Potential fitness tradeoffs for thermal tolerance in the intertidal copepod *Tigriopus californicus.*

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ABSTRACT

Thermal adaptation to spatially varying environmental conditions occurs in a wide range of species, but what is less clear is the nature of fitness tradeoffs associated with this temperature adaptation. Here, populations of the intertidal copepod *Tigriopus californicus* are examined at both local and latitudinal scales to determine whether these populations have evolved differences in their survival under high temperature stress. A clear pattern of increasing high temperature stress tolerance is seen with decreasing latitude, consistent with temperature adaptation. Additionally, there is also evidence for significant variation in thermal tolerance on a smaller scale. The competitive fitnesses of pairs of northern and southern copepod populations were also examined under a series of lower, more moderate temperatures. These fitness assays show that the southern populations that have the best survival under extreme high temperatures have lowered competitive fitness at the lower temperatures tested, while the fitness of the southern populations exceeded that of the northern populations at the highest temperatures tested. Combined, these results suggest that there may be evolutionary tradeoffs between performance at high and stressful temperatures and fitness at moderate temperatures in this species.

INTRODUCTION

Exposure to extreme temperatures is likely to be a source of strong selective pressure for many ectothermic animal species, but it is often difficult to determine the impacts of this selection. Many studies have shown a correlation between the environmental conditions experienced by organisms and their performance and/or fitness at those temperatures suggestive of temperature adaptation (Huey and Kingsolver 1989; Angilletta et al. 2002; Hochachka and Somero 2002; Hoffmann et al. 2003; Angilletta 2009). Patterns of adaptive variation have been

found within species that are consistent with adaptation to extreme temperatures associated with differences in environmental gradients such as latitude (Hallas et al. 2002; Hoffmann et al. 2003; David et al. 2003; Castañeda et al. 2004; Barhndorff et al. 2006), altitude (Sørensen et al. 2005; Collinge et al 2006; Karl et al. 2008), and tidal height (Davenport and Davenport 2005). Simple predictions from these environmental gradients for thermal tolerance can be confounded in many species by factors such as behavioral thermoregulation, temperature-stress resistant life stages such as diapause, or small scale spatial variation in local environmental conditions. These factors likely contribute to observations in insects that patterns of cold temperature tolerance often show a stronger relationship to latitude than do patterns of high temperature tolerance (Addo-Bediako et al. 2000; Hoffmann et al. 2003; Ragland and Kingsolver 2008), and that other species do not always differ predictably in thermal tolerance over these environmental gradients (Coyne et al. 1983; Mitchell and Lampert 2001; Winne and Keck 2005; Angilletta 2009).

Temperature adaptation to stressful temperatures may result in tradeoffs with performance or fitness at different temperatures, tradeoffs that could take a number of different forms. Several different evolutionary scenarios have been proposed previously for tradeoffs between adaptation to extreme temperatures and performance at moderate temperatures (Huey and Kingsolver 1993). A population that has evolved to handle higher temperatures might have lost the ability to handle cold temperatures, i.e. there has been a shift in the thermal niche without an expansion of breadth. Alternatively, a population that has evolved to handle higher temperatures might still be able to handle cold temperatures, i.e. the thermal breadth expands, but this expansion results in lowered fitness at intermediate temperatures. The latter example is a generalist-specialist tradeoff, a situation that fits the expression, “a jack-of-all-temperatures is a master of none” (Huey and Hertz 1984). If no tradeoff in thermal performance exists, but

adaptation to extreme temperatures incurs an energetic cost to the organism, then there are likely to be other costs to the organism mediated via the differential acquisition or allocation of resources (Huey and Hertz 1984; Angilletta et al. 2003). Although there is evidence in a number of species for presumably adaptive differences in extreme temperature tolerance among populations, the empirical evidence for tradeoffs in fitness at moderate temperatures for these populations is mixed (Angilletta et al. 2003; Angilletta 2009).

Genetically divergent populations of the upper intertidal copepod *Tigriopus californicus* provide a useful model with which to examine the evolution of thermal tolerance at extreme temperatures and potential fitness tradeoffs at moderate temperatures. This species occurs in splash pools at the edge of the intertidal zone in rocky outcrops from central Baja California, Mexico to Alaska, along the west coast of North America. The pools that this species inhabits in the intertidal vary temporally in factors such as salinity and temperature, and pools can even dry up completely on occasion (Dybahl 1994; Powlick 1998). However, in spite of this temporal variability, *T. californicus* has strongly genetically structured populations, indicating that copepod populations are able to persist for long periods of time on individual rocky outcrops with little genetic exchange with neighboring outcrops (Burton and Feldman 1981; Burton 1997; Willett and Ladner 2009). In fact, populations across southern California can differ by more than 20 percent in mtDNA-encoded genes such as cytochrome b (Willett and Ladner 2009). These studies suggest that *T. californicus* does not have high levels of gene flow between neighboring populations that could swamp out the ability of local populations to differentially adapt to the local environmental conditions.

In studies conducted to date there is only limited evidence for local adaptation among the genetically divergent populations of *T. californicus*. Variation in the allozyme glutamate-

pyruvate transaminase (GPT) appears to be maintained by selection stemming from salinity stress, a pattern that could reflect locally varying salinity adaptation (Burton and Feldman 1983). Allele frequencies for GPT show a perfect rank order correlation with maximal pool salinity across outcrop populations in the Santa Cruz, CA region (Burton 1986). In contrast, in a study testing for local temperature adaptation among populations at moderate temperatures, Edmands and Deimler (2004) found no evidence for population differences among three *T. californicus* populations (two from California, and a third population from Oregon). They examined survival and metamorphosis number at 25˚C versus 15˚C under non-competitive conditions with two different salinity regimes and did not find a significant temperature-by-environment interaction, suggesting that these populations were not responding differently to these two temperature treatments for these measures of fitness. They did not examine divergence in high temperature tolerance in their study.

In the genus *Tigriopus* there are patterns of upper temperature tolerance among species suggestive of extensive temperature adaptation. Kontogiannis (1975) found that 50 percent of *T. californicus* adults from Flat Rock Point near Los Angeles, CA survived for 1 hour at 38˚C. Similar studies on congeners found upper lethal temperatures from 44˚C for *T. fulvus* (Caniço, Madiera), to 35˚C for *T. brevicornis* (Isle of Cumbrae, Scotland), and 22˚C for *T. angulatus*, (South Geogia in southern Atlantic Ocean) (Damgaard and Davenport 1994; Davenport et al. 1997). These interspecific comparisons show a general correspondence between each species' thermal tolerance and the temperature environment that it inhabits. These studies showed that all of these species survived temperatures at or below 0˚C, with *T. brevicornis* from Scotland having the remarkably low median lethal temperature of -16˚C. Although these studies did not test for

adaptation within species, the striking differences between species suggest that extreme temperature thermal tolerance is likely to be an important trait in this genus.

In this study, I examine the variation between geographically and genetically isolated *T. californicus* populations in upper thermal temperature for measures of acute and chronic stress. Previous studies in this species have looked at variation between populations in measures of fitness under moderate temperatures but not extreme temperatures. I also look at a measure of competitive fitness for sets of populations from southern and more northern regions of the geographical range of this species. I show that there is evidence for both a latitudinal gradient and some local differentiation for acute and chronic upper temperature tolerance. Interestingly, under competitive conditions the more heat tolerant southern populations do more poorly at low to moderate temperatures than the less heat tolerant northern populations. Only at relatively high temperatures do these southern populations have higher competitive ability, suggesting that there is a tradeoff between fitness at moderate temperatures and upper temperature thermal tolerance in this species.

MATERIALS AND METHODS

Copepod collection and propagation

For this study a set of *T. californicus* populations were chosen to study variation in patterns of thermal tolerance over both a latitudinal gradient and at a more local scale. The selected populations differed in their wave exposure environment and the levels of genetic divergence between populations. Copepods were collected from several pools from a single outcrop at each of 11 sites (Table 1 and Figure 1); copepod samples from these pools were then combined and used to found laboratory populations to use for temperature analyses. These

populations include genetically proximate (BC/VI), geographically and genetically proximate (AB/FLR1, and BHB/BHH), geographically proximate populations but genetically divergent populations (SD/LJS, and FLR1/PTD), and genetically and geographically distant populations (remaining comparisons). The two sets of northern sites were selected to maximize local environmental differences: VI is a site on the outer-exposed side of Vancouver Island while BC is a site on the Burrard Inlet off of the Strait of Georgia well away from the open ocean. On a smaller scale BHB is an exposed site, while BHH is a sheltered harbor location; copepod pools from these two sites have been shown to differ substantially in environmental conditions (Dybdahl 1995).

Once in the laboratory, copepod populations were maintained at 20˚C with a 12:12 L:D daily light cycle in both 400-ml beakers (100-plus founding parents) and in petri dishes with 25 founding adult females; female *T. californicus* only mate a single time and store the sperm to produce multiple clutches of offspring (Burton 1985). To maintain moderate population sizes, when new petri dishes or beakers were started, the founding females were collected from across multiple (four or five) source petri dishes/beakers to start the next set of cultures. To establish subsequent generations in petri dishes, females were removed from petri dishes once they had produced progeny and progeny were left to mature in the dish. Each petri dish was given a unique source designation that was included as an effect in the subsequent analyses. Cultures were maintained at concentration of 35 parts per thousand in artificial seawater (Instant Ocean, Aquarium Solutions). They were fed with commercial flake fish food, but copepods also consumed natural algal growth and detritus in the petri dishes and beakers.

The different experimental populations of copepods that were used in temperature stress experiments had the following generation designations: Generation 1 were the copepods

collected directly from the field, with subsequent generations labeled sequentially (Gen 2, 3…). For acute and chronic temperature stress experiments, generation 2 copepods from 400 ml beaker populations (2B) were used for some experiments, while generations 1-5 were all collected from petri dish populations. To test for whether inbreeding could influence patterns of thermal tolerance in later generation petri dish cultures, crosses were set up between independent lineages of the same population and the progeny of these crosses were used in thermal tolerance experiments. These crosses were between lineages maintained in beakers and lineages from the same populations maintained in petri dishes and would be expected to relieve any lab-generated inbreeding depression. To initiate these crosses, males from 3+ generation petri dishes from the AB, SD, and SCN populations were crossed to virgin females from 2+ generation beakers from the same populations. The progeny of these three different crosses were used in temperature stress experiments (these copepods were called BP).

Chronic and acute temperature stress experiments

The ability of copepods from different *T. californicus* populations to handle high temperature stress was examined under both short-term, acute exposure to extreme high temperatures, and long-term, chronic exposure to constant high temperatures. The temperatures to conduct these acute (35-39˚C) and chronic (32˚) temperature stress trials were determined by preliminary experiments and are likely to be in the range of temperatures experienced by these copepods in the field. The actual temperature that intertidal organisms experience in the natural environment vary depending upon their position in the intertidal, air temperature, sun exposure, water temperature, and timing of low tides (Helmuth and Hoffman 2001; Helmuth et al. 2002; Gilman et al. 2006). For *T. californicus* in the upper intertidal splash pools it inhabits, the

ambient air temperature and sun exposure generally will play the largest roles in determining pool temperature except at the highest of tides or during high wave conditions when the ocean temperature will be more important. Temperature data collected by Egloff (1966) in Pacific Grove, CA, from pools containing *T. californicus* during the summer showed that the average daily maximal pool temperature was slightly warmer (+1.1˚C) than the average maximal air temperature. Examination of weather records from a number of weather stations directly on the coast show a gradual increase in both the mean daily maximal temperature and the extreme high temperatures with decreasing latitude (Figure 2). The temperatures used in the temperature stress experiments appear to be within the range of temperatures experienced by these copepod populations; this is corroborated by fairly extensive (but limited duration) temperature samples taken from *T. californicus* pools from a small number of populations along the range of this species (Egloff 1966; Vittor 1971; Dybdahl 1995; Powlik 1998). Based on the air temperature records (Figure 2), a long-term exposure to a constant 32˚C of the type used in the chronic stress assay is unlikely to be experienced by any population for a substantial period of time, but does this assay does give a different measure of thermal tolerance that should reflect longer-term performance under elevated temperatures.

Acute temperature stress assays were conducted by collecting 10 males and 10 females from a population/generation treatment and placing them into a single 50-ml centrifuge tube with 25 ml of artificial seawater. For a small number of replicates only one sex was abundant and 20 individuals of the same sex were used in a single tube. Up to eight tubes (a batch) were then immersed in a water bath at a single temperature between 35˚C and 39˚C for one hour. At the end of the hour the tubes were immersed in a large beaker of water at 20˚C to bring the temperature back down in a consistent fashion. After 20 minutes the copepods were transferred

to petri dishes with a small amount of food and returned to 20˚C. Survivors were scored after 3 days. Preliminary experiments indicated that mortality from the heat stress had declined dramatically by this point. The number of replicates performed for each population/generation and temperature are listed in Table 1. Survival for the acute temperature stress experiments was modeled as binomial using generalized linear mixed models (function lmer, lme4 package; Bates et al. 2008) with R (version 2.8.1; R Development Core Team 2008). 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. The same statistical framework was used to test the significance of the population effect for smaller subsets of populations including only geographically proximate populations.

Chronic temperature stress experiments were performed by placing either 10 males or 10 females from a population/generation source into a petri dish and maintaining the dish at a constant temperature of 32˚C. A small amount of commercial flake fish food was added to the dishes at the start and food and water would be replenished as needed through the course of the experiment. Petri dishes were monitored daily and the number of surviving copepods recorded. The number of replicates performed for each population and generation is listed in Table 1. Survival analyses for the chronic temperature stress experiments were performed by using a proportional hazards model in the program JMP (version 5.0, SAS Institute, Cary, NC). An analogous procedure to that used for acute stress tolerance was followed to determine the best proportional hazard model, i.e. effects were added sequentially and the nested models tested using likelihood ratio tests. In these analyses, the number of days survived is a minimum; i.e. a copepod that died between the checks on the 2^{nd} and 3^{rd} days would have a value of 2.

Competition experiments at varying temperatures

Competition assays between pairs of populations were conducted to look at fitness differences between these populations under different temperature conditions. Pairs of populations were tested with one southern California population (SD or LJS) and a second central/northern Californica population (SCN or BHB). Competition assays between these populations pairs were conducted under either three (LJS/BHB) or four (SD/SCN) different thermal regimes. The experiments were carried out over several years for the different temperature regimes: For the SD/SCN population pairing, two assays were conducted in 2001, the first at a 16˚C constant temperature with an 8 h:16 h L:D daily cycle, and the second with a 25˚C:16˚C daily temperature cycle and corresponding 16 h:8 h L:D cycle. Different photoperiods were used initially to reflect seasonal differences at a northern site; however, photoperiod was not manipulated in subsequent experiments when it appeared that temperature was having a large impact by itself. A third assay for SD/SCN was done in 2003 at a constant 20˚C with a 12 h:12 h L:D cycle, and a fourth assay was done in 2006 with a 20˚C 12 h:28˚C 12 h daily temperature cycle and 12 h:12 h L:D. The LJS/BHB population pairing assays were all conducted in 2008 under three sets of conditions, 16˚C and 20˚C constant temperatures and a 20˚C:28˚C daily cycling temperature regimes all with 12 h:12 h L:D. Trial experiments determined that females from the BHB, SCN, and SD populations could all survive and reproduce when reared at the highest temperature regime (20-28˚C daily cycle) and that first stage nauplii also could develop to adults under this same regime (C.S.W. unpublished results).

Competition assays were performed by mixing equal numbers of fertilized females from the two populations in a single petri dish and allowing them to reproduce and the progeny to

develop into adults. Before initiating a competition assay, petri dishes were set up with pure population copepods (~20 mature females) to generate a population of young copepods from which pairs could be collected. To standardize the life stage of the female copepods in these assays, pairs of copepods were collected from these petri dishes and placed into new petri dishes to allow mating with males from the same population to go to completion. Once pairs had separated, females were collected and matched with females of the other population to make replicate dishes of each competition assay. Equal numbers of females from each population were added to a competition petri dish (generally 8 or 10 of each population depending on the number of newly mated females available at the right time) and allowed to produce offspring. Three replicate dishes of each population pair/temperature regime were set up (with two separate sets of three replicates set up for BHB/LJS at 20˚C). Founding females were transferred to a new petri dish when the progeny in the dish reached the copepodid stage. Up to five total transfer plates were generated from each original replicate dish in an assay (parental females were transferred until either all died or they were collected when they reached very low numbers). In most petri dishes large numbers of progeny were produced by the females but only a small fraction of these would develop to adults.

To determine the results of the competition assays, the progeny were collected and genotyped and the proportion of progeny produced by each parental population calculated. The surviving adults were collected from each petri dish and placed into 20 μl of lysis buffer in 96-well plates and DNA prepared for PCR-based genotyping (Willett 2006). Genotyping was PCR-based using either population-specific PCR primers (based on fixed nucleotide differences between populations) or primers that amplified across fixed insertion/deletion length differences between populations to amplify a unique size PCR fragment for each population. These

population-specific PCR fragments were scored by running them on agarose gels and visualizing bands after ethidium bromide staining (see Supplemental Table T1 for more details on genotyping). This genotyping determined the percent of adult progeny produced from each parental-type population in each transfer plate and replicate. For a few petri dishes large numbers of progeny developed into adults and only a subset of at least 100 copepods were collected as a representative sample of the total. The number of founding females that started each replicate and transfer dish and the numbers of progeny genotyped are listed in Supplemental Table T2. Competition results were fit to a generalized linear model as a binomial (function glm) with R (version 2.8.1: R Development Core Team 2008). For this model the numbers of progeny that were northern genotype versus southern genotype were summed within each replicate across transfer plates and these numbers were used to compare across temperature treatments and population pairs.

RESULTS

Survival under acute temperature stress

Tigriopus californicus populations differ dramatically in how well individuals from these populations survive acute, high temperature stress (Figure 3). One common treatment across all populations, a temperature of 37˚C for one hour, killed nearly all copepods from the more northern populations while causing much more modest mortality in the most southern populations. Modeling survival with a generalized linear model as a binomial revealed that a model incorporating population, sex, temperature, and sex-by-population as fixed effects and source and batch as random effects fit the data best (Table 2). Dropping population, sex, temperature, and sex-by-population effects from this main model results in a significantly worse

fit to the data. More complex models including other interaction effects, generation, or year-collected do not improve the model. The estimated effects from this main model show a clear latitudinal cline from south to north in the ability of populations to survive acute, high temperature stress (Supplemental Table T3). Males do not survive high temperature stress as well as females across most populations (see Supplemental Figure S1). Year-collected significantly impacted the thermal tolerance for only one population when populations were analyzed individually (SD had higher tolerance in 2003; see Supplemental Figure S2 for more details), but overall the impacts of generation and year-collected were limited. To examine the effect of latitude alone, the latitudinal value of a population was used in place of population in the models. There is a large impact of latitude evident when latitude is dropped from the model either across temperatures (χ^2=79.1, d.f.=1, P<0.0001) or within the single temperature of 37˚C (χ^2=73.6, d.f.=1, P<0.0001). These models incorporating latitude included sex and temperature as fixed effects and batch and source as random effects, but a sex-by-latitude effect was not included and did not improve the fit of these models.

A comparison of geographically and/or genetically proximate populations reveals some evidence for differentiation in acute temperature stress tolerance at a more local scale. For comparisons within the two sets of southern California populations, the inclusion of population in a generalized linear mixed model with sex, source, and batch improves the model significantly: the SD and LJS populations are geographically proximate (χ^2=5.48, d.f.=1, P=0.019), and the AB, FLR1, and PTD populations are geographically proximate with the AB and FLR1 populations being genetically similar as well (χ^2=6.87, d.f.=2, P=0.032). From these populations the SD copepods (particularly males) survive worse than LJS copepods under acute stress, while FLR1 copepods survive worse than AB and PTD copepods. For the two sets of

northern populations, the inclusion of a population effect did not improve the fit of models for the geographically and genetically proximate BHH and BHB populations (χ^2=0.70, d.f.=1, P=0.44), and the genetically similar BC and VI populations (χ^2=0.04, d.f.=1, P=0.84; the sex effect was not significant and was not included in this model comparison). Therefore, the two sets of southern populations showed significant local heterogeneity in acute thermal tolerance while the two sets of more northern populations did not.

Survival under chronic temperature stress

Under high temperature chronic stress *T. californicus* populations show a south to north gradient in ability to survive at a constant 32˚C (Figure 4). Across all populations a chronic stress temperature regime results in substantial mortality after only a few days, but populations further to the south have a larger percentage of the population that survives longer. Examination of the data with a proportional hazards model including population, sex, year collected, generation, sex-by-population, and sex-by-generation effects provides the best fit to the data. These effects are all significant with the exception of sex in this model (Table 3A). The risk ratios from this model show an increasing risk of mortality for the more northern populations (risk ratio details can be found in Supplemental Figure S3). The collection year 2003 survived better than 2004 or 2006, and while the effect of generation is significant, there is not a clear pattern for the impact of specific generations (except that the first generation copepods survived worse; see Supplemental Figure S4). If the latitudinal value of a population is used in a proportional hazards model instead of population, there are significant effects of latitude, sex, year collected, generation, and source, but sex-by-latitude and sex-by-generation are no longer significant effects (Table 3B). Using 3rd and 4th generation, 2004 copepods (Figure 4C, D), which represent the largest, most homogenous

dataset, there is also a significant effect of latitude (χ^2=16.6, d.f.=1, P<0.0001) in a model with sex (χ^2=44.3, d.f.=1, P<0.0001), generation (χ^2=16.6, d.f.=1, P<0.0001), and source (χ^2=13.0, d.f.=1, P=0.0003).

Similar to the results seen for acute stress, there is evidence for population differences among geographically and/or genetically proximate southern populations but not northern populations in tolerance to chronic stress. For a proportional hazards model using only the southern California SD and LJS populations (including the factors population, sex, year collected, generation, sex-by-population, and sex-by-generation), population is a significant effect [Likelihood ratio (L-R) χ^2=63.1, d.f.=1, P<0.0001] with the LJS population surviving better than SD. For models with the southern California AB, FLR1, and PTD populations (including the factors population, sex, and year collected), population is a significant effect (L-R χ^2=48.4, d.f.=2, P<0.0001) with the AB population surviving better and FLR1 worse. For models with the northern Calfornia BHB and BHH populations (including the factors sex, population, sex-by-population) neither population nor sex-by-population have a significant effect (population L-R χ^2=0.55, d.f.=1, P=0.46; sex by population L-R χ^2=2.49, d.f.=1, P=0.11). The same was true for the most northern VI and BC populations for models including population and sex-by-population effects (population L-R χ^2=0.49, d.f.=1, P=0.48; sex-by-population L-R χ^2=1.87, d.f.=1, P=0.17).

Competition assays for fitness at varying temperatures

Competition assays were set up to compare the relative fitnesses of pairs of northern versus southern *T. californicus* populations under different temperature regimes. These assays were started by combining equal numbers of fertilized females from the two populations and

measuring the proportion of the adult progeny that were of each parental genotype. Competition assays were done under four different temperatures for the southern California population of SD and central California population of SCN and three temperatures for the southern California population of LJS and the northern California population of BHB (Figure 5). A striking pattern emerges for the SCN versus SD comparison where SCN progeny dominate the surviving adult progeny in the lowest temperature regime, and SD progeny dominate the surviving adult progeny in the highest temperature regime. For intermediate temperatures there is more scatter, but the SCN copepods generally do better (more detailed results from individual replicates can be found in Supplemental Table S2). A similar pattern was seen for competition assays between the southern California LJS population and the northern California BHB population for three different temperature regimes (Figure 5). LJS clearly does better than BHB at the highest temperature and BHB does better at the lowest temperature utilized. More variation was seen for the intermediate 20˚C temperature with neither population clearly doing better. Fitting a generalized linear model as a binomial to these competition data reveal that there are significant effects of temperature (with four discreet states χ^2=1746, d.f.=3, P<0.0001), population pair used (χ^2=28.6, d.f.=1, P<0.0001), and population-by-temperature effects (χ^2=123, d.f.=2, P<0.0001).

DISCUSSION

The evolution of upper thermal temperature tolerance

The populations of *T. californicus* show a clear pattern of increasing high temperature tolerance with decreasing latitude for both acute (Figure 3) and chronic thermal stress (Figure 4). Coupled with the environmental gradient in both extreme temperatures and moderate temperatures over this same range of latitudes (Figure 2), this suggests that they are adapting to

latitudinal differences in temperature. Several components of the life-history of *T.californicus* suggest that such adaptation is likely to be important for the continued persistence of populations of this copepod. First, *T. californicus* is not known to have a subtidal refuge from which it could recolonize outcrops if it were to be eliminated from its upper intertidal splash pools by an extreme temperature event (Dethier 1980). Second, *T. californicus* does not have a resting or diapause stage of the sort seen in other copepods and arthropods, life stages that often have greater tolerance to extreme environmental conditions (Edmands and Deimler 2004). Third, although they are clearly capable of occasionally recolonizing outcrops from which they have been extirpated, levels of gene flow between even nearby outcrops are extremely limited, suggesting that migration levels must be very low (Burton and Feldman 1981; Burton 1997; Willett and Ladner 2009). These factors suggest that thermal tolerance in *T. californicus* is likely to be an important component of fitness, and may help determine whether populations can persist over time on individual outcrops.

Several of the sampled populations were selected to help determine the degree to which locally spatially varying environmental conditions could impact the patterns of upper temperature thermal tolerance between *T. californicus* populations. Temperature monitoring of *T. californicus* pools by Dybdahl (1995) found more variable and higher extreme temperatures for the more wave-exposed BHB site versus BHH. The BHH site is unusual in its sheltered nature and is likely to have a greater connection to the ocean . Based on the available air temperature data (Figure 2), the more inland BC site might be expected to be warmer than VI. However, these expected environmental differences did not result in any significant differences in our measures of temperature tolerance. In contrast, in southern California, two set of geographically proximate sites (SD/LJS and AB/FLR1/PTD) did show some evidence for

differences in thermal tolerance with both acute and chronic stress assays, suggesting that local differentiation is possible for thermal tolerance. Better field temperature data at a local outcrop or even pool to pool scale would be useful in determining whether there are indeed local environmental temperature differences between these sites. One possible explanation for the lack of apparent thermal tolerance divergence in northern populations versus southern populations is that in general the northern populations are much less genetically divergent from one another, potentially reflecting recolonization after the last ice age (Edmands 2001).

Inbreeding and rapid evolution are two factors that could impact the patterns of thermal tolerance within and between populations in these experiments, but these factors do not appear to alter the overall latitudinal pattern. Inbreeding in laboratory cultures might be expected to decrease the thermal tolerance over time, but no significant effect of generation was seen for the acute stress assays, while no clear pattern of decrease with increasing generation was found for the chronic stress assays. In addition, the treatment that was set up to test for these inbreeding effects, the BP treatment, showed no consistent pattern of higher tolerance than other treatments in both types of stress assays (higher tolerance would be expected if this BP cross relieved inbreeding depression). Another potential complication could stem from the rapid evolution of thermal tolerance over the course of a season or multiple seasons within a population. The relatively short generation time of this species [egg to adult times of about 20 days under summer conditions (Powlik et al. 1997)] could facilitate such rapid evolution. The present experiment was not explicitly designed to answer questions about the potential for rapid intra-population evolution, but the modest effects of year of collection suggest that such rapid evolution does not undermine the overall conclusions about the patterns of thermal tolerance in this species.

Potential tradeoffs of high temperature tolerance with fitness at lower temperatures

The evolution of thermal tolerance in *T. californicus* populations is associated with differences in competitive fitness at moderate temperatures. Upper temperature tolerance was lower for northern California populations than for southern California populations. In contrast, in competition experiments northern California populations were able to out-compete the populations from southern California at lower temperatures (16˚C) but not at higher temperatures (a 20-28˚C cycle). These competitive assays reveal differences between populations in fitness at moderate temperatures that are not apparent under non-competitive conditions using other fitness proxies. Edmands and Deimler (2004) did not find any population or population-by-environment interaction effects for juvenile survival or developmental rate at 15˚C and 25˚C for three different *T. californicus* populations. Both competitive and non-competitive measures of fitness are likely to be ecologically relevant for this species given what is known about the nature of population size, growth, and recolonization in the field. Under high density conditions maximizing competitive ability should be favored over higher intrinsic growth rates; *T. californicus* populations can reach densities as high as 20 000 copepods per liter under favorable environmental conditions (Powlik 1998). In contrast, when copepod populations grow from a relatively small number of founding copepods, optimal population growth could be favored over competitive ability. Extreme wave action, physiological stress, or complete drying can result in the extinction or dramatic reductions in population size of copepod populations that subsequently begin to expand once conditions improve (Dethier 1980; Dybdahl 1994; Powlik 1998).

The differences in fitness between northern populations and paired southern populations are consistent with a tradeoff between thermal tolerance at extreme high temperatures and

competitive fitness at more moderate temperatures; in addition, there are also apparent tradeoffs between competitive fitness under the lowest temperatures used in the competition assays and competitive fitness for the highest temperatures used in these assays (Figure 5). The differences in competitive abilities among these populations may be related to differences in copepod size, temperature dependent fecundity differences, or other life history characteristics. Edmands and Harrison (2003) found significant variation in body size and life history traits between copepod populations from northern copepod populations (Vancouver and Puget Sound) and southern California populations. They found that northern populations tended to be larger and develop faster when all populations were maintained at a constant 20˚C. Although Edmands and Harrison used some different populations from those used the current study, these measures may hint at why the northern populations have higher competitive fitness at the lower temperatures. At the higher end of the temperature spectrum, it is possible that a heat stress response could be triggered in northern populations at a lower temperature than in southern populations. In a congener, *T. japonicus,* a temperature of 30˚C for 90 minutes is sufficient to induce some response in HSP70, with much higher induction at 35˚C (Rhee et al. 2009). Although no results have been published regarding the induction of heat-shock proteins in *T. californicus*, it is possible that such a differential induction of heat stress response could lower the fitness of northern populations at higher temperatures relative to the southern populations.

Previous studies have found mixed evidence for tradeoffs between fitness or performance at moderate temperatures and adaptation to extreme temperatures with some support for each of the three models of evolution discussed in the introduction, generalist/specialist, horizontal niche shift, or no apparent tradeoff (Angilletta et al. 2003; Angilletta 2009). For performance traits such as locomotion, performance ability at extreme temperatures and performance at moderate

temperatures are often uncorrelated (Huey and Hertz 1984). Experimental laboratory selection experiments in *Drosophila* that involve long-term evolution to new temperature conditions have provided evidence that some measures of performance at extreme temperatures are correlated with shifts in performance at moderate temperatures (Huey and Kingsolver 1993; Gilchrist et al. 1997; Hoffmann et al. 2003). Other studies of tradeoffs have employed laboratory experimental evolution studies in microbes, with some of these studies finding generalist/specialist tradeoffs or horizontal niche shifts (Cooper et al. 2001; Knies et al. 2006) and other studies not showing such tradeoffs (Bennett and Lenski 1993; Knies et al. 2009). Finally, adding to this contradictory pattern, evidence for generalist specialist tradeoffs has been found from studies of genetic correlations for genetic variation within natural populations in some animal species (Gilchrist 1996; Izem and Kingsolver 2005), but not others (Palaima and Spitze 2004).

Do these results from *T. californicus* suggest that there are generalist-specialist tradeoffs for temperature adaptation among populations of this species? Under this scenario the northern populations would be specialized for intermediate temperatures while the southern populations would be able to handle a broader range of temperatures but have the tradeoff of lowered fitness at intermediate temperatures. Potentially supporting this scenario, the 16˚-25˚C cycling environment reflects a slightly warmer than average daily summer temperature range for southern California (based on air temperatures in Figure 2), but the SD population is out-competed by the SCN population at this temperature. However, a horizontal shift in the thermal niche and not a "jack-of-all-temperatures is a master of none"-style tradeoff is another potential explanation if southern populations have degraded thermal tolerance at lower extreme temperatures. Therefore, at this point although either a generalist-specialist tradeoff or a shift in thermal niche are possible explanations for the patterns of thermal performance seen among

these *T. californicus* populations, the results suggest that there are indeed fitness tradeoffs that accompany the evolution of improved high temperature performance and survival in populations of this species.

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FIGURE LEGENDS

Figure 1. The location and phylogenetic relationships of *T. californicus* copepod populations used in temperature assays. Sampling locations along the Pacific coast of North America are indicated by arrows. Coastal weather stations used for temperature data are indicated with an * with numbers corresponding to weather stations in Figure 2. The phylogenetic tree of copepod populations used in this study is based upon the relationships between individuals from these populations determined by Willett and Ladner (2009) with a Bayesian analysis of the mtDNA-encoded cytochrome b gene. The tree is shown rooted with cytochrome b sequences from a congener, *T. japonicus*. Further details of both the phylogenetic methods and locations and nature of sampling sites can be found in Willett and Ladner (2009).

Figure 2. Air temperature data for coastal temperature stations along the Pacific coast of North America. The weather stations were selected to be as close to the coast and sampled copepod populations as possible (locations shown on Figure 1). The squares show the yearly extreme highs, which are the average of the extreme high temperature for each single year over the last 40 years of available data for each station (standard deviation of these temperatures and the all-time highs over this period are also shown). Average daily maximum (diamonds) and minimum temperatures (triangles) over the period of June to October are shown (data from 1970-2000). The yearly ranges in average daily highs are depicted on the daily maximum points (i.e. the range between the highest average daily highs during the summer and the lowest average daily high temperatures during the winter). Temperature monitoring of copepod pools has suggested that the copepod pools are slightly warmer on average than the air temperature (Egloff 1966).

Climate data for this figure came from the Western Regional Climate Center (http://www.wrcc.dri.edu/Climsum.html) and the National Climatic Data Center (http://www.ncdc.noaa.gov/oa/ncdc.html) for the United States and the National Climate Data and Information Archive from Environment Canada (http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html) for Canada.

Figure 3. Box plots of *T. californicus* survival under acute stress temperature exposures. The southern populations start on left with northern populations towards the right of each box plot. Temperatures used in assays were as follows: (a.) 35˚C, (b.) 36˚C, (c.) 37˚C, (d.) 38˚C, and (e.) 39˚C. Note that not all populations were tested at each temperature. Bold line in each plot indicates median value, box, box indicates first and third quartiles, dots indicate outliers, and whiskers indicate largest and smallest non-outlier values.

Figure 4. Box plots of *T. californicus* survival under the chronic high temperature stress condition of constant 32˚C. The results are broken up by sex, year of collection, and population of origin. Box plots are constructed as in Figure 3 with the addition of means indicated by dark ovals.

Figure 5. Competitive fitness assays for northern versus southern *T. californicus* populations under varying temperature regimes. For these assays progeny from females of northern populations (SCN or BHB) compete with progeny from females from southern populations (SD or LJS) under a series of different temperature regimes and those progeny surviving to adult are collected and genotyped to determine parentage. The percentage of the genotyped progeny that

are from the northern population (SCN or BHB) is shown on the y-axis while the temperature regime is shown on the x-axis. The symbols depict the mean over each replicate and all transfer plates for the pair of populations tested under that temperature regime (bars depict standard errors). Supplemental table S3 has the results broken down by replicate and transfer.

Table 1. Sampling years, sites and number of replicates for acute and chronic stress trials for *T. californicus*.

Site	Year	˚N	˚W	Gen	32˚C assay		35˚C 1 hr		36˚C 1 hr		37˚C 1 hr		38˚C 1 hr		39˚C 1 hr	
					f	m	f	m	f	m	f	m	f	m	f	m
SD	2003	32.7457	117.2550	3	5	5					2	2	4	4		
	2004			3	4	4					12	12	2	2		
	2004			4	10	10					27	27	4	4		
	2004			5									6	6		
	2004			BP	17	17					17	17	24	24		
	2006			1	10	10							15	15	3	3
	2006			2	24	20					16	16	12	12		
	2006			3	6	6					5	6	21	20		
	2006			2B	4	4					1	2	4	4		
LJS	2006	32.8434	117.2808	1	10	10					8	8	15	15	3	3
	2006			2	10	10					8	8	28	28	16	16
	2006			3									1	1		
	2006			2B	11	11					2	2	4	4		
AB	2003	33.7377	118.3753	3	5	5					2	2	4	4		
	2004			3	14	14					24	24				
	2004			4	12	11					10	10	11	10		
	2004			BP	7	6							12	8		

FLR1	2004	33.7971	118.4083	3	4	4					7	7		
	2004			4	10	9					9	9	8	8
PTD	2004	34.0003	118.8068	3	12	12					40	20		
	2004			4									8	8
SS	2004	35.5816	121.1212	3	20	20					36	36	6	6
SCN	2003	36.9495	122.0470	3	8	6					2	2	4	4
	2004			3							4	3		
	2004			4	22	17	4	4	28	28	15	16		
	2004			BP	15	13					8	7		
BHH	2004	38.3046	123.0562	4	10	10			22	22	8	8		
BHB	2004	38.3051	123.0654	3	10	10			24	24	8	8		
VI	2006	48.9335	125.5413	3	33	18	8	3	19	15	4	4		
BC	2006	49.3381	123.2502	3	38	26	12	12	21	22	14	13		

The locations of the collection sites are listed in degrees N and degrees W in first row for each site. Chronic stress, 32˚C constant replicates consisted of either 10 males or 10 females. Acute stress, 35-39˚C replicates were generally conducted with 10 males and 10 females but in a few cases different numbers of males and females were used. Gen is generation or source of copepods as follows: 1 is 1st generation (collected from field), 2 is 2nd, 3 is 3rd, 4 is 4th, and 5 is 5th generation reared in the lab. 2B are 2nd generation copepods reared in beakers rather than Petri dishes. BP are the progeny of crosses between independent copepod lineages from beakers and plates.

Table 2. Acute temperature stress model comparisons for assays using *T. californicus* populations.

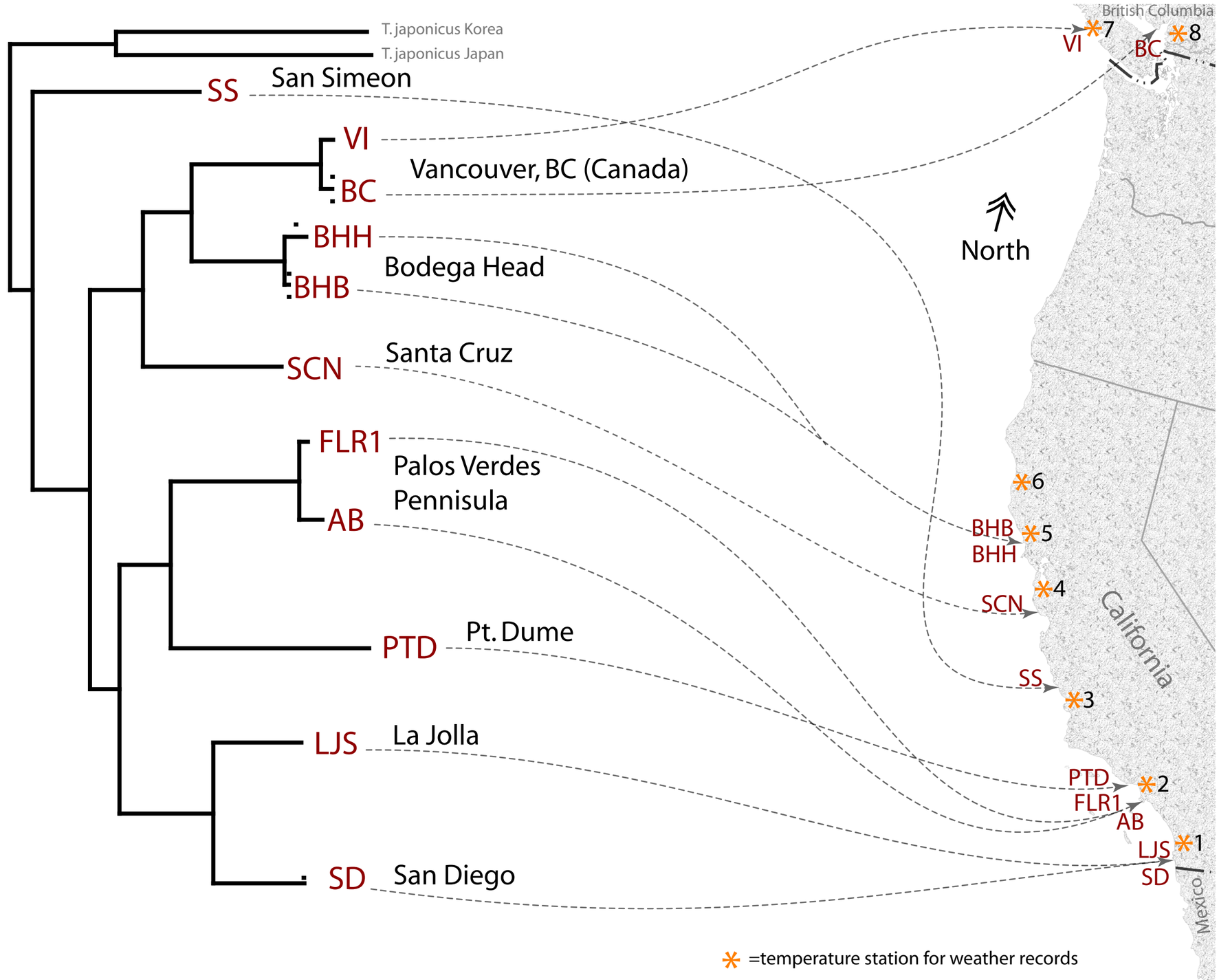
Model #	Model	Effect	DF	AIC	χ^2 difference	Pr(>χ^2)
1	Main		25	3526.9		
2	-Sex x Pop.	fixed	15	3591.5	84.5	>0.000001
3	-Pop.	fixed	5	3710.9	223.9	>0.000001
4	-Temp	fixed	24	3643.7	118.8	>0.000001
5	-Sex	fixed	14	3852.1	347.2	>0.000001
6	-Source	random	24	4712.8	1187	>0.000001
7	-Batch	random	24	4293.6	768.7	>0.000001
8	+Sex x Temp.	fixed	26	3528.5	0.40	0.52
9	+Temp. x Pop.	fixed	35	3710.6	14.2	0.16
10	+Gen.	fixed	31	3684.4	12.0	0.06
11	+Year Col.	fixed	26	3657.4	3.67	0.053

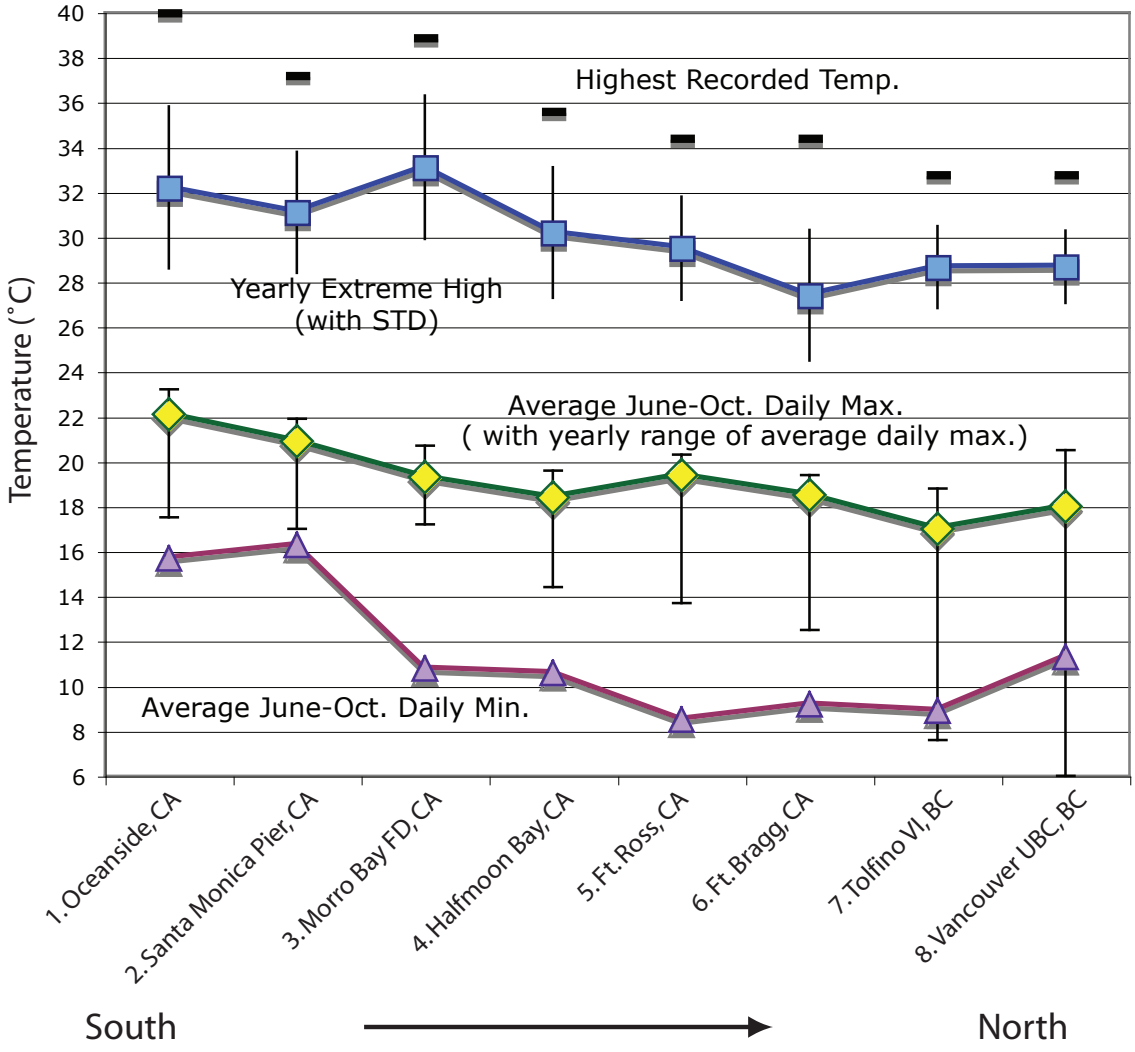
Main model (#1) incorporates population (pop.) sex, temperature (temp.), and sex–by-population (Sex x Pop.) as fixed effects and source and batch as random effects. Models 2-7 show the impact of dropping these effects from the main model (e.g. –pop. would be the same model without population and sex-by-population as fixed effects). Models 8-11 are not significantly better than the main model with the addition of sex-by-temperature (Sex x Temp.), temperature-by-population (Temp. x Pop.), or generation (gen.) or year collected (year col.) as fixed effects to the model. The AIC is the Akaike's Information Criterion which indicates a model with a better fit to the data with a lower number. χ^2 difference is in comparison with the main model.

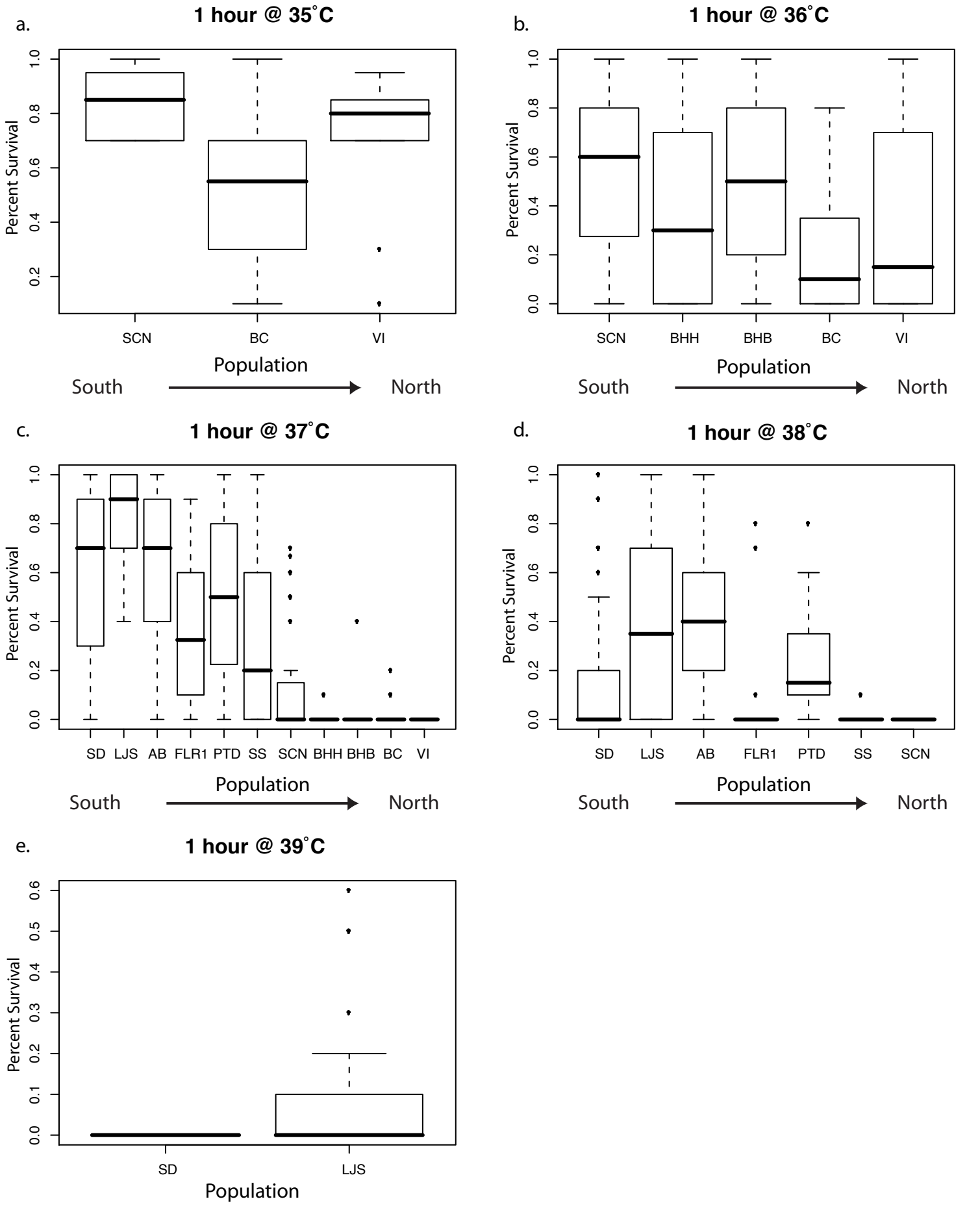
Table 3. Significance of effects in a proportional hazards model for analysis of chronic temperature stress mortality in *T. californicus* populations.

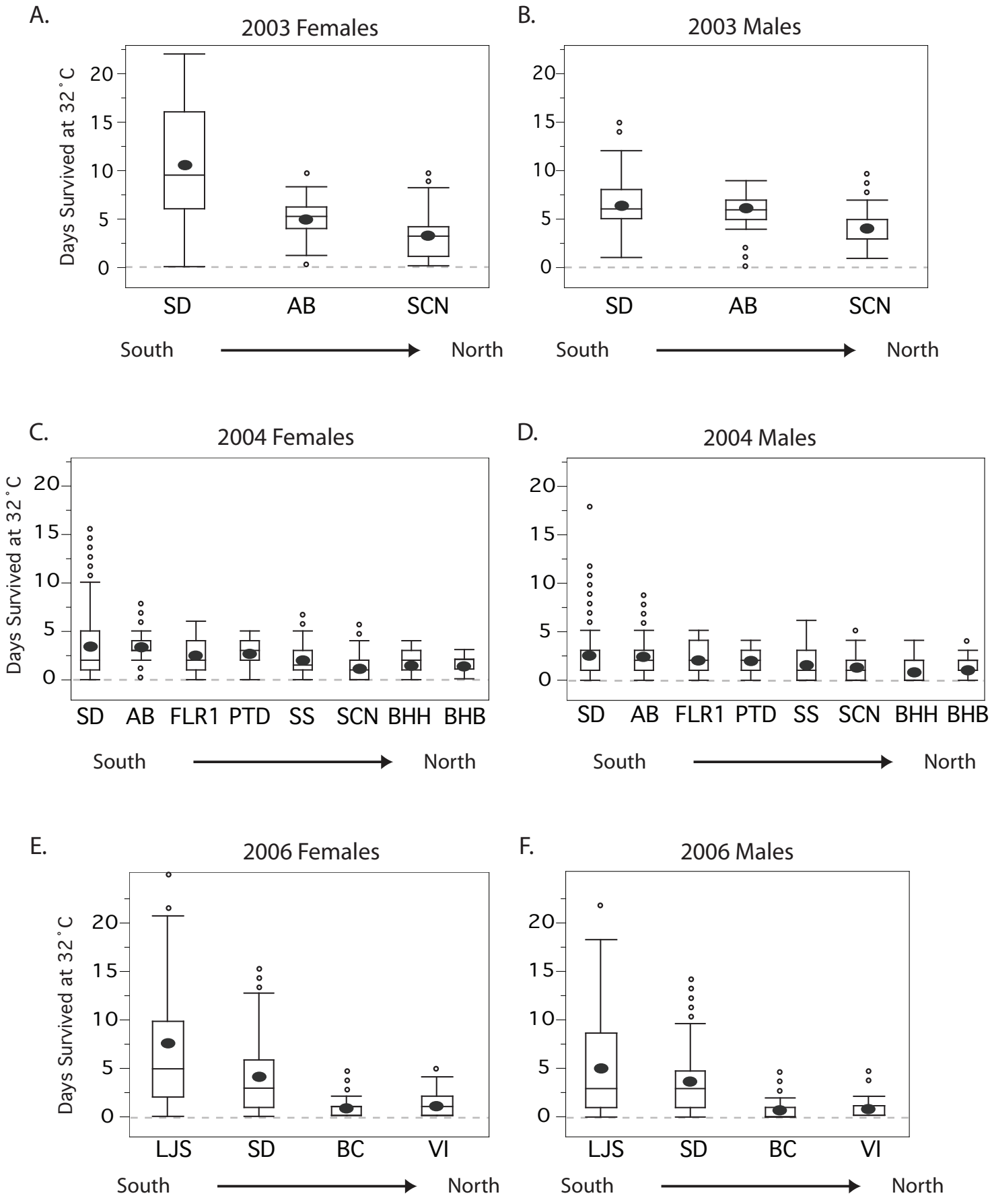
A. Effect with Population	L-R χ^2	DF	P value
Population	645.0	10	>0.0001
Sex	1.314	1	0.25
Sex x Pop.	51.04	10	>0.0001
Year-Collected	158.2	2	>0.0001
Gen.	123.8	5	>0.0001
Sex x Gen.	19.93	5	0.0013
B. Effect with Latitude			
Latitude	378.5	1	>0.0001
Sex	49.6	1	>0.0001
Year-Collected	133.7	1	>0.0001
Generation	188.1	5	>0.0001
Sex x Latitude	0.621	1	0.43
Sex x Gen.	6.99	5	0.22
Source	43.3	1	>0.0001

Models used either Population (Pop.) as an effect (A.) or Latitude as an effect (B.). Models were run on full dataset including 6161 mortality events. L-R χ^2 is the likelihood ratio χ^2.


T. japonicus Korea
T. japonicus Japan
SS
San Simeon
VI
BC
Vancouver, BC (Canada)
BHH
BHB
Bodega Head
SCN
Santa Cruz
FLR1
Palos Verdes
Pennisula
AB
PTD
Pt. Dume
LJS
La Jolla
SD
San Diego
British Columbia
VI
7
BC
8
North
6
BHB
BHH
5
4
SCN
California
SS
3
PTD
2
FLR1
AB
LJS
1
SD
Mexico
=temperature station for weather records


Temperature (˚C)
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
Highest Recorded Temp.
Yearly Extreme High
(with STD)
Average June-Oct. Daily Max.
(with yearly range of average daily max.)
Average June-Oct. Daily Min.
1. Oceanside, CA
2. Santa Monica Pier, CA
3. Morro Bay FD, CA
4. Halfmoon Bay, CA
5. Ft. Ross, CA
6. Ft. Bragg, CA
7. Tolfino VI, BC
8. Vancouver UBC, BC
South
North


a.
1 hour @ 35˚C
Percent Survival
0.2
0.4
0.6
0.8
1.0
SCN
BC
VI
Population
South
North
b.
1 hour @ 36˚C
Percent Survival
0.0
0.2
0.4
0.6
0.8
1.0
SCN
BHH
BHB
BC
VI
Population
South
North
c.
1 hour @ 37˚C
Percent Survival
0.0
0.2
0.4
0.6
0.8
1.0
SD
LJS
AB
FLR1
PTD
SS
SCN
BHH
BHB
BC
VI
Population
South
North
d.
1 hour @ 38˚C
Percent Survival
0.0
0.2
0.4
0.6
0.8
1.0
SD
LJS
AB
FLR1
PTD
SS
SCN
Population
South
North
e.
1 hour @ 39˚C
Percent Survival
0.0
0.1
0.2
0.3
0.4
0.5
0.6
SD
LJS
Population


A.
2003 Females
Days Survived at 32˚C
0
5
10
15
20
SD
AB
SCN
South
North
B.
2003 Males
SD
AB
SCN
South
North
C.
2004 Females
Days Survived at 32˚C
SD
AB
FLR1
PTD
SS
SCN
BHH
BHB
South
North
D.
2004 Males
SD
AB
FLR1
PTD
SS
SCN
BHH
BHB
South
North
E.
2006 Females
Days Survived at 32˚C
LJS
SD
BC
VI
South
North
F.
2006 Males
LJS
SD
BC
VI
South
North

Supplemental Figures Legends

Supplemental Figure S1: Average survival of copepods from different *T. californicus* populations under acute stress temperature exposures. Solid lines connect average values for males at indicated temperatures, while dashed lines are for females. Southern-most population start on left to northern populations on right.

Supplemental Figure S2: The effects of year collected and generation on acute temperature stress tolerance in *T. californicus* populations. These are the populations with multiple generations or year-collected sources used in acute stress assays. Populations are as follows: (a-d) SD, (e) LJS, (f-i) AB, and (j-l) SCN. For plots a, b, e, f, g, j, and k, the mean percent survival is plotted on the y-axis and temperature of the assay is plotted on x-axis with generations or year collected broken out as shown in the legend on each plot. The effects of generation within a single temperature are shown in plots c and h (37˚C) and d and i (38˚C) with mean percent survival again on the y-axis and year-collected on the x-axis.

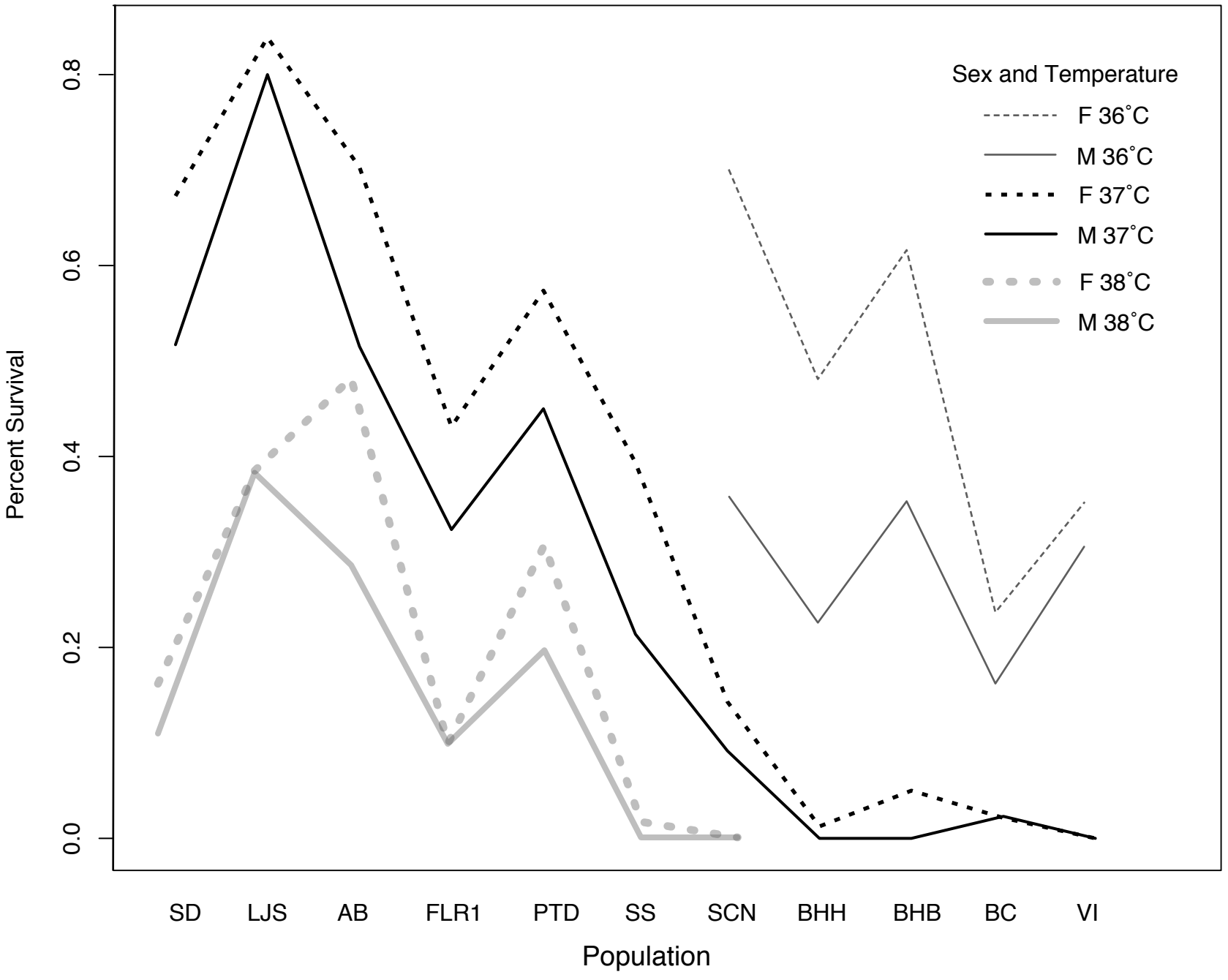
Analyses of single populations for the effects of year-collected and generation using generalized linear mixed models were conducted for the SD, AB, SCN, and LJS populations. Models for the SD population incorporating sex, temp as fixed effects and batch and source as random effects was improved slightly by the addition of year as a fixed effect (χ^2=4.11, d.f.=1, P=0.046) but not generation (χ^2=5.05, d.f.=6, P=0.53); see Supplemental Figure S2 for impacts of year and generation. The 2003 year class did the best across temperature treatments [see (a)-(d)], but had a small sample size (N=12),

followed by the 2004 class (N=184), with the 2006 class the worst (N=155). For the other populations in which multiple generations or years were tested (AB, LJS, and SCN), only the inclusion of generation as a fixed effect for the SCN populations improved the logistic regression models for these populations (χ^2=16.1, d.f.=2, P=0.003), but this appears to be an artifact of the non-overlapping generation assays performed for this population [see (e)-(l) for data broken down by year and generation for these populations].

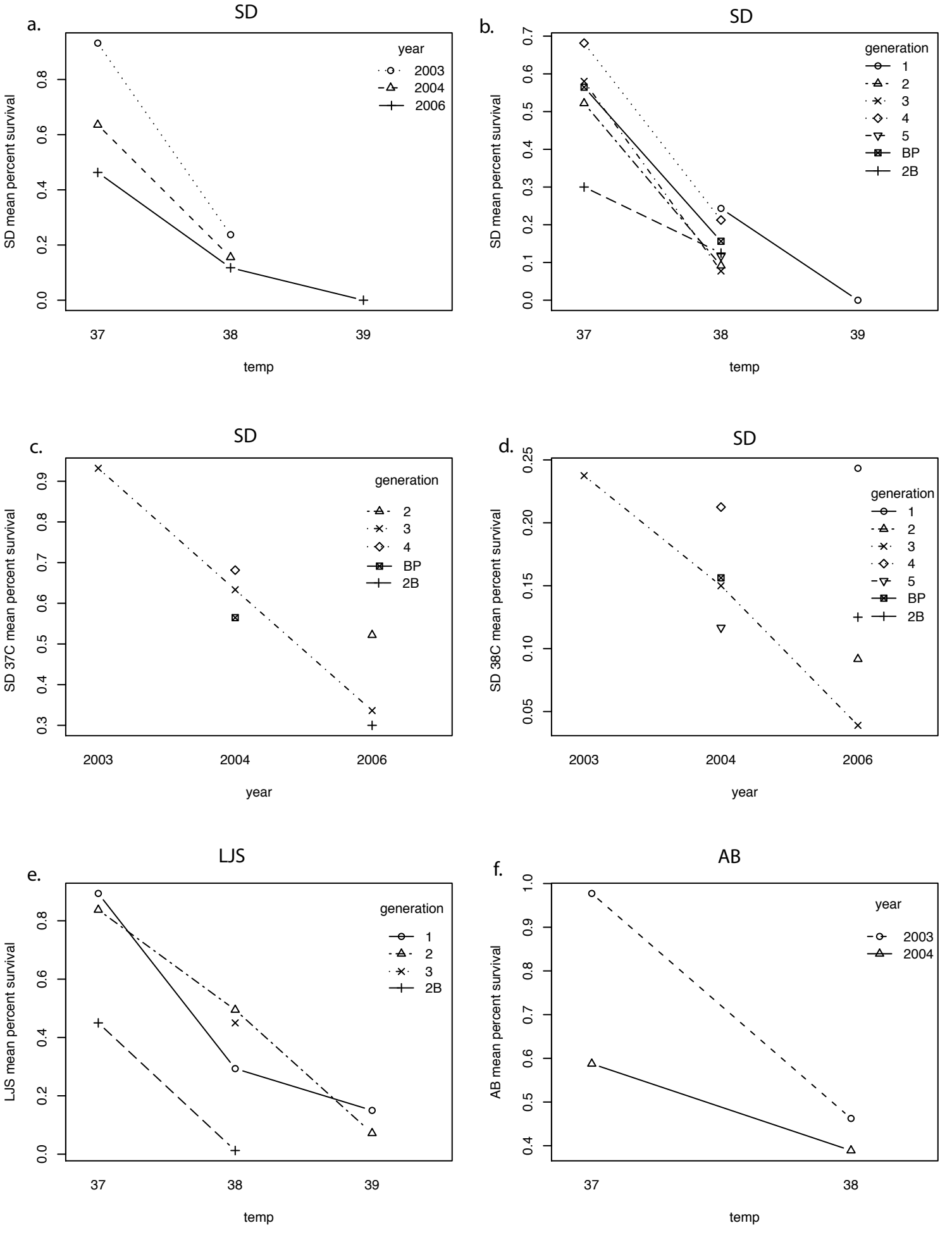
Supplemental Figure S3. Plot of risk ratios from a logistic regression model of *T. californicus* survival under chronic high temperature stress conditions. Higher risk ratios indicate a higher chance of dying under these conditions for indicated factor. Error bars indicate the upper and lower 95% confidence limits on the risk ratio. Results are shown in figure for interactions that did not have confidence limits that overlapped with one.

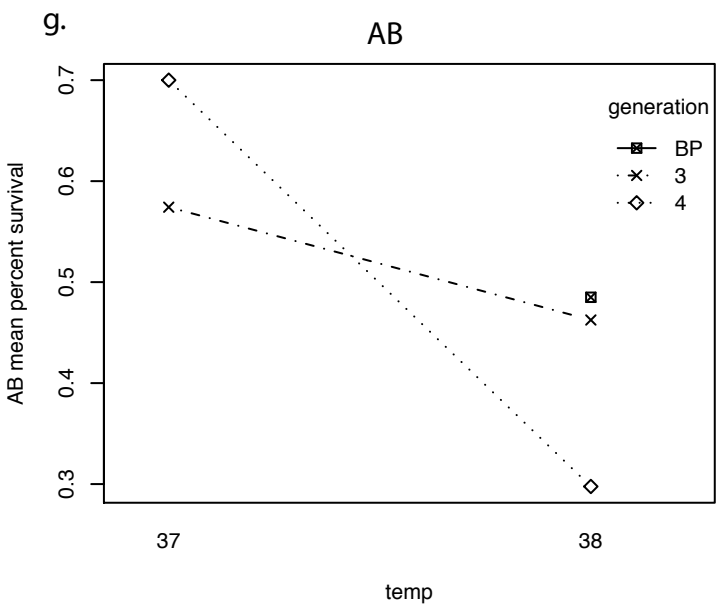
Supplemental Figure S4: The effects of year-collected and generation on chronic temperature stress survival in *T. californicus* populations. These are the populations with multiple generations or year-collected sources used in chronic stress assays. Populations are listed above each plot with females on the left and males on the right. These are box plots with the y-axis showing the number of days survived at 32˚C. Generation and year-collected are given below each box plot.

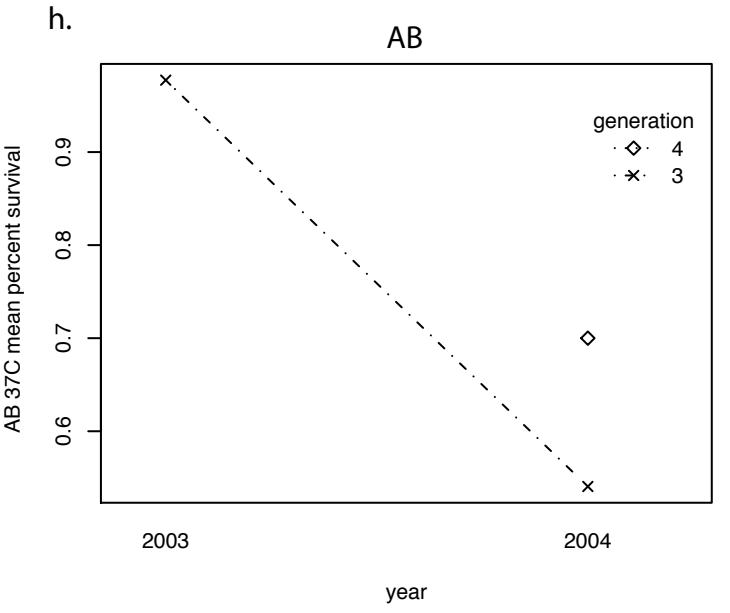
Supplemental Figure S1



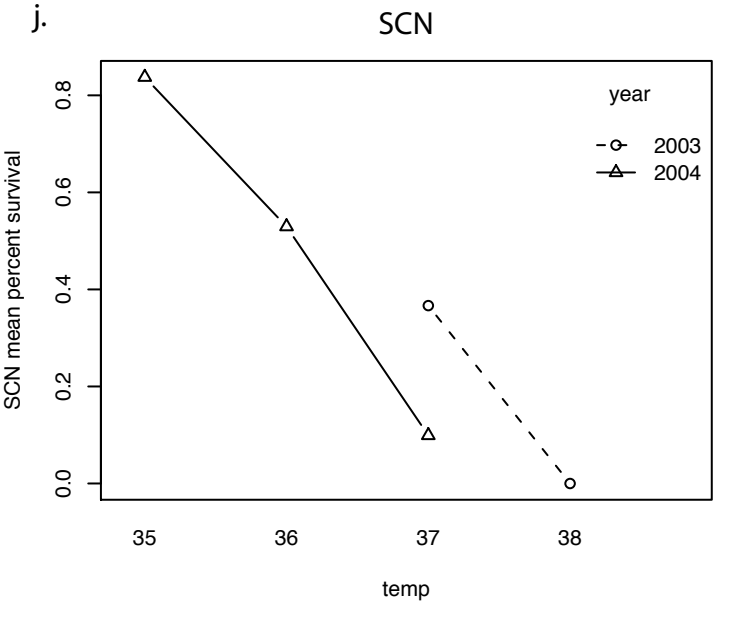
Supplemental Figure S2

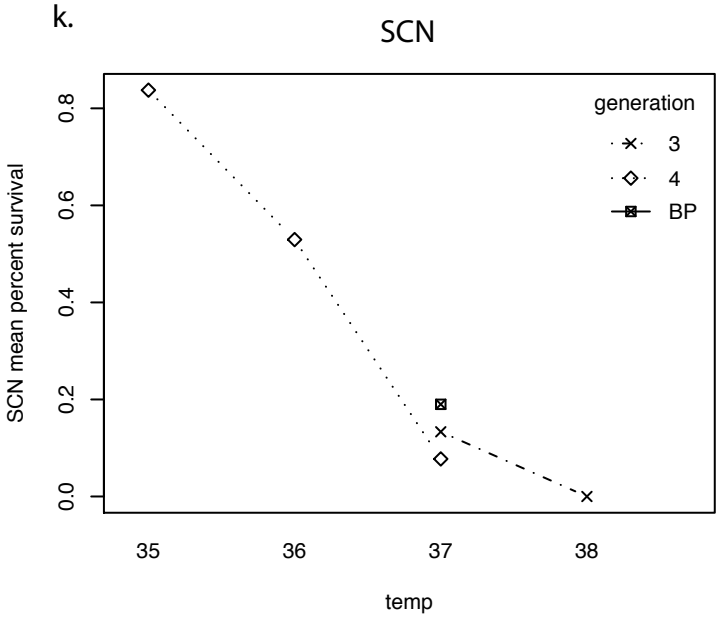


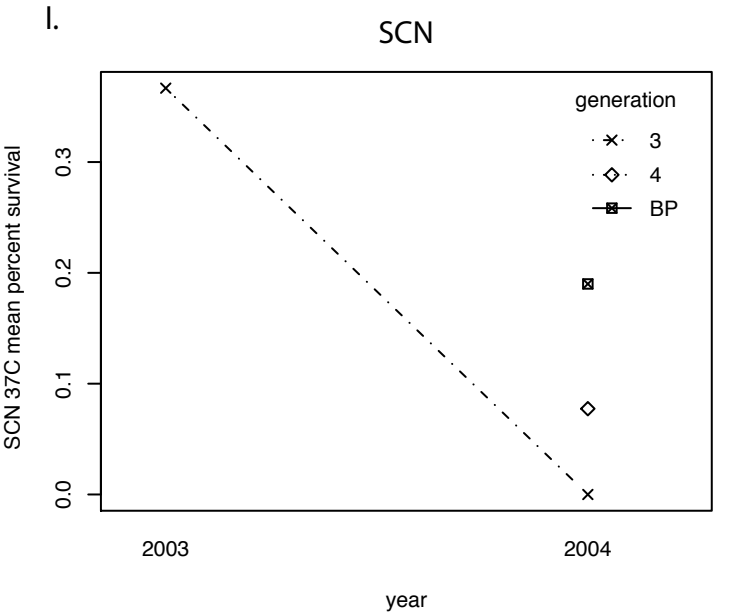

g.
AB
AB mean percent survival
0.3
0.4
0.5
0.6
0.7
generation
BP
3
4
37
38
temp


h.
AB
AB 37C mean percent survival
0.6
0.7
0.8
0.9
generation
4
3
2003
2004
year

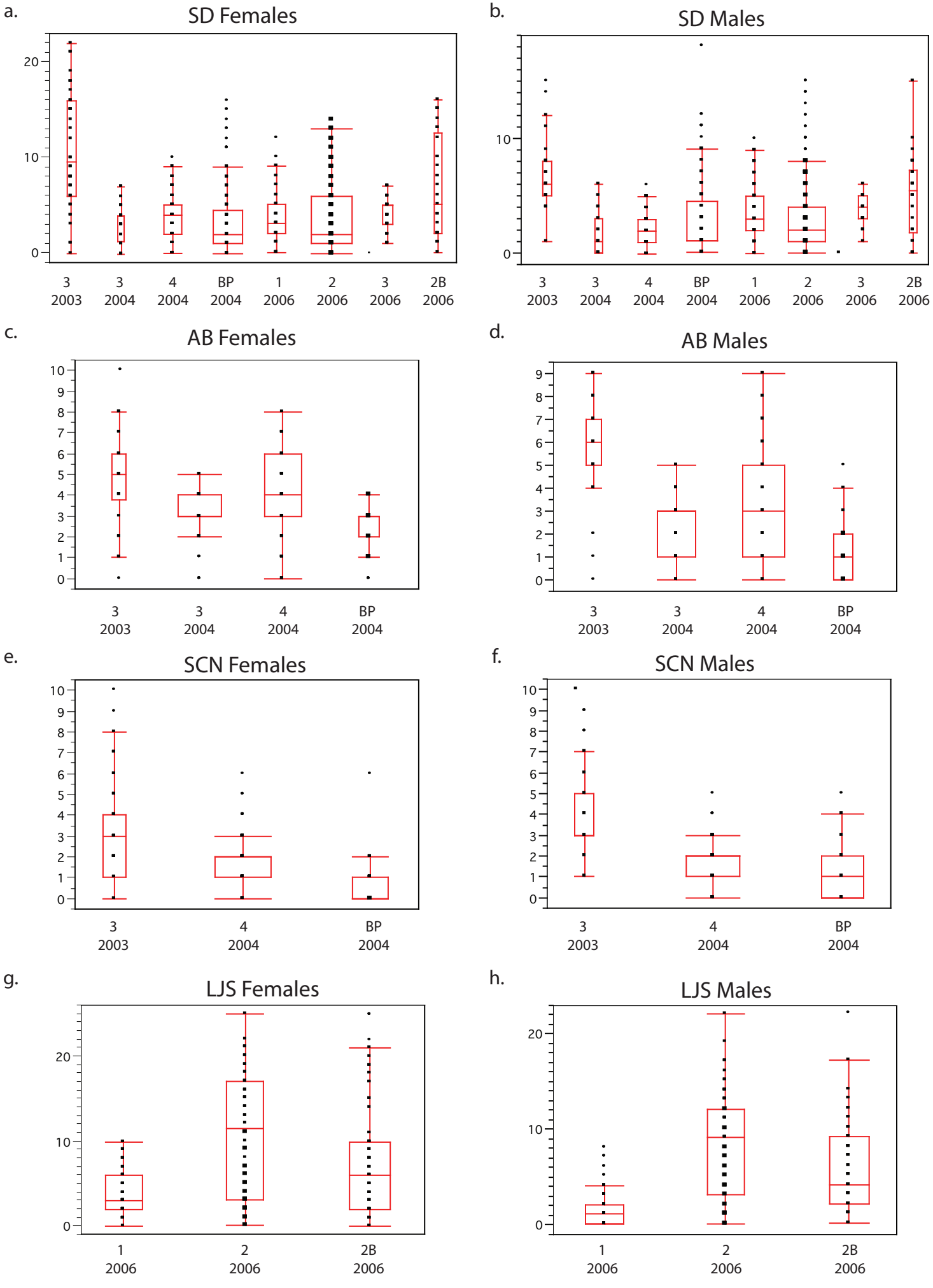

i.
AB
AB 38C mean percent survival
0.30
0.35
0.40
0.45
generation
BP
3
4
2003
2004
year


j.
SCN
SCN mean percent survival
0.0
0.2
0.4
0.6
0.8
year
2003
2004
35
36
37
38
temp

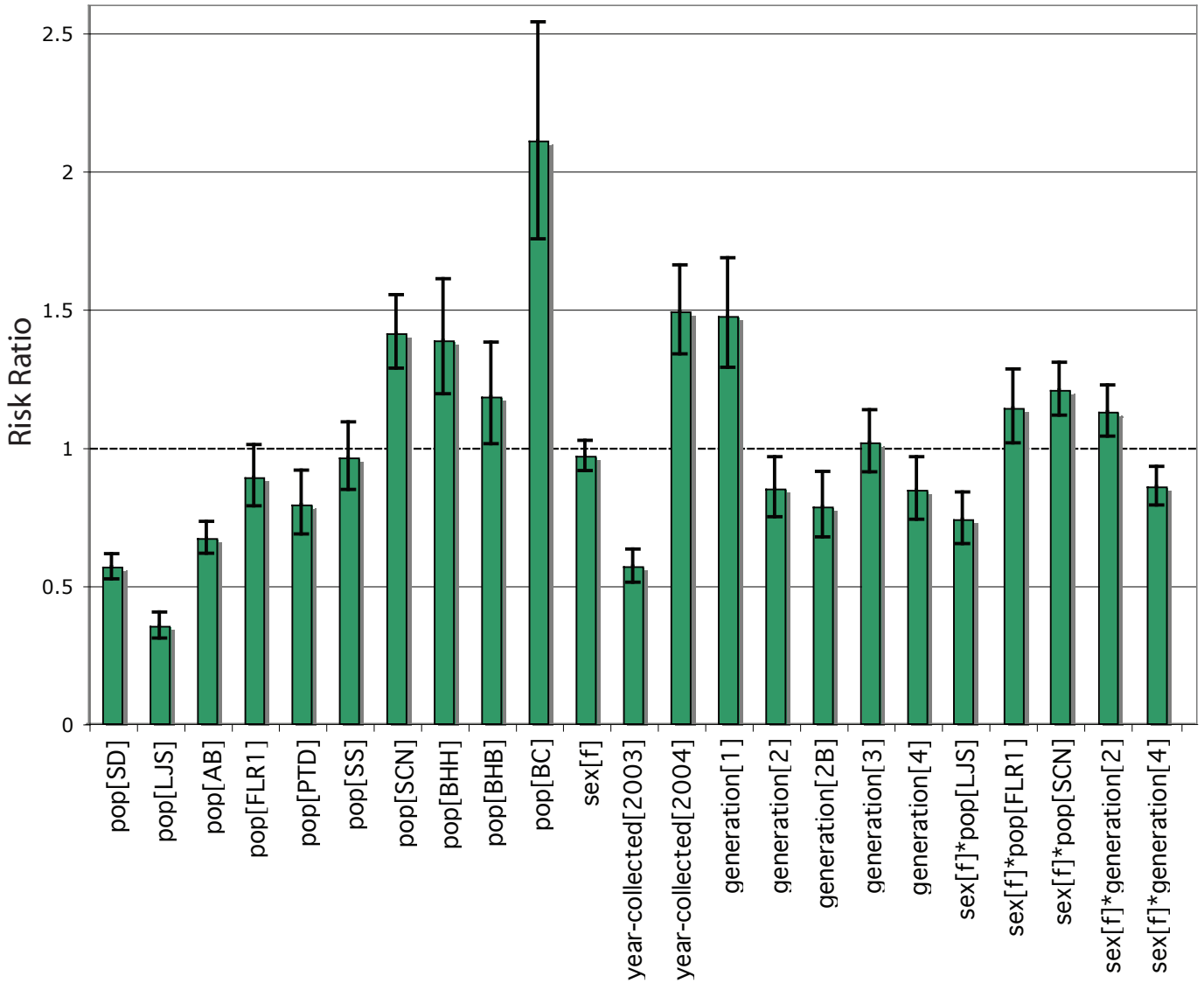

k.
SCN
SCN mean percent survival
0.0
0.2
0.4
0.6
0.8
generation
3
4
BP
35
36
37
38
temp


l.
SCN
SCN 37C mean percent survival
0.0
0.1
0.2
0.3
generation
3
4
BP
2003
2004
year

Supplemental Figure S3



Supplemental Figure S4



Supplemental Table T1. Primer combinations used for genotyping competition assay progeny.

Population pair-primer	Primer Name	Sequence (5' to 3')
<u>BHB and LJS pops</u>		
BHB/LJS-conserved	CYC1ex3bcon.f	GCGGATTTGACCTTGCACCCAC
BHB/LJS-BHB specific	CYC1BHBin3.r	TAGTCTAGGGTTAGCCAGATCAGCCA
BHB/LJS-LJS specific	CYC1SDin3.r	TCTGGGAGGACACCCATGATCCC
<u>SCN and SD pops</u>		
SCN/SD-conserved	RISPcon.r2	CATTGATTGTTCCGGACCAAAAACTC
SCN/SD-SCN specific	RISPSCN.f	GAAGCCTTGAAACAATATACCACTAAC
SCN/SD-SD specific	RISPSD.f	ACATACGTCCTGAGTTGGTGTTCAG

Primers listed above for a pair of populations were combined into a single PCR reaction to generate population-specific sized fragments. Conditions for BHB/LJS were 30 cycles of 95˚C for 25s, 64˚C for 30s, and 72˚C for 2m. This amplifies a 324 bp fragment of the BHB *CYC1* gene and a 235 bp fragment of the LJS *CYC1* gene. Conditions for SCN/SD were 35 cycles of 95˚C for 25s, 58˚C for 30s, and 72˚C for 1m. This reaction amplifies a 311 bp fragment of the *RISP* gene for the SCN population and a 501 bp fragment of the SD *RISP* gene.

Supplemental Table T2. Competition assay results

BHB and LJS competition assays

Numbers of scored progeny are listed split by sex from the founding female's population. The number of founding females for each replicate dish is listed as the #in, while the number of females surviving to be transferred to a new dish is listed as #out.

LJS vs BHB 16˚C assays

Rep4	Lj(m)	Lj(f)	Bh(f)	Bh(m)	Lj(total)	Bh(total)	%BHB	# in	#out
4a	0	0	2	1	0	3	100	20	19
4b	0	3	57	21	3	78	96.296	19	15
4c	0	4	25	5	4	30	88.235	15	15
4d	0	0	4	0	0	4	100	15	12
4e	6	4	20	7	10	27	72.973	12	
4total	6	10	108	34	16	142	89.873		
Rep5									
5a	1	1	1	0	2	1	33.333	20	20
5b	13	15	37	36	28	73	72.277	20	17
5c	5	5	23	14	10	37	78.723	17	17
5d	0	0	27	12	0	39	100	17	>4
5e	37	43	2	1	80	3	3.6145	>4	
5total	56	64	90	63	120	153	56.044		
5a-d total	19	21	88	62	40	150	78.947		
Rep6									
6a	1	3	11	5	4	16	80	20	19
6b	0	0	33	7	0	40	100	19	14
6c	3	0	31	5	3	36	92.308	14	>10
6d	1	0	11	6	1	17	94.444	>10	10
6e	3	4	28	16	7	44	86.275	10	
6total	8	7	114	39	15	153	91.071		
16 degrees tot	70	100	312	136	170	448	72.492		
16 degrees av	23.333	27	104	45.333	50.333	149.33	78.996		

LJS vs BHB 20˚C assays

plt id	Lj(m)	Lj(f)	Bh(f)	Bh(m)	Lj(total)	Bh(total)	%BHB		
rep1								# in	#out
1	5	4	1	1	9	2	18.182	20	19
1a	0	0	0	0	0	0		19	17
1b	1	4	0	0	5	0	0	17	1
1c	0	0	0	0	0	0		1	
1 total	6	8	1	1	14	2	12.5		
rep2									
2	47	22	18	20	69	38	35.514	20	18
2a	1	0	1	1	1	2	66.667	18	16
2b	0	0	0	0	0	0		16	
2 total	48	22	19	21	70	40	36.364		

rep3									
3	11	9	4	1	20	5	20	20	13
3a	0	0	0	0	0	0		13	8
3b	0	0	0	0	0	0		8	
3 total	11	9	4	1	20	5	20		
Rep1-2								**# in**	**#out**
1a	1	0	0	3	1	3	75	20	19
1b	1	1	9	7	2	16	88.889	19	16
1c	0	0	12	8	0	20	100	16	16
1d	0	0	0	0	0	0		16	0
1 total	2	1	21	18	3	39	92.857		
Rep2-2									
2a	3	0	8	14	3	22	88	20	20
2b	12	1	32	14	13	46	77.966	20	14
2c	0	0	1	3	0	4	100	14	>5
2d	0	0	0	0	0	0		>5	0
2total	15	1	41	31	16	72	81.818		
Rep3-2									
3a	1	2	2	1	3	3	50	20	19
3b	0	0	6	6	0	12	100	19	13
3c	1	0	0	0	1	0	0	13	13
3d	0	0	0	0	0	0		13	0
3total	2	2	8	7	4	15	78.947		
overall 20˚ tot	84	43	94	79	127	173	57.667		
overall 20˚ av	14	7.1667	15.667	13.167	21.167	28.833	53.748		

LJS vs BHB 20-28˚C cycle

Rep4	**Lj(m)**	**Lj(f)**	**Bh(f)**	**Bh(m)**	**Lj(total)**	**Bh(total)**		**# in**	**#out**
4	29	27	0	1	56	1	1.7544	20	13
4a	2	1	0	0	3	0	0	13	2
4b	0	0	0	0	0	0		2	0
4 total	31	28	0	1	59	1	1.6667		
Rep5									
5	18	16	0	0	34	0	0	20	15
5a	0	0	0	0	0	0		15	0
5 total	18	16	0	0	34	0	0		
Rep6									
6	4	6	0	0	10	0	0	20	11
6A	0	0	0	0	0	0		11	0
6 total	4	6	0	0	10	0	0		
24˚C total	53	50	0	1	103	1	0.9615		
24˚C average	17.667	16.667	0	0.3333	34.333	0.3333	0.5556		

SCN and SD competition assays

16° constant (8h:16h LD)

replicate 1 IIA	SDm	SDf	SCm	SCf	SD	SC	%SC	# in	# out
plate 2	0	0	22	39	0	61	100	14	7
plate 3	4	0	41	40	4	81	95.294	7	7
total r1-16	4	0	63	79	4	142	97.26		

replicate 2 IIA	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	0	0	37	32	0	69	100	14	14
plate 2	0	0	38	39	0	77	100	14	12
total r2-16	0	0	75	71	0	146	100		

replicate 3 IIA	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	0	0	34	31	0	65	100	20	11
plate 4	0	0	41	47	0	88	100	8	7
total r3-16	0	0	75	78	0	153	100		

16° total	4	0	213	228	4	441	99.101		
16° average	1.3333	0	71	76	1.3333	147	99.087		

16-25 cycle environment (16h:8h))
19°C average temp (12:12 LD cycle)

replicate 1 IAE	SDm	SDf	SCm	SCf	SD	SC	%SC	# in	# out
plate 2	20	19	45	29	39	74	65.487	13	9
plate 3	6	27	33	19	33	52	61.176	9	5
total r1-25	26	46	78	48	72	126	63.636		

replicate 2 IAE	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	1	4	93	82	5	175	97.222	20	18
plate 2	0	0	9	1	0	10	100	18	9
plate 3	0	0	28	26	0	54	100	9	0
total r2-25	1	4	130	109	5	239	97.951		

replicate 3 IAE	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	1	1	42	39	2	81	97.59	14	13
plate 2	1	2	41	33	3	74	96.104	13	12
plate 3	3	23	22	17	26	39	60	12	0
total r3-25	5	26	105	89	31	194	86.222		

25cycle total	32	76	313	246	108	559	83.808		
25cycle averag	10.667	25.333	104.33	82	36	186.33	82.603		

20° constant
SD and SCN

replicate 1	SDm	SDf	SCm	SCf	SD	SC	%SC	# in	# out
plate 1	26	8	3	9	34	12	26.087	16	14
plate 2	8	48	0	6	56	6	9.6774	14	2
plate 3	0	25	15	28	25	43	63.235	2	ted (SD)
total r1-20	34	81	18	43	115	61	34.659		

replicate 2	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	0	11	0	8	11	8	42.105	16	15
plate 2	6	8	1	12	14	13	48.148	15	13
plate 3	4	11	4	3	15	7	31.818	13	13
plate 4	3	32	13	83	35	96	73.282	13	, 7 SCN)
total r2-20	13	62	18	106	75	124	62.312		

replicate 3	SDm	SDf	SCm	SCf	SD	SC	%SC		
plate 1	1	4	38	19	5	57	91.935	16	8
plate 2	6	1	0	0	7	0	0	8	3
unk	1	1	20	43	2	63	96.923		
total r3-20	8	6	58	62	14	120	89.552		

20°C total	55	149	94	211	204	305	59.921		
25°C average	18.333	49.667	31.333	70.333	68	101.67	62.174		

2006 SD vs. SCN 20-28 cycling

replicate 1	SDm	SDf	SCm	SCf	SD	SC	%SC	# in	# out
1	68	18	16	19	86	35	28.926	16	15
1A	89	61	14	2	150	16	9.6386	15	5
1B	83	45	0	0	128	0	0	5	2
total r1-28	240	124	30	21	364	51	12.289		

replicate 2	SDm	SDf	SCm	SCf	SD	SC	%SC		
2	88	94	3	8	182	11	5.6995	16	10
2A	39	50	0	0	89	0	0	10	8
2B	140	23	0	0	163	0	0	8	3
total r2-28	267	167	3	8	434	11	2.4719		

replicate 3	SDm	SDf	SCm	SCf	SD	SC	%SC		
3	22	22	12	5	44	17	27.869	16	15
total r3-28	22	22	12	5	44	17	27.869		

28cycle total	529	313	45	34	842	79	8.5776		
28cycle averag	176.33	104.33	15	11.333	280.67	26.333	14.21		

Supplemental Table T3. Detailed description of acute temperature stress main model.

Random Effects				
	Variance	Std. Dev.		
Source (intercept)	1.3655	1.1685		
Batch (intercept)	1.3751	1.1727		
Fixed Effects				
	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	106.20511	7.93316	13.387	**< 0.000001**
popSD	-1.21609	0.51729	-2.351	**0.019**
popLJS	-0.02579	0.61211	-0.042	0.97
popFLR1	-2.44064	0.75341	-3.239	**0.0012**
popPTD	-1.20042	0.66215	-1.813	0.07
popSS	-2.75773	0.67903	-4.061	**0.00005**
popSCN	-4.37605	0.59236	-7.388	**< 0.000001**
popBHH	-5.30652	0.82034	-6.469	**< 0.000001**
popBHB	-4.81934	0.76727	-6.281	**< 0.000001**
popBC	-7.07864	0.77151	-9.175	**< 0.000001**
popVI	-7.0642	0.81669	-8.65	**< 0.000001**
sexM	-0.95181	0.14878	-6.397	**< 0.000001**
temp	-2.81786	0.21285	-13.239	**< 0.000001**
popSD:sexM	0.04664	0.1801	0.259	0.8
popLJS:sexM	0.80277	0.19612	4.093	**0.00004**
popFLR1:sexM	0.79705	0.25427	3.135	**0.0017**
popPTD:sexM	0.11908	0.23726	0.502	0.62
popSS:sexM	-0.16512	0.24155	-0.684	0.49
popSCN:sexM	-0.38681	0.22448	-1.723	0.085
popBHH:sexM	-0.64907	0.29469	-2.203	**0.028**
popBHB:sexM	-0.51631	0.263	-1.963	**0.05**
popBC:sexM	0.84464	0.23617	3.576	**0.00035**
popVI:sexM	0.29983	0.31018	0.967	0.33

Model incorporates population, sex, temperature, and sex*temperature as fixed effects, with source and batch as random effects. AIC value is 3527, Log Likelihood -1738, and deviance 3477 for this model. Model is based upon 1190 observations with 184 batches and 126 sources.