UNIVERSITY OF CALIFORNIA, SAN DIEGO

Potential for Adaptation to Climate Change in Interpopulation Hybrids of the Copepod *Tigriopus californicus*

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Biology by Summer Rae Maga

Committee in Charge:

Professor Ronald Burton, Chair
Professor Kaustuv Roy, Co-Chair
Professor Jonathan Shurin

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The Thesis of Summer Rae Maga is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

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Table of Contents

Signature Page........................................................................................................iii

Table of Contents.................................................................................................iv

List of Figures.......................................................................................................v

List of Tables.........................................................................................................vi

Acknowledgements..............................................................................................vii

Abstract of the Thesis..........................................................................................viii

Introduction...........................................................................................................1

Materials and Methods.........................................................................................7

Results..................................................................................................................13

Discussion............................................................................................................19

Bibliography..........................................................................................................24

Supplemental Materials.........................................................................................27
List of Figures

Figure 1 Phylogeography of *T. californicus* ........................................4

Figure 2 Parental survivorship .........................................................14

Figure 3 Extrinsic fitness of hybrids ...............................................15

Figure 4 Intrinsic fitness of hybrids ...............................................16

Figure 5 Extrinsic fitness as a function of first clutch size ...............17
List of Tables

Table 1 Survivorship percentage of parentals

Table 2 Survivorship percentage of hybrids

Table 3 List of p-values
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There are a number of Professors at the University of California at San Diego that both challenged and guided me. Dr. Roy and Dr. Shurin are included. There are too many others to list here, but I am no less grateful for each one.
Abstract of the Thesis

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by

Summer Rae Maga

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Professor Ron Burton, Chair
Professor Kaustuv Roy, Co-Chair

Accurately predicting how species respond to climate change is increasingly important as current models suggest most will be unable to adapt.
But can organisms respond to climate change faster than studies of selection in the laboratory suggest? Here we investigate potentially extreme phenotypes of heat tolerance resulting from complementary gene action. We do so by hybridizing two populations of *Tigriopus californicus* with high genetic divergence that naturally inhabit similar temperature regimes. Parentals as well as F1, F2 and F6+ generations were subjected to acute heat stress at a sublethal and lethal temperature. We also investigate life history traits of these hybrids to better understand the viability of these populations. Eight of the twenty F6+ lines had survivorships significantly higher than the parental mean after being stressed at the sublethal temperature. Three of those eight had significantly higher survivorship after being stressed at the lethal temperature. Breakdown in life history traits is observed across all hybrid lines, including those exhibiting extreme thermal tolerance. These results suggest that hybrids between allopatric populations may be able to adapt to climate change faster than populations adapting by selection on standing genetic variation alone.
Introduction

Potential for Adaptation to Climate Change

The global climate is warming quickly and we have seen a 0.2°C increase in average temperature per decade for the past 30 years (Hansen et al 2006). The resulting shifts in selection pressures impact the survival and range distributions of many species (Parmesan 2003, Thomas et al 2004, Perry et al 2005). Predicting such impacts is increasingly important as it is widely regarded that most species will not be able to adapt to rapid climate change and will ultimately face extinction (Lynch and Lade 1993, Chevin et al 2010). However, most assessments of species vulnerability limit their scope of adaptation to selection acting on extant genetic variation alone.

Although within-population genetic variation may limit selective response to the changing environment, species with strong population structure possess a wealth of between-population genetic variation. Here we suggest that hybridization between these allopatric populations may provide novel genetic trajectories by which species can adapt.

Warming global climate is forcing many species to shift their ranges northward in an attempt to remain in habitats with temperature regimes resembling those to which they are adapted (Parmesan, C., Yohe, G. 2003). During these range shifts, not only are community structures predicted to change, but also many conspecific populations will encounter each other for the first time (Walther et al 2002). These first encounters could result in novel
hybridization events and genetic combinations that can result in novel phenotypes. The environmental tolerances of these potential novel populations are unknown. Populations that share ancestral history and have diverged in allopatry evolve alternate genetic solutions to common environmental challenges. If they come into secondary contact following a range shift or other geographic change, viable interpopulation offspring may be produced with unique genomic compositions. Such hybrids may express extreme environmental tolerances as a result of complementary gene action, these environmental tolerances can exceed any observed in the parental populations and are therefore termed “transgressive” (Rieseberg et al 1999, Pereira et al 2013). Literature reviews suggest that the production of extreme phenotypes by transgressive segregation is not uncommon ( Tanksley 1993; Rieseberg et al. 1999).

Transgressive hybrids have successfully pioneered habitats and niches inaccessible to their parents. For example, the hybridization of two North American sunflower species (Helianthus annuus and H. petiolaris) has resulted in three recognized diploid hybrid species, all of which have extreme phenotypes compared with their parents for either flowering time or morphology. Additionally, H. paradoxus has an expanded range of environmental tolerance, able to thrive in drier ecological conditions than either of its parent species: H. anomalus and H. deserticola (Rieseberg 1991, Rieseberg et al 2003). In vertebrates, a group of invasive sculpin species in European rivers are found to be of hybrid origin. The parents, Cottus perifretum and C. rhenanus, are found in clear, cold, highly oxygenated waters. The hybrid offspring are able to occupy warmer and larger
bodies of water downstream that are inaccessible to their parents. It is documented that this novel adaptation has emerged quickly (Nolte et al. 2005).

Not all extreme phenotypes can be attributed to complementary gene action. Hybrids frequently exhibit expanded tolerances in their first generation, a phenomenon known as hybrid vigor. This is typically due to heterosis, where heterozygosity at all loci silences deleterious recessive alleles. This phenomenon is short lived, disappearing in the next generation as heterozygosity is cut in half. In contrast, transgression by complementary gene action persists in subsequent generations and can be driven to fixation by selection.

Despite being common, transgression is not expressed in all hybrids. So, which conditions give rise to transgressive segregation? Can we predict which hybrids will exhibit transgression? Can transgressive segregation facilitate adaptation to climate change faster than selection on standing variation alone? We investigate by testing thermal tolerance in interpopulation hybrids of the copepod species *Tigriopus californicus*.

*T. californicus* as a model organism

*T. californicus* is an ideal model organism for investigating potential for adaptation to climate change because of its wide geographic range along the Pacific coast of North America from Alaska to Baja California, Mexico. Across this range, there are many isolated populations, each having evolved a unique
genetic makeup. Even proximal populations experience little to no gene flow (Tangwancharoen and Burton 2014, Edmands 2001). Also along this range, there is local adaptation to temperature, with southern populations more tolerant to warm temperatures and northern populations more sensitive (Willet 2010). The splash pools that they inhabit often have wide daily temperature fluctuations, subjecting the organism to regular extreme changes in temperature (Kelly et al 2011).

Figure 1

Phylogeography of *T. californicus*. The populations of AB and SD are highly genetically divergent despite geographic proximity and similar thermal adaptation.
Selection and transgression in *T. californicus*

A recent study compared the incidence of transgression for thermal tolerance among recombinant inbred lines (RILs) produced by crossing two pairs of *T. californicus* populations. Crosses between two geographically distant populations (San Diego and Santa Cruz) failed to produce any transgressive RILs, while crosses between two neighboring populations (San Diego and Bird Rock) produced several RILs with extreme thermal tolerance phenotypes. In the former cross, the *T. californicus* populations are both genetically and ecologically divergent (one cold and one warm water adapted) while the latter cross included closely related populations collected from similar temperature regimes (both warm water adapted) (Pereira et al. 2013). Which factor determines the occurrence of transgression: similar ecological adaptation or close genetic relatedness? Here by examining RILs between genetically divergent but ecologically similar populations, we make a first effort to distinguish the roles of these factors.

Additionally, it has been shown that strong selection for increased heat tolerance in laboratory populations of *T. californicus* across 10 generations does not induce a tolerance in northern populations comparable to that of southern populations. In fact, after 5 generations, most lines tested showed no additional increase in tolerance, suggesting that standing genetic variation for this trait had disappeared by that time (Kelly et al 2011). We predict that hybridization between
allopatric *T. californicus* populations may increase their ability to respond to climate change by transgressive segregation.
Materials and Methods

Population Sampling

*T. californicus* were collected from the wild at two locations: Ocean Beach (San Diego County, CA, 32°44’41.17 N, 11°15’19.43 W; abbreviated SD) and Abalone Cove (Los Angeles County, CA, 33°44’30.8”N 118°22’38.9”W; abbreviated AB). These habitats yield two populations that are both similarly warm adapted with high genetic divergence despite their proximity (Willett 2006). Each population was collected from high intertidal splash pools and maintained in the lab in several replicate 400ml beakers. Copepods were fed ground algal wafers (Top Fin brand algae thins fish food). Stock populations were kept at 20°C with a 12:12 hour L:D photoperiod for one month in lab (approximately one generation) to establish a “common garden” environment. These beakers were used as parental populations.

Local Adaptation Between Parental Populations

To measure thermal tolerance of the two parental populations, we used the acute temperature stress assay previously used to measure the gradient of thermal tolerance along the Pacific coast of North America (Willett 2010). Ten males and ten females from the target population were placed into a 15 mL Falcon tube with 10 mL of filtered seawater. After an acclimatization period
of 1 hour in a beaker of water at 20°C, the tubes were immersed in a water bath, preheated to a stress inducing temperature, for 1 hour. Following heat stress, the Falcon tubes were immersed in 20°C water for 1 hour of recovery. Copepods were then transferred to a culture dish with food at 20°C for 3 days. Surviving animals were counted at that time with males and females counted separately.

To test for differences between parental taxa in heat tolerance, we performed this heat stress for temperatures between 35 and 38°C at half degree intervals, with at least 9 replicates at each target temperature. Both populations were measured for heat stress tolerance at the same time to account for potential temporal differences of the assays.

Nonparametric tests are appropriate to assess statistical significance, as survivorship to heat-stress is not expected to follow a normal distribution. For each temperature, we test for differences between taxa using a Wilcoxon pairwise comparison. Statistical analyses were performed in JMP. Based on studies in other T. californicus populations, we expect both parental populations to have a similar tolerance to heat stress as they are both southern latitude, heat adapted populations.

Experimental Hybridization

Clasped pairs from each population were separated with a fine needle. Females from each of the two populations are mated with males from the other. Males were subsequently removed when females developed an egg sac. The
resulting larvae were removed and cultured separately to maintain non-overlapping generations. F2 and F3 individuals are also cultured in this way, resulting in outbred individuals with various recombinant genotypes with high heterozygosity. Animals were kept in petri dishes with identical water, temperature and food conditions at all stages of hybridization. Full sib matings were then carried out for three generations to establish RILs with somewhat reduced heterozygosity. This was done by isolating F3 females with egg sacs, eventually moving paired sibs to their own culture dish. In total, 114 gravid females were isolated for SD♀xAB♂ and 108 for AB♀xSD♂. Following the three generations of sib mating, lines were moved to beakers and maintained with overlapping generations for approximately 3 generations. This strategy results in F6+ RILs with unique recombinant nuclear genotypes with ∼40% of their original heterozygosity (Hedrick 2011). The amount of time in which the F6+ RILs were allowed to breed freely should produce approximately F9 individuals. Because generations are overlapping, however, we cannot be certain of this and so the approximate F9 individuals are here referred to as F6+.

The number of RILs reaching F6+ is expected to be reduced from the original number of started lines. Also, this process may select for lines with higher survivorship (lines carrying alleles causing outbreeding or inbreeding depression are expected to die off before reaching F6+). To assess the fitness of these RILs compared to their parental populations, 15 RILs were established for SD♀xAB♂ and 5 for AB♀xSD♂.
Extrinsic Selection of Hybrids

To measure the heat tolerance of recombinant genotypes compared to parentals, the aforementioned assay was performed at two temperatures: sublethal and lethal. Sublethal is defined as the lowest temperature at which average parental survival was ~50%. Lethal is defined as the lowest temperature at which average survival of either population is <5%. Interpopulation hybrids of copepods often show hybrid breakdown (Burton et al. 2006) therefore the number of replicates among population crosses varied with the availability of adults for testing. For F1, F2 and F6+ RILs, we replicated the assay 8, 6 and 4 times respectively.

Mean parental survivorship (±1 SE) was used to define parental fitness. Mean survivorship of hybrid genotypes above parental fitness is indicative of increased fitness while those below suggest a decrease. As before with comparisons among parental populations, we tested for overall differences in survivorship using a Wilcoxon pairwise comparison.

Intrinsic Selection of Hybrids

Interpopulation hybrids of *T. californicus* exhibit breakdown in fecundity and survivorship (Edmands 1999; Burton et al. 2006). We measured the effect of hybridization on intrinsic fitness in F1, F2 and F6+ RILs and compared this with
parentals. Pairs with the genotype of interest were isolated in culture dishes and observed. Pairs were used rather than gravid females to ensure that the egg clutch analyzed was the female’s first, avoiding natural variation in subsequent clutch size. All nauplii were counted the day they hatched. Survivors were counted 14 days later and used to calculate percent survivorship.

Mean parental survivorship was used to define parental fitness. Intrinsic hybrid breakdown is not expected to follow a normal distribution, therefore nonparametric analyses are appropriate. We tested for overall differences in clutch size and survivorship using Wilcoxon pairwise comparisons. Based on studies with other *T. californicus* populations (Edmands 1999), we expect F1 hybrids to show increased fitness whereas F2 and inbred lines are expected to exhibit a decrease.

Impact of Extrinsic and Intrinsic Selection on Different RILs

Interpopulation hybrids of *T. californicus* are affected by both intrinsic selection (Burton et al. 2006) and extrinsic selection (Willet 2010). It has been suggested that the loci determining these selective regimes segregate independently, rather than being physically linked (Pereira et al. 2013). To investigate this possibility in our cross, we use F6+ RILs and standardize both intrinsic and extrinsic fitness by subtracting the respective parental mean. Positive values indicate higher relative fitness and negative values indicate lower
relative fitness. To investigate extrinsic survival, we consider only the sublethal temperature of 36°C. To investigate intrinsic fitness, we consider first clutch size.
Results

Local Adaptation Between Parental Populations

No significant differences in survivorship between sexes were observed at any temperature. (Wilcoxon pairwise comparison; all \( P > 0.392 \)).

Significant differences in tolerance between the two populations were observed only at 35°C with AB being the more tolerant population. This difference was not seen in higher temperatures. Both populations experienced 100% mortality at 38°C. The sublethal temperature, where the average survival of both populations was approximately 50%, was 36°C. The lethal temperature, where the average survival of either populations was <5% was 37°C.

Experimental Hybridization

The majority of inbred lines did not survive to the F6+ generation. The number of RILs that survived to F6+ was higher for SD♀xAB♂ (15) than those of AB♀xSD♂ (5). This difference in productivity between the two lines is significant (0.050\( > P > 0.025 \)).

Extrinsic Selection of Hybrids

There was no significant difference in survivorship between the two parent populations (\( P = 0.7209 \)) at either the sublethal or the lethal temperature. Therefore, the two were combined for a cumulative parental mean. There is an increase in mean percent survival of AB♀xSD♂ F1s above the parental mean.
In contrast, neither F2 cross showed an increase in mean survival over the parentals. Extreme phenotypes were observed among the RILs. At the sublethal temperature, SD♀xAB♂ produced 4 RILs and AB♀xSD♂ produced 4 RILs that were more heat tolerant than the parentals. SD♀xAB♂ produced 2 RILs more heat sensitive than parentals at the sublethal temperature. No AB♀xSD♂ RILs were significantly more heat sensitive than the parentals.

![Figure 2](image)

**Figure 2**

Parental Survivorship. Mean values are shown for AB and SD populations at temperatures ranging from 35°C to 38°C.
Figure 3

Extrinsic fitness of hybrids. The bars refer to survivorship to heat stress (mean ±1 SE) of various generations of hybrids at a sublethal temperature of 36°C (A) and a lethal temperature of 37°C (B). “Parental fitness” is defined by the mean survivorship (±1 SE) of parental taxa. Higher survivorship is indicative of “increased fitness” while lower survivorship is indicative of “decreased fitness.”
Mean F2 survivorship for SD♀xAB♂ at the lethal temperature was higher than the parental mean. At the lethal temperature, SD♀xAB♂ produced 1 RIL and AB♀xSD♂ produced 2 RILs significantly more heat tolerant than the parental mean. All 3 RILs transgressive at the lethal temperature were also transgressive at the sublethal temperature. Transgression was expressed evenly by both sides of the cross for the sublethal temperature and asymmetrically at the lethal temperature.

Intrinsic Selection of Hybrids
No significant difference in first clutch size was seen between the parental populations, so the two are combined into a cumulative parental mean. Both F1 and F2 crosses had a slightly lower first clutch size than parentals, though none of these are significant. Three of the five RILs tested have significantly fewer nauplii in their first clutch when compared to parental lines, however, none of the three have clutch sizes significantly different from either of the F2 generations.

Both F1s and F2s have a lower mean percent survival of nauplii 14 days after hatching than parentals, though none of these differences are significant. All

Figure 5
Extrinsic fitness as a function of first clutch size. Each dot represents a recombinant inbred hybrid line. Data were standardized with parental means so that a zero value represents equivalence with the parental mean. For extrinsic survival, only the sublethal temperature treatment is considered. $r^2=0.142$
RILs also have a lower mean percent survival when compared to all other stages of hybridization and to parentals, though these differences are not significant.
Discussion

Based on their results employing an artificial selection experiment, Kelly et al. 2012 concluded that the ability of *T. californicus* populations to adapt to elevated thermal regimes was limited. Although some response to selection was observed, northern populations could not achieve the tolerances observed in southern populations. Furthermore, selective response did not extend past generation 5, as standing genetic variation in the population was apparently exhausted by that time.

Here we examine the potential role of interpopulation variation which is known to be very high among *T. californicus* populations. Although gene flow among populations is highly restricted, the fact that *T. californicus* has been able to colonize essentially all habitat patches across its range indicates that some dispersal does occur so interpopulation hybridization may play a role in evolutionary adaptation.

The populations chosen for this study had previously been shown to hybridize to produce offspring that exhibit hybrid breakdown (Willett 2006) but had not yet been tested for transgressive segregation for thermal tolerance. Hybrids of Abalone Cove and San Diego populations, which are distantly related but adapted to a similar thermal regime, exhibit extreme phenotypes. Because transgression was not seen in the genetically divergent populations from different thermal regimes hybridized by Pereira and Burton 2013, our results suggest similar ecological adaptation determines the occurrence of this phenomenon.
Most of the recombinant inbred lines cultured and tested were not transgressive. Of the twenty RILs, eight were transgressive at the sublethal temperature and three were transgressive at the lethal temperature. This differs from previous work where most lines tested were transgressive (Pereira et al. 2013). This may be a function of the genetic incompatibilities between the more divergent populations chosen for this study. The two lines that were more heat sensitive than their parents at the sublethal temperature may also suffer from detrimental combinations of complementary gene action.

More lines were produced by SD♀xAB♂ than by AB♀xSD♂. Perhaps development time was shorter for SD♀xAB♂ resulting in the production of more lines in the time allotted. Investigating development time for hybrids of AB and SD has been attempted (Burton 1990) though too few F2’s made it to maturity to test this.

It has been shown that F1 hybrids tend to have an increase in mean and decrease in variance while F2 hybrids show the converse (Burton 1990, Edmands 1999). Despite the expectation of hybrid vigor for both F1 generations, we see an increase in tolerance at only the sublethal temperature for only AB♀xSD♂ F1s. If SD♀xAB♂ F1s were more influenced by incompatibilities between the parental genomes then we would expect to see a similar influence on the F2s, which we do not. Perhaps there is more variation in the survivorship of F2s of very divergent parents. This could be tested by culturing F2s and testing their survivorship across a range of temperatures. These results could
then be compared to survivorship of F1s and parentals at the F2 sublethal and lethal temperatures.

The individuals tested in this study were all adult *T. californicus*. It has been shown, however, that survivorship to heat stress varies across life stages (Tangwancharoen and Burton 2014). Measuring and comparing survivorship of hybrids across different life stages would further inform whether this extreme thermal adaptation benefits the organism across its life cycle.

Fecundity and overall survivorship are important influences on the health of a population. In order for increased environmental tolerance to benefit a population, it must continue to be viable. Hybridization often reduces fitness in these life history traits in *T. californicus* (Edmands 1999, Burton et al. 2006, Willett 2010). Based on the results here, there appears to be no relationship between transgression and first clutch size or survivorship suggesting that the two traits are independent of one another rather than linked. We conclude that hybridization alone results in hybrid breakdown with no further degradation of life history traits as a result of transgression.

Additional opportunities for investigation include hybridizing many of the other *T. californicus* populations. Notably the populations of Santa Cruz and Pescadero, which share similar ecological adaptation much like the cross used in our project. Rather than both being warm water adapted, as in this study, both are heat sensitive. The thermal tolerance of the resulting F6+ phenotypes would further inform our understanding of transgressive segregation.
We have seen *T. californicus* hybrids, apparently by way of complementary gene action, adapt to higher temperatures more quickly than by selection on standing variation alone. This was seen without the effects of selection. The addition of selective force on the complementary gene action seen here could result in rapid organismal adaptation. Additionally, transgression is expressed in hybrid offspring of parent populations with extreme genetic divergence, pointing to the importance of their ecological similarity. Hyper-expression of thermal tolerance by complementary gene action facilitates a rapid organismal response to a changing climate. Many species are experiencing northward range shifts and interpopulation hybrids are in a position to respond to a changing climate much faster than by selection within a single population. These results seen here have implications for the adaptation of many species to climate change.
Bibliography


Tangwancharoen, S., Burton, R.S. 2014. Early life stages are not always the most sensitive: heat stress response in the copepod Tigriopus californicus. Marine Ecology Progress Series. 517, 75-83.


Table 1

Survivorship percentage of parentals.

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Table 2

Survivorship percentage of hybrids.

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Table 2
Survivorship percentage of hybrids, continued.
Table 2

Survivorship percentage of hybrids, continued.

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Table 3

List of p-values. All lines with mean stress survivorship higher than parental mean are shown.

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