number, developmental time, or naupliar (larval copepods) ability to tolerate salinity stress (Burton 1986, 1987, 1990; Edmands 1999; Edmands and Deimler 2004; Ellison and Burton 2008). It is possible then that many of the F2 hybrids with the lowest fitness are eliminated during development and never reach the adult stage. However, I have done preliminary experiments that suggest that this hypothesis may not completely explain the differences in patterns among these studies. I tested nauplii from crosses of the SD and SCN populations and found that patterns of heat tolerance do not differ appreciably from the results seen in adult copepods. In these assays, for chronic stress on nauplii, the F2 nauplii have higher heat tolerance than the less-tolerant SCN population and are lower (but not significantly) than the SD population. Results for F1 nauplii were not obtained (Willett CS, unpublished data). Although these experiments were preliminary, they do hint that the F2 nauplii may not perform dramatically differently from F2 adults in this cross of populations. If these results held up in a larger sample and for more crosses, they would suggest that the F2 adults are not greatly improved in their temperature tolerance in comparison to the nauplii.

Let us now turn to the second hypothesis, could the impacts of hybrid breakdown become less substantial in hybrids that are exposed to stressful temperatures as opposed to more benign temperatures? The results of the current study would be consistent with this idea as there is little evidence from acute and chronic temperature stress assays for lowered resistance of F2 hybrids in comparison to the midparental value. Edmands and Deimler (2004) previously found that in a cross of a northern population from Oregon with the SD population from southern California there was no reduction in F2 hybrid fitness from the midparental value for measures of metamorphosis fraction and larval survival when copepods were reared at 25 °C. In contrast under both low and high salinity conditions with constant 15 °C rearing conditions, there was a decrease in F2 hybrid fitness below the midparental value. Under all 3 sets of conditions in this study, the F1 had substantially higher fitness measures than the midparental values and often higher than either parental population. From studies in other systems, there is clear evidence that advanced generation hybrids with recombinant genotypes can have higher fitness under unique types of stressful environmental conditions (Rieseberg et al. 2003; Martin et al. 2006), but in these cases, these hybrid individuals are a subset of hybrids with unique genotypes (sometimes even showing transgressive segregation) and not the average values for the hybrid generation as a whole. Armbruster et al. (1997) found that under the stressful conditions of high density, crosses between genetically divergent populations of pitcher plant mosquitoes had reduced breakdown in F2 hybrid fitness as compared with lower density conditions. They suggested that the positive impacts of heterosis could be offsetting the negative impacts of deleterious gene combinations under stressful conditions. Overall then, the results of studies on the impacts of stress on hybrid breakdown are still a bit thin to make definitive conclusions at this point, but there is at least a suggestion that stress could result in a reduction in hybrid breakdown. For these *T. californicus* populations, it would be useful to have results from the same cross (performed at the same time) that looked at both fitness under highly stressful and nonstressful conditions.

What conclusions can be drawn about the potential for extrinsic and intrinsic reproductive isolation to contribute in general to reproductive isolation in hybrids from crosses of populations of *T. californicus*? First, the caveat must be made that given the apparently low dispersal abilities for this species (Burton 1997; Willett and Ladner 2009), hybridization between these populations in nature at the present time is likely to be a relatively rare phenomenon even for populations that are relatively geographically close together. That said, these results could serve as a model for secondary contact between populations that are diverging in allopatry. Temperature records from copepod pools and the results of thermal tolerance assays suggest that the high temperatures used in the acute stress assays are likely to be rare but potentially very important selective events and that the highest temperatures occur in the southern populations (Willett 2010; Kelly et al. 2011). *Tigriopus californicus* does not have any stress-resistant resting stages nor any known subtidal refuge from which it could recolonize intertidal pools if it were wiped out from a severe high temperature event. The results of our temperature tolerance studies with hybrids show that F1 hybrids would not generally have lower resistance under high temperature stress conditions but F2 hybrids could be lower than at least one parental population in a number of cases. If we were to consider the case of rare migrants, backcross progeny would probably be more common than F1 × F1 hybrid progeny. Backcross hybrids were not tested in these studies but would likely be closer in tolerance to the parental population that comprises on average 75% of the genome. These results suggest that extrinsic postzygotic reproductive isolation based on thermal tolerance would probably be a fairly weak barrier to gene flow between populations of this species and may be weaker than the largely intrinsic reproductive isolation resulting from DM incompatibilities in advanced generation hybrids in these same population crosses.

**Supplementary Material**


**Funding**

National Science Foundation (DEB-0516139 and DEB-0821003); University of North Carolina, Chapel Hill.
Acknowledgments

Thanks to T. Lima for helpful comments on the manuscript. A number of UNC undergraduates performed the assays and helped organize the data including H. Leasy, Q. Qian, E. Washburn, L. Todd, W. McGee, J. Michalak, S. Underwood, M. Smith, S. Patel, C. Desai, N. Gindele, and J. Yanik. Thanks to R. Burton for collecting some of the copepod samples (with J. S. Harrison).

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J. Yanik. Thanks to R. Burton for collecting some of the copepod samples


Received July 1, 2011; Revised September 7, 2011; Accepted September 21, 2011

Corresponding Editor: Stephen Karl