

# INTERPOPULATION HYBRID BREAKDOWN MAPS TO THE MITOCHONDRIAL GENOME

Christopher K. Ellison<sup>1,2</sup> and Ronald S. Burton<sup>1</sup>

<sup>1</sup>Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093

<sup>2</sup>E-mail: cellison@ucsd.edu

Received August 13, 2007

Accepted November 7, 2007

Hybrid breakdown, or outbreeding depression, is the loss of fitness observed in crosses between genetically divergent populations. The role of maternally inherited mitochondrial genomes in hybrid breakdown has not been widely examined. Using laboratory crosses of the marine copepod *Tigriopus californicus*, we report that the low fitness of F<sub>3</sub> hybrids is completely restored in the offspring of maternal backcrosses, where parental mitochondrial and nuclear genomic combinations are reassembled. Paternal backcrosses, which result in mismatched mitochondrial and nuclear genomes, fail to restore hybrid fitness. These results suggest that fitness loss in *T. californicus* hybrids is completely attributable to nuclear–mitochondrial genomic interactions. Analyses of ATP synthetic capacity in isolated mitochondria from hybrid and backcross animals found that reduced ATP synthesis in hybrids was also largely restored in backcrosses, again with maternal backcrosses outperforming paternal backcrosses. The strong fitness consequences of nuclear–mitochondrial interactions have important, and often overlooked, implications for evolutionary and conservation biology.

**KEY WORDS:** Copepod, intergenomic coadaptation, intrinsic postzygotic isolation, mitochondrial function, nuclear–mitochondrial epistasis.

Evolution in isolated populations can result in genetic differentiation that negatively impacts the fitness of interpopulation hybrids. Although first generation hybrids are frequently characterized by hybrid vigor, later generations often suffer dramatically reduced fitness (Dobzhansky 1970; Armbruster et al. 1997; Galloway and Fenster 1999; Hall and Willis 2005). This pattern of F<sub>2</sub> hybrid breakdown can be regarded as an early stage of isolation between species and is most commonly attributed to negative interactions between loci known as Dobzhansky–Muller incompatibilities (Dobzhansky 1936; Muller 1942; Coyne and Orr 2004). Although several genes involved in Dobzhansky–Muller incompatibilities have been identified via genetic mapping approaches (Ting et al. 1998; Barbash et al. 2003; Presgraves et al. 2003; Brideau et al. 2006), the specific interacting loci and physiological processes driving hybrid breakdown remain largely unexplained.

To date, most genetic analyses of hybrid incompatibility have focused on interactions between nuclear genes. However,

an increasing number of studies have suggested that nuclear–mitochondrial gene interactions may be particularly susceptible to disruption by hybridization (Breeuwer and Werren 1995; Edmands and Burton 1999; Sackton et al. 2003; Perrot-Minnot et al. 2004; Zeyl et al. 2005). In fact, the often rapid rate of mitochondrial DNA (mtDNA) evolution in animals combined with the essential function of nuclear–mitochondrial gene interactions in cellular energy metabolism may predispose this system to dysfunction in hybrids, where the mitochondrial genome interacts with nuclear genes derived from a different population. Rand and colleagues (2004) have suggested a model of nuclear–mitochondrial interaction in which the accumulation of deleterious mutations in mtDNA within a population is tolerated due to compensatory mutations in interacting nuclear genes. Under this model, F<sub>1</sub> hybrids contain a full haploid complement of nuclear genes coadapted to the mtDNA and suffer no loss of fitness, whereas recombination yields F<sub>2</sub> and later hybrids lacking the

full set of compensatory mutations and, consequently, these later generation hybrids exhibit reduced fitness.

Populations of the marine copepod *Tigriopus californicus* exhibit high levels of mtDNA sequence divergence but retain the ability to produce viable hybrid offspring (Burton 1986; Edmands 1999). Laboratory crosses between *T. californicus* populations show a consistent pattern of F<sub>1</sub> hybrid vigor and F<sub>2</sub> hybrid breakdown for many metrics of physiological performance and fitness (Burton 1986, 1990; Edmands 1999; Burton et al. 2006). We hypothesize that the pervasive nature of hybrid breakdown in *T. californicus* may result from the dysfunction of some fundamental cellular process resulting in broad pleiotropic effects. As discussed above, the interaction between nuclear and mitochondrial genomes that underlies mitochondrial energy production (Burton et al. 2006) is an intriguing candidate system for such dysfunction in hybrids. The mitochondrial genome of *T. californicus* has been sequenced (Burton et al. 2007) and, like most animal mtDNA, encodes 13 polypeptides, all of which are subunits of the enzyme complexes comprising the mitochondrial electron transport system (ETS). ETS activity requires functional interactions between each of the mitochondrial-encoded components and multiple nuclear-encoded ETS subunits. Specific functional interactions between nuclear-encoded cytochrome *c* and cytochrome *c* oxidase (ETS Complex IV) have demonstrated that *T. californicus* populations harbor coadapted sets of nuclear and mitochondrial loci and that mismatches in hybrids can lead to disruption of physiological processes and reduced fitness (Rawson and Burton 2002; Harrison and Burton 2006). At the organellar level, mitochondrial energy production is reduced in *T. californicus* interpopulation hybrids, as is the activity of those ETS enzyme complexes composed of both nuclear and mitochondrial subunits (Ellison and Burton 2006). Together, these studies suggest that mitochondrial energy production plays an important role in hybrid breakdown, although it has been difficult to assess the quantitative contribution of nuclear-mitochondrial genomic interactions to reduced hybrid fitness.

Here we report the first direct test of the hypothesis that disruption of nuclear-mitochondrial gene interactions can account for the reduced fitness of interpopulation hybrids. The experimental strategy is simple: hybrids with low fitness are backcrossed to both maternal and paternal parental lines. Because mtDNA is maternally inherited, reciprocal backcrosses can be used to generate hybrids that, in the absence of strong selection, have identical average nuclear gene composition, but different mtDNA. We hypothesize that wild-type fitness requires the mtDNA and, at minimum, a full haploid nuclear genome from the same population. This hypothesis is rejected if both reciprocal backcrosses, or neither, result in the recovery of wild-type fitness and gains support if fitness recovery is observed only in the maternal backcross. Using this approach, we can quantitatively assess the role of nuclear-mitochondrial interactions in interpopulation hybrid breakdown.

## Methods

### TIGRIOPUS CULTURE CONDITIONS

*Tigriopus californicus* populations were sampled at three locations: Santa Cruz, California, USA (SCN: 36°57'N, 123°03'W, collected April 2006), Abalone Cove, Palos Verdes Peninsula, California, USA (AB: 33°44'N, 118°22'W, collected May 2006), and San Diego, California, USA (SD: 32°45'N, 117°15'W collected June 2006). Stock cultures of each population were kept in beakers containing 200 mL seawater at 20°C and fed dried *Spirulina* algae. All experimental crosses were completed in 100 mm diameter petri dishes containing 0.1 mg ground *Spirulina* per liter filtered seawater. Animals were transferred to fresh dishes with each generation.

Mature male *T. californicus* clasp virgin females with their antennae until the females are reproductively mature; each female mates only once. Virgin females were separated from clasped males using a fine needle, then mated with males from the required population. Six interpopulation crosses were undertaken comprising all pairwise comparisons of the three populations listed above and their reciprocal crosses. For generations beginning with F<sub>1</sub> hybrids, pairs were removed from culture and crossed with individuals in a replicate petri dish containing the same cross and same generation. All crosses were sufficiently replicated to employ a noninbreeding strategy through the F<sub>3</sub> generation. Backcrosses were performed by crossing virgin F<sub>3</sub> hybrid females to males from either the original maternal or paternal population.

### LIFE HISTORY AND MITOCHONDRIAL MEASUREMENTS

For each generation in this study (parental controls, F<sub>1</sub> hybrids, F<sub>2</sub> hybrids, F<sub>3</sub> hybrids, maternal backcrosses, and paternal backcrosses), fecundity, survivorship, and metamorphosis data were collected. Clasped pairs were placed in individual petri dishes and the male was removed from the dish once the pair separated. Fecundity was measured as the number of nauplii hatching from a female's first eggsac. These nauplii were transferred to a new dish and observed again after 14 days; both the number of surviving individuals and the number of copepodids (juveniles) were counted. Survivorship was calculated as the fraction of first clutch nauplii surviving to 14 days and metamorphosis fraction was calculated as the percentage of first clutch nauplii that had developed into copepodids after 14 days. All data were collected for 10 replicate clutches for each cross at each generation. Animals were cultured under common garden conditions at 20°C.

ATP production by isolated mitochondria was determined for each of the clutches according to the protocol of Ellison and Burton (2006). Briefly, all adult individuals from a clutch were pooled 18 to 24 days after hatching. Intact, functional mitochondria were extracted from these pooled animals and used to measure rate of ATP production in a 5-min end point assay with exogenous

ADP, pyruvate, and malate substrates (compared to a blank with no added substrate). Data were normalized to protein content in the mitochondrial preparation.

**STATISTICAL ANALYSIS**

All statistical analyses were completed using the SPSS 11.0 statistics package. One-way analysis of variance (ANOVA) was used for each comparison with a post hoc Bonferroni test using an alpha value of 0.05 for five comparisons within each of the six interpopulation hybrid classes for fecundity, survivorship, metamorphosis fraction, and ATP production rate data: maternal population backcross versus F<sub>3</sub> hybrid, paternal population backcross versus F<sub>3</sub> hybrid, maternal population backcross versus paternal population backcross, maternal population backcross versus maternal population, and paternal population backcross versus maternal population. Comparisons of backcrosses versus midparent values were calculated, but were not qualitatively different than the maternal

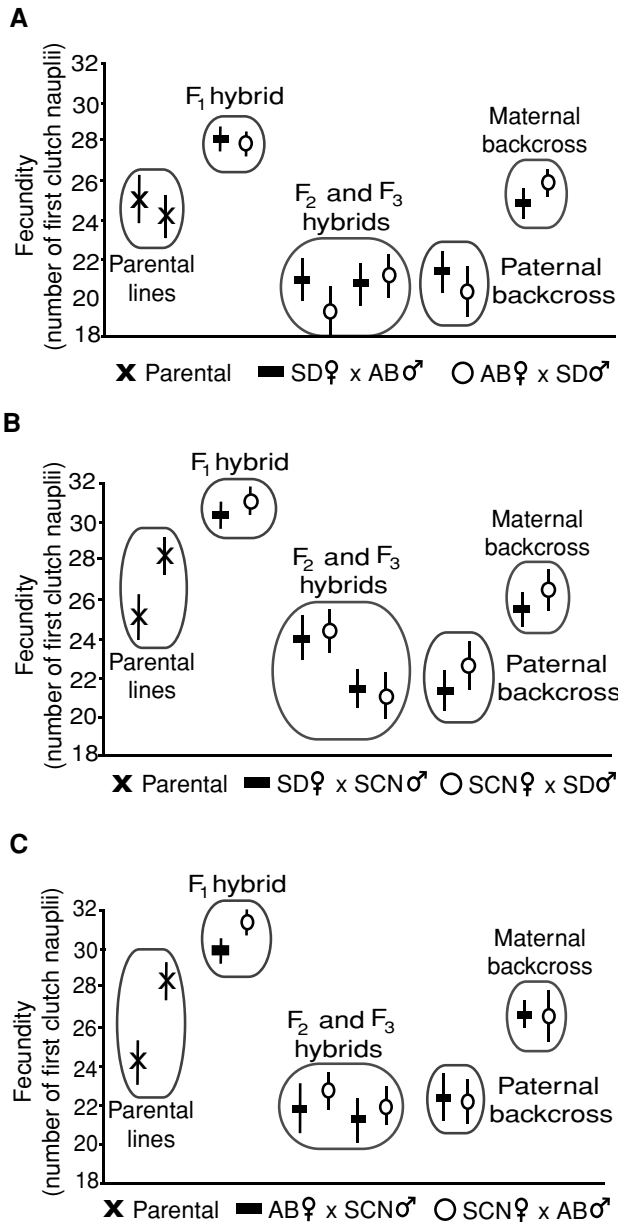
population comparisons (Table 1) and are not reported. All measures were analyzed with the units described above. *n* = 10 for each category of data (i.e., 10 replicates of each measure for each cross in every generation). Equality of variance was tested using Levene’s test of homogeneity of variances; no significant deviations were found (alpha = 0.05) Normal distribution of data was tested using a Kolmogorov–Smirnov test for goodness of fit with Gaussian parameters; no significant deviations from normality were found (alpha = 0.05).

*Results*

All six pairwise crosses were initiated among three natural populations of *T. californicus*. Fecundity, survivorship, and metamorphosis rate were recorded for each of these crosses at the F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> generations to determine fitness, then F<sub>3</sub> females were backcrossed to both maternal and paternal parental populations

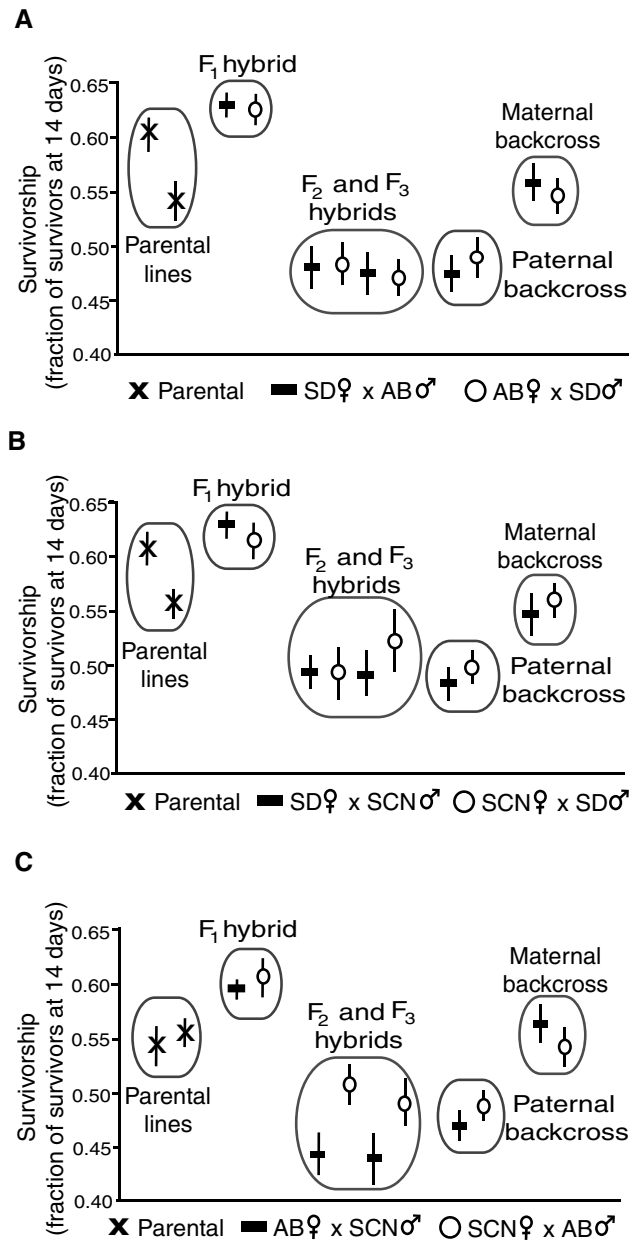
**Table 1.** Comparison of hybrid and hybrid backcross fitnesses and mitochondrial ATP production for six interpopulation crosses. Significance values are given for ANOVA addressing five hypotheses: (1) does the maternal backcross outperform hybrid animals? (F<sub>3</sub> hybrids vs. maternal backcross, Bm), (2) does the paternal backcross outperform hybrid animals? (F<sub>3</sub> hybrids vs. paternal back cross, Bp), (3) do maternal and paternal backcrosses result in differential fitnesses? (Bm vs. Bp), (4) is fitness recovered to the level of the maternal parent in the hybrid maternal backcrosses? (maternal backcross, Bm, vs. maternal parent P), and (5) is fitness recovered to the level of the maternal parent in the hybrid paternal backcross? (paternal backcross, Bp, vs. maternal parent, P). *n*=10 for each category of data. An asterisk denotes significance at alpha=0.05

Maternal parent:	SD	AB	SD	SCN	AB	SCN
Paternal parent:	AB	SD	SCN	SD	SCN	AB
<b>Fecundity</b>						
F <sub>3</sub> vs. Bm	0.0346*	0.0194*	0.0757	0.0124*	0.0054*	0.0375*
F <sub>3</sub> vs. Bp	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Bm vs. Bp	0.0281*	0.0044*	0.0638	0.1482	0.0474*	0.0016*
Bm vs. P	1.0000	1.0000	1.0000	1.0000	0.6164	1.0000
Bp vs. P	0.0797	0.0909	0.0757	0.0050*	1.0000	0.0023*
<b>Survivorship</b>						
F <sub>3</sub> vs. Bm	0.0081*	0.0148*	0.2018	1.0000	<0.0001*	0.5759
F <sub>3</sub> vs. Bp	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
Bm vs. Bp	0.0074*	0.0188*	0.0627	0.5296	0.0020*	0.1123
Bm vs. P	0.2282	1.0000	0.0820	1.0000	1.0000	1.0000
Bp vs. P	<0.0001*	0.1473	<0.0001*	0.5849	0.0168*	0.0270*
<b>Metamorphosis</b>						
F <sub>3</sub> vs. Bm	0.1408*	0.0824	0.6661	0.7253	<0.0001*	0.2961
F <sub>3</sub> vs. Bp	1.0000	1.0000	1.0000	1.0000	0.2260	1.0000
Bm vs. Bp	0.2255	0.1961	0.3702	0.8992	0.0361*	0.4835
Bm vs. P	1.0000	1.0000	1.0000	1.0000	0.9833	1.0000
Bp vs. P	0.248	1.0000	0.5137	1.0000	0.8572	0.6036
<b>ATP production</b>						
F <sub>3</sub> vs. Bm	0.0167*	0.0137*	0.0006*	0.1533	0.0002*	0.5677
F <sub>3</sub> vs. Bp	1.0000	1.0000	0.0721	1.0000	0.5031	1.0000
Bm vs. Bp	0.3035	0.2242	0.5581	1.0000	0.2086	1.0000
Bm vs. P	0.0521	1.0000	0.1717	0.0050*	0.3595	0.0001*
Bp vs. P	0.0002*	0.0401*	0.0018*	0.0002*	0.0012*	<0.0001*



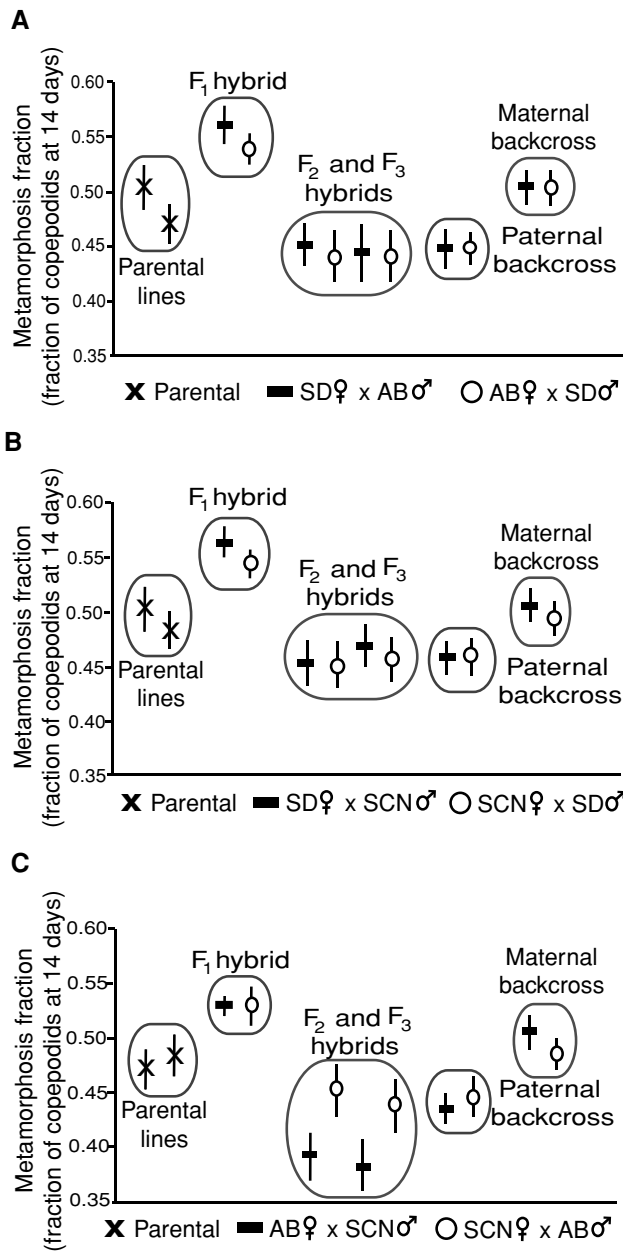
**Figure 1.** Maternal backcrossing rescues fecundity. Fecundity (mean  $\pm$  1 SEM) for parental lineages, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> hybrids, and maternal and paternal backcrosses for three interpopulation hybridizations and their reciprocals. Panel A = SD and AB hybrids, panel B = SD and SCN hybrids, panel C = AB and SCN hybrids.  $n = 10$  for each point.

and fitness measures were repeated. In all six cases, fitnesses of F<sub>1</sub> offspring were equal to or greater than that of either parental lineage. In contrast, fitnesses declined significantly in the F<sub>2</sub> generation and showed no recovery in the F<sub>3</sub> generation (Figs. 1–3). *T. californicus* have 12 chromosomes and lack recombination in females (Ar-Rushdi 1963; Burton et al. 1981), so the combined effect of independent assortment and recombination effectively ensured that none of the F<sub>3</sub> offspring would have a full haploid



**Figure 2.** Maternal backcrossing rescues survivorship. Survivorship fractions (mean  $\pm$  1 SEM) for parental lineages, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> hybrids, and maternal and paternal backcrosses for three interpopulation hybridizations and their reciprocals. Panel A = SD and AB hybrids, panel B = SD and SCN hybrids, panel C = AB and SCN hybrids.  $n = 10$  for each point.

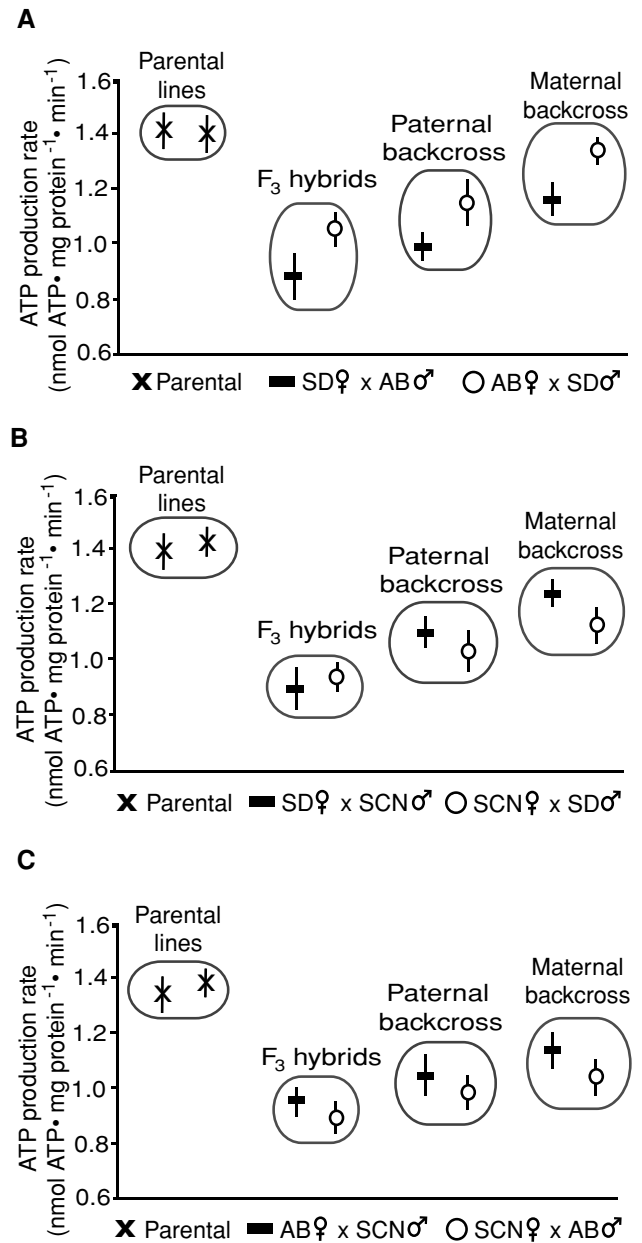
complement of alleles from either parental population. Hybrid females (F<sub>3</sub>) were then backcrossed to males of either the maternal or the paternal lineage. The pattern of fitness recovery in the backcrossed animals was striking. Although no fitness recovery was observed in any of the six paternal backcrosses, maternal backcrosses resulted in nearly complete recovery to maternal parental fitness in every case (Table 1, Figs. 1–3). The differential effect of paternal versus maternal backcross was significant in all six



**Figure 3.** Maternal backcrossing rescues metamorphosis rate. Metamorphosis fraction (mean  $\pm$  1 SEM) for parental lineages, F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> hybrids, and maternal and paternal backcrosses for three interpopulation hybridizations and their reciprocals. Panel A = SD and AB hybrids, panel B = SD and SCN hybrids, panel C = AB and SCN hybrids. *n* = 10 for each point.

crosses for the fecundity measure and in 11 of 18 cases across all fitness measures for all crosses (three fitness measures for six crosses); maternal backcross hybrids always outperformed paternal backcross hybrids (Figs. 1–3).

As a measure of overall mitochondrial function, we assayed the rate of mitochondrial ATP production in intact mitochondria isolated from parental lineages, F<sub>3</sub> hybrids, and maternal and pa-



**Figure 4.** Mitochondrial ATP production rate is partially rescued in all backcrosses. Mitochondrial ATP production rate (mean  $\pm$  1 SEM) for parental lineages, F<sub>3</sub> hybrids, and maternal and paternal backcrosses for three interpopulation hybridizations and their reciprocals. Panel A = SD and AB hybrids, panel B = SD and SCN hybrids, panel C = AB and SCN hybrids. *n* = 10 for each point.

ternal backcrosses. Consistent with previous results (Ellison and Burton 2006), mitochondrial ATP production in F<sub>3</sub> hybrids was lower than that of the parental lineages. Rates of ATP production by mitochondria isolated from paternal backcrosses tended to be slightly higher, but not significantly different, than F<sub>3</sub> hybrids, whereas mitochondrial ATP production rate in maternal backcrosses increased significantly relative to F<sub>3</sub> hybrids in four of six crosses (Fig. 4). Although the increased rate observed in maternal

backcrosses was generally greater in magnitude than that in paternal backcrosses, this difference was not significant (Table 1), likely due to the partial recovery of mitochondrial ATP production rate in paternal backcross animals (Fig. 4). Importantly, although both fitness and mitochondrial ATP synthetic capacity recovered in maternal backcross hybrids, they show slightly different patterns of recovery.

## Discussion

Comparisons of reciprocal crosses show that fitness recovery in *T. californicus* hybrids was not achieved by restoring a full nuclear genetic complement alone. The backcross experimental design generates four unique nuclear and mitochondrial genotypes. Reciprocal F<sub>3</sub> hybrid pairs have, on average, identical nuclear genetic composition prior to being backcrossed, but different maternally derived mtDNA. Hence, the maternal backcross (hybrid female × maternal population male) offspring of one interpopulation hybrid has, on average, the same nuclear genetic composition as the paternal backcross (hybrid female × paternal population male) offspring of the reciprocal interpopulation hybrid. The genetic differences between the maternal and paternal backcrosses are then restricted to cytoplasmic genetic elements such as the mtDNA. Two caveats with this design are: (1) although the nuclear genetic composition of reciprocals is expected to be identical; any strong selection in the F<sub>1</sub> and F<sub>2</sub> generations may result in deviations from this expectation, and (2) although identical in expected genetic composition, gene expression in hybrids may be biased (Landry et al. 2007; Ortíz-Barrientos et al. 2007). Despite identical nuclear genetic composition, only the maternal backcross offspring were found to have wild-type fitness. Remarkably, fitness recovery was essentially complete in every maternal backcross, whereas fitness recovery was absent in paternal backcrosses. Although previous studies have shown that nuclear–mitochondrial interactions can impact enzyme activities and the relative fitnesses of different hybrid genotypes, the results presented here are the first clear demonstration that nuclear–cytoplasmic interactions play a dominant role in hybrid breakdown in *T. californicus*.

Rates of ATP synthesis in isolated mitochondria were measured to assess how nuclear–cytoplasmic interactions were manifested at the level of cellular physiology. Mitochondria isolated from F<sub>3</sub> hybrids showed significant reduction in ATP biosynthetic capacity relative to parental lineages. Mitochondrial ATP production was partially rescued in maternal backcrosses. However, in contrast to fitness measures, recovery of ATP synthesis was not complete in maternal backcrosses. Also contrasting with the fitness measures, some recovery of ATP synthesis was observed in paternal backcrosses. This pattern of recovery of mitochondrial function is likely complicated by the presence of nuclear–nuclear interactions in addition to nuclear–mitochondrial interactions. The

mitochondrial ETS contains 13 mitochondrial-encoded and over 70 nuclear-encoded polypeptides (Sackton et al. 2003), so it is reasonable to expect that some coadaptation of nuclear-encoded subunits may evolve within populations. A recent study of ETS complex III interactions in *T. californicus* hybrids, found evidence for nuclear–nuclear, but not nuclear–mitochondrial, interactions among three components of complex III (Willett 2006). Although our data strongly suggest that nuclear–mitochondrial interactions are the primary component of interpopulation hybrid breakdown in fitness, it seems likely that interpopulation hybridization also results in disruption of nuclear–nuclear interactions within the ETS.

Maternal backcross hybrids show recovery of mitochondrial ETS function and concomitant recovery of fitness. ATP production capacity might reasonably be expected to have beneficial effects on fitness, and previous work (Burton et al. 2006; Ellison and Burton 2006) has suggested that mitochondrial function may, in fact, be positively correlated with fitness. These studies report such findings in the context of inbred hybrid lines however, and the high homozygosity harbored in such lines may exaggerate fitness effects. In this study, partial recovery of mitochondrial function in paternal backcross hybrids without a corresponding recovery of fitness suggests that there is little correlation between mitochondrial ATP production rate and organismal fitness. This may be a consequence of statistical power. Maternal backcrosses were found to have fecundities approximately 20% greater than those of either F<sub>2</sub> and F<sub>3</sub> hybrids or paternal backcross hybrids across six crosses. Although the fitness and ATP production rate recovery of maternal backcross hybrids was far greater than that of paternal backcross hybrids, the observed variance of our fitness measures far exceeded that of our measurements of ATP production in isolated mitochondria. Consequently, the resolution afforded by this study may have been unable to detect a partial recovery of fitness on the order of 5% to 10%, corresponding to the partial recovery of mitochondrial ATP production rate in paternal backcross hybrids. Thus, apparent differences of recovery patterns in fitness and mitochondrial ATP production do not necessarily preclude a functional relationship between these parameters.

Alternatively, differences in patterns of fitness recovery and recovery of mitochondrial ATP biosynthetic capacity in backcrosses may reflect disruption of other aspects of mitochondrial function. Mitochondria are rightfully regarded as the powerhouses of the cell due to their central role in cellular energy production in the form of ATP. However, the performance of alternative mitochondrial functions may also contribute to hybrid fitness. At the organellar level, mitochondria perform DNA replication, transcription, and translation and are involved in lipid metabolism in addition to the production of ATP (Scheffler 1999). Further, the function of mitochondria in the cell is known to extend well beyond such metabolic and biosynthetic capacities to include metabolic

regulation (Das 2006), apoptosis (Newmeyer and Ferguson-Miller 2003), antiviral response mechanisms (Seth et al. 2005), and control of development and the cell cycle (Mandal et al. 2005). Although intuition may suggest that interaction between nuclear and mitochondrial genomes must necessarily involve protein-coding regions, and thus the mitochondrial ETS, the multifarious functions of the mitochondria suggest that impacts on other processes are also probable.

The evidence presented here strongly suggests that the interaction of nuclear and mitochondrial genomes from distinct populations may have serious consequences for mitochondrial function and fitness of hybrids. Although previous studies have found evidence of mitochondrial dysfunction in interspecific nucleocytoplasmic hybrids of both mammals (Schmidt et al. 2001) and amphibians (Liepins and Hennen 1977), here we found that similar processes may act at the level of conspecific populations, as well. This intraspecific break is a consequence of extremely high mitochondrial divergence among *T. californicus* populations. It must be noted, however, that high levels of mitochondrial DNA divergence have been reported in a growing number of taxa, including both invertebrates (e.g., the snail *Cepaea nemoralis*, Thomaz et al. 1996) and vertebrates (e.g., the freshwater fish *Galaxias maculatus*, Waters and Burrige (1999), and the green python, *Morelia vidis* (Rawlings and Donnellan 2003)). Our results have important implications for conservation efforts in which anthropogenic enhancement of depleted populations might introduce nuclear and/or mitochondrial genomes that are incompatible with resident genomes. Although the dilemma posed by inbreeding depression is often a design consideration for conservation management plans, the parallel danger of outbreeding depression is less frequently an explicit concern (Edmands 2007). Our results demonstrate not only that such oversight could have marked consequences for the long-term success of interpopulation hybrid individuals, but also that the role of the mitochondrial genome, often overlooked with regard to outbreeding depression, cannot be ignored.

The search for “speciation” or “barrier” genes has undergone a recent resurgence (see Noor and Feder 2006 for a review of several loci associated with reproductive incompatibility). However, pinpointing the ultimate origin of reproductive isolation between species can be a difficult undertaking, as many barriers have arisen between most species pairs and those most readily mapped may not represent the original causal barriers delineating species (Coyne and Orr 2004). Interpopulation hybrid breakdown represents an intermediate point between reproductively isolated biological species and a single, panmictic population. As a result, species with populations exhibiting hybrid breakdown, such as *T. californicus*, are uniquely suited to examine not only the process of species formation, but also the underlying mechanisms that drive it. Our data demonstrate that there is a significant cytoplasmic

contribution to hybrid breakdown in *T. californicus* and that possession of a complete haploid nuclear genome and the mitochondrial genome from the same parent is sufficient to restore fitness in hybrids. Interestingly, the pattern of recovery of mitochondrial ATP production is not fully congruent with that of fitness, possibly due to the presence of nuclear–nuclear interactions or the action of alternative mitochondrial functions. We conclude that interactions between nuclear and mitochondrial genomes represent an important, and often under appreciated, component of hybrid breakdown and species formation with potential effects in a variety of applications.

#### ACKNOWLEDGMENTS

We thank M.E. Hellberg, B. Palenik, C.S. Willett, D. Rand, and one anonymous reviewer for helpful comments and A. Ho and S. Hsu for *T. californicus* culture assistance. This research was supported in part by grants from the National Science Foundation to RSB (DEB-0236363) and a National Institutes of Health graduate training grant to CKE.

#### LITERATURE CITED

- Armbruster, P., W. E. Bradshaw, and C. M. Holzapfel. 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. *Evolution* 51:451–458.
- Ar-Rushdi, A. 1963. The cytology of achiasmatic meiosis in the female *Tigriopus* (copepoda). *Chromosoma* 13:526–539.
- Barbash, D. A., D. F. Siino, A. M. Tarone, and J. Roote. 2003. A rapidly evolving MYB-related protein causes species isolation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 100:5302–5307.
- Breeuwer, A. J., and J. H. Werren. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution* 49:705–717.
- Brideau, N. J., H. A. Flores, J. Wang, S. Maheshwari, X. Wang, and D. A. Barbash. 2006. Two Dobzhansky-Muller genes interact to cause hybrid lethality in *Drosophila*. *Science* 314:1292–1295.
- Burton, R. S. 1986. Evolutionary consequences of restricted gene flow in the intertidal copepod *Tigriopus californicus*. *Bull. Mar. Sci.* 39:526–535
- . 1990. Hybrid breakdown in developmental time in the copepod *Tigriopus californicus*. *Evolution* 44:1814–1822.
- Burton, R. S., M. W. Feldman, and S. G. Swisher. 1981. Linkage relationships among five enzyme-coding loci in the copepod *Tigriopus californicus*: a genetic confirmation of achiasmatic meiosis. *Biochem. Genet.* 10:1237–1245.
- Burton, R. S., C. K. Ellison, and J. S. Harrison. 2006. The sorry state of F<sub>2</sub> hybrids: consequences of rapid mitochondrial DNA evolution in allopatric populations. *Am. Nat.* 168:S14–S24.
- Burton, R. S., R. J. Byrne, and P. D. Rawson. 2007. Three divergent mitochondrial genomes from California populations of the copepod *Tigriopus californicus*. *Gene* 403:53–59.
- Coyne, J., and H. A. Orr. 2004. *Speciation*. Sinauer Associates, Inc. Sunderland, MA.
- Das, J. 2006. The role of mitochondrial respiration in physiological and evolutionary adaptation. *BioEssays* 28:890–901.
- Dobzhansky, T. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* 21:113–135.
- . 1970. *Genetics of the evolutionary process*. Columbia University Press, New York, NY.
- Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53:1757–1765.

- . 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* 16:463–475.
- Edmunds, S., and R. S. Burton. 1999. Cytochrome c oxidase activity in interpopulation hybrids of a marine copepod: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. *Evolution* 53:1972–1978.
- Ellison, C. K., and R. S. Burton. 2006. Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution* 60:1382–302.
- Galloway, L. F., and C. B. Fenster. 1999. The role of nuclear and cytoplasmic factors in the adaptive evolution of populations of *Chamaecrista fasciculata* (Fabaceae). *Evolution* 53:1734–1743.
- Hall, M. C., and J. H. Willis. 2005. Transmission ratio distortion in intraspecific hybrids of *Mimulus guttatus*. *Genetics* 170:375–386.
- Harrison, J. S., and R. S. Burton. 2006. Tracing hybrid incompatibilities to single amino acid substitutions. *Mol. Biol. Evol.* 23:559–564.
- Landry, C. R., D. L. Hartl, and J. M. Ranz. 2007. Genome clashes in hybrids: insights from gene expression. *Heredity* 99:483–493.
- Liepins, A., and S. Hennen. 1977. Cytochrome oxidase deficiency during development of amphibian nucleocytoplasmic hybrids. *Dev. Biol.* 57:284–292.
- Mandal, S., P. Guptan, E. Owusu-Ansah, and U. Banerjee. 2005. Mitochondrial regulation of cell cycle progression during development as revealed by the *tenured* mutation in *Drosophila*. *Dev. Cell* 9:843–854.
- Muller, H. J. 1942. Isolating mechanisms, evolution and temperature. *Biol. Symp.* 6:71–125.
- Newmeyer, D. D., and S. Ferguson-Miller. 2003. Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* 112:481–490.
- Noor, M. A. F., and J. L. Feder. 2006. Speciation genetics: evolving approaches. *Nat. Rev. Genet.* 7:851–861.
- Ortiz-Barrientos, D., B. A. Counterman, and M. A. F. Noor. 2007. Gene expression divergence and the origin of hybrid dysfunctions. *Genetica* 129:71–81.
- Perrot-Minnot, M.-J., A. Migeon, and M. Navajas. 2004. Intergenomic interactions affect female reproduction: evidence from introgression and inbreeding depression in a haplodiploid mite. *Heredity* 93:551–558.
- Presgraves, D. C., L. Balagopalan, S. M. Abmayr, and H. A. Orr. 2003. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. *Nature* 423:715–719.
- Rand, D. M., R. A. Haney, and A. J. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol. Evol.* 19:645–653.
- Rawlings, L. H., and S. C. Donnellan. 2003. Phylogeographic analysis of the green python, *Morelia viridis*, reveals cryptic diversity. *Mol. Phylogenet. Evol.* 27:36–44.
- Rawson, P. A., and R. S. Burton. 2002. Functional coadaptation between cytochrome c and cytochrome c oxidase withing allopatric populations of a marine copepod. *Proc. Natl. Acad. Sci. USA* 99:12955–12958.
- Sackton, T. B., R. A. Haney, and D. M. Rand. 2003. Cytonuclear coadaptation in *Drosophila*: disruption of cytochrome c oxidase activity in backcross genotypes. *Evolution* 57:2315–2325.
- Scheffler, I. E. 1999. *Mitochondria*. John Wiley & Sons, New York, NY.
- Schmidt, T. R., W. Wu, M. Goodman, and L. I. Grossman. 2001. Evolution of nuclear- and mitochondrial-encoded subunit interaction in cytochrome c oxidase. *Mol. Biol. Evol.* 18:563–569.
- Seth, R. B., L. Sun, C.-K. Ea, and Z. J. Chen. 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappa B and IRF3. *Cell* 122:669–682.
- Ting, C.-T., S.-T. Tsaur, M.-L. Wu, and C.-I. Wu. 1998. A rapidly evolving homeobox at the site of a hybrid sterility gene. *Science* 282:1501–1504.
- Thomaz, D., A. Guiller, and B. Clarke. 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. *Proc. R. Soc. Lond. B* 363–368.
- Waters, J. M., and C. P. Burrige. 1999. Extreme intraspecific mitochondrial DNA sequence divergence in *Galaxias maculatus* (Osteichthys: Galaxiidae), one of the world's most widespread freshwater fish. *Mol. Phylogenet. Evol.* 11:1–12.
- Willett, C. S. 2006. Deleterious epistatic interactions between electron transport system protein-coding loci in the copepod *Tigriopus californicus*. *Genetics* 173:1465–1477.
- Zeyl, C., B. Andreson, and E. Weninck. 2005. Nuclear-mitochondrial epistasis for fitness in *Saccharomyces cerevisiae*. *Evolution* 59:910–914.

Associate Editor: O. McMillan