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CYTOCHROME *C* OXIDASE ACTIVITY IN INTERPOPULATION HYBRIDS OF A MARINE COPEPOD: A TEST FOR NUCLEAR-NUCLEAR OR NUCLEAR-CYTOPLASMIC COADAPTATION

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Abstract.—The respiratory enzyme cytochrome *c* oxidase (COX) is composed of subunits encoded by both nuclear and mitochondrial genes; thus, COX activity reflects, to some extent, the coordinated function of the two genomes. Because extensive mtDNA differentiation exists between populations of the copepod *Tigriopus californicus*, we hypothesized that laboratory hybridizations that disrupt natural combinations of nuclear and mitochondrial genes might negatively impact COX activity. Although experimental results varied greatly among different crosses, replicate sets of crosses between two particular populations showed consistent evidence for nuclear-cytoplasmic coadaptation.

Key words.—Coadaptation, copepod, cytochrome *c* oxidase, enzyme activity, introgression, nuclear-cytoplasmic interactions, *Tigriopus californicus*.

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Several proteins functioning in the mitochondrial electron-transport chain are composed of subunits encoded by both nuclear and mitochondrial genomes. Proper function of such proteins presumably requires regulatory and functional coordination among subunits and may therefore result in selection favoring intergenomic coadaptation (Attardi and Schatz 1988). The enzyme cytochrome *c* oxidase (COX), which catalyzes the final step in the electron transport chain, is an example of such a protein. In eukaryotes, COX is composed of three subunits encoded by mitochondrial DNA (mtDNA) and numerous subunits (10 in mammals; Capaldi 1996) encoded by nuclear DNA. A number of lines of evidence suggest that coadaptation has occurred between nuclear and mitochondrial subunits and/or between the COX subunits and cytochrome *c* (nuclear encoded). For example, Liepens and Hennen (1977) transplanted single nuclei from one frog species into enucleated eggs of a congener and found that normal COX activity required mtDNA plus at least a haploid complement of nuclear DNA from the same species. Work by Osheroff et al. (1983; reviewed by Cann et al. 1984) showed that primate cytochrome *c* reacts better with primate COX than with nonprimate COX. King and Attardi (1989) introduced mitochondria from different human cell lines into a common host cell line depleted of mitochondria and found that the level of COX activity varied with the source of the mitochondria. This latter study was the first to suggest that nuclear/mitochondrial coadaptation may occur within a species, but the experimental analysis was far removed from the intact organism.

In this study, we test for gene interactions affecting COX function by manipulating cytonuclear genotypes via laboratory crosses between conspecific populations. The study system consists of a series of genetically differentiated populations of the marine copepod *Tigriopus californicus*. Both allozyme and DNA studies have shown extensive genetic differentiation among *T. californicus* populations, even over distances as small as 10 km (e.g., Burton and Feldman 1981; Burton 1998). This differentiation appears to be temporally stable (Burton 1997), suggesting that natural populations of this species have long, independent evolutionary histories. Such highly structured populations provide the opportunity for the evolution of coadapted gene complexes (Burton et al. 1999).

When *T. californicus* populations are hybridized in the laboratory, F_1 offspring are typically similar to their parents for a variety of physiological and developmental traits, whereas F_2 offspring show reduced resistance to osmotic stress (Burton 1986, 1990a) and increased development time (Burton 1987, 1990b). These results suggest that populations have evolved different systems of positively interacting genes (e.g., coadapted gene complexes) that are disrupted by segregation and recombination in the F_2 generation. Interpopulation comparisons of both COXI (Burton and Lee 1994) and cytochrome *c* (Burton et al. 1999) show extensive DNA sequence divergence and moderate levels of inferred amino acid divergence. Using a simple spectrophotometric assay, Edmands and Burton (1998) showed that isofemale lines of *T. californicus* cultured under common-garden conditions had significant variation in COX activity levels both within and between populations, indicating a heritable component to COX activity variation. However, reciprocal crosses between lines indicated that patterns of inheritance of COX activity were complex. In interpopulation crosses, F_1 offspring generally had COX activities similar to or higher than the parents, whereas F_2 offspring had enzyme activities below the parents, suggesting that strong nuclear-nuclear or nuclear-mitochondrial interactions affect COX function.

To better understand the source of gene interactions affecting COX function, we have transplanted nuclear genes from one cytoplasmic background to another by hybridization followed by repeatedly backcrossing females to males from

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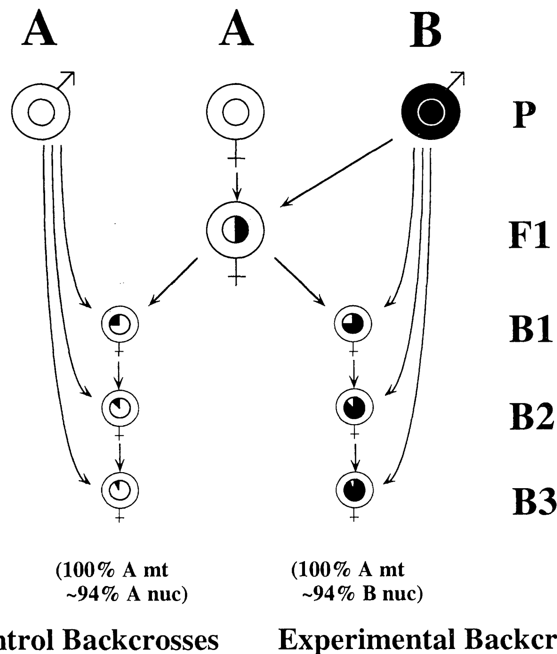

FIG. 1. Crossing scheme used to effectively move nuclear genes from one population onto the mitochondrial background of another population. In this example, population A (white) females were crossed with population B (black) males. Female F_1 hybrids were then repeatedly backcrossed to males from population B, resulting in individuals with population A mitochondria and primarily population B nuclear genes. As a control, F_1 females were repeatedly backcrossed to males from population A, effectively returning population A nuclear genes to their original mitochondrial background. (Figure modified from Breeuwer and Werren 1995.)

the paternal population. In theory, each generation of backcrossing will replace half of the maternal nuclear genome with the paternal nuclear genome (Fig. 1). If nuclear-nuclear interactions predominate, then deviations from expectations based on purely additive gene action should be greatest in the earliest generations of hybridization because animals will lack complete nuclear sets of coadapted alleles from either parental population. In contrast, if nuclear-mitochondrial interactions predominate, then deviations should be greatest in the later generations of hybridization, when the entire nuclear genome has been replaced by a foreign nuclear genome to which the mitochondrial genome is not coadapted. As a control, F_1 hybrid females were also repeatedly backcrossed to males from the maternal population, effectively restoring the original maternal cytonuclear environment. The five populations used in this study are substantially different genetically, with up to 23% sequence divergence for a segment of the mitochondrial COI gene (see Burton and Lee 1994; Burton 1998). Because hybridization among these populations may have consequences for many traits besides COX function, we also estimated total fitness for a subset of the lines.

MATERIALS AND METHODS

Copepod Cultures

Natural populations of *T. californicus* were sampled from high intertidal rock pools in five locations in California: Santa Cruz (SC, 36°57′N, 122°03′W), Abalone Cove (AB, 33°44′N, 118°22′W), Royal Palms (RP, 33°43′N, 118°19′W), Laguna Beach (LB, 33°33′N, 117°47′W), and San Diego (SD, 32°45′N, 117°15′W). Animals were maintained as mass cultures (300 or more adults) in 400-ml beakers. All cultures were kept at 15 ± 2°C with a 12:12 L:D photoperiod. The cross between populations SC and AB appeared to show strong nuclear-mitochondrial interactions. To test for the effects of within-population mitochondrial variation, isofemale/isomitochondrial lines from the AB population were established by isolating single gravid females in petri dishes (15 mm × 100 mm) and inbreeding the resulting offspring which are full-siblings because females mate only once (Burton, 1985). All copepods were maintained in natural seawater supplemented with commercial flake-type fish food.

Backcrosses

Interpopulation crosses followed methods in Burton et al. (1981). Crosses were conducted in petri dishes (15 mm × 100 mm) that were monitored three times per week. For each cross, multiple males and virgin females were united in each dish. When F_1 juveniles (copepodids) were first observed, parents were removed from the dish. To distinguish males from females, offspring were left in the same dish until they formed clasped pairs (adult male + virgin female). The pairs were then dissected apart. For the experimental backcross lines, F_1 virgin females were mass mated to males from the paternal population. For the control backcross lines, F_1 virgin females were mass mated to males from the maternal population. Initially, seven crosses were conducted between outbred populations (SC female × AB male and reciprocal crosses between LB and SD, RP and SD, and SC and SD). The choice of crosses was limited by the number of animals in culture; therefore, all reciprocal crosses could not be done. Because the SC × AB cross showed the strongest evidence for nonadditive gene action for COX activity, we focused on further crosses between these two populations. Five isofemale lines from population AB were backcrossed to the SC population. Isofemale lines were used as the maternal parent in these crosses to restrict the effects of mtDNA variation within populations. However, all backcrosses were done to populations (instead of isofemale lines) to avoid confounding the effects of outbreeding with the effects of progressive inbreeding. Crosses were maintained for two to eight backcross generations. The duration of the cross depended on the number of copepods in culture available for mating. For all crosses, COX activity measurements were taken every generation when possible. In some cases, the lines died out prematurely or there were insufficient numbers of animals to allow individuals to be sacrificed in each generation.

COX Activity Assays

Activity of cytochrome *c* oxidase (EC 1.9.3.1) was measured by a standard spectrophotometric assay based on the oxidation of cytochrome *c* (Simon and Robin 1971). A solution of 400 mg cytochrome *c* (Type VI from horse heart, Sigma Chemical Co., St. Louis, MO) and 2.4 ml phosphate buffer (50 mM NaH_2PO_4, pH 7.1) was reduced with 40 mg ascorbic acid. The ascorbic acid was then removed by dialysis

(Slide-A-Lyzer® cassette, Pierce Chemical Co., Rockford, IL) against phosphate buffer (10°C, overnight, three changes of buffer). The reduced cytochrome *c* was frozen at −20°C and used within two weeks. Adult copepods were sexed (three categories: male, female, and female with egg sac) and rinsed in filtered seawater. Individual copepods were then homogenized in microcentrifuge tubes in 200 μL ice-cold homogenization buffer (freshly mixed phosphate buffer and 0.05% Tween 80 detergent [polyoxyethylene-20-sorbitan monooleate, Fisher Scientific, Pittsburgh, PA]) for approximately 10 sec using a motorized Delrin plastic pestle fitted to 1.5-ml microcentrifuge tubes. After centrifugation for 5 min at 2000 G, two 40 μL aliquots of the supernatant were distributed into each of two 96-well microtitre plates; one plate was then used for the COX assay and the other was used for the total protein assay. For the COX assays, 160 μL of 51 μM reduced cytochrome *c* (calculated using the millimolar extinction coefficient of 29.5 at 550 nm) was added to each well and OD_{550} was measured using a microtitre plate reader (UVmax®, Molecular Devices Corporation, Menlo Park, CA). Plates were incubated at 23 ± 1°C for 30 min and OD_{550} was remeasured. The duration of the endpoint assay and the concentrations of homogenate and substrate were chosen to yield linear changes in absorption over time. For the total protein assays, 160 μL bicinchoninic acid reagent (BCA; Pierce Chemical Co.; Smith et al. 1985) was addd to each well. Plates were incubated overnight at 37°C and OD_{562} was measured. Total protein content was determined from comparisons with bovine serum albumin standards run on each plate.

Fitness Assays

To estimate fitness, females were removed from the mating dishes as soon as they produced their first egg sac. Each female was placed in a petri dish containing 30 ml filtered seawater and excess food (24 mg finely ground Tetramin fish food). After 30 days at 15°C all surviving nauplii (larvae), copepodids (juveniles), and copepods (adults) were counted.

Statistical Analyses

Although enzyme activities are often reported as on a per unit protein basis, this implicitly assumes that a doubling in protein content results in a doubling in enzyme activity. Instead, we used analysis of covariance (SUPERANOVA, Abacus Concepts, Berkeley, CA) to adjust for protein content of individual copepods. Analysis of covariance was also used to factor out the effects of sex and microtitre plate. Cytochrome oxidase activity was the dependent variable, sample type (i.e., isofemale line 1, line 1 × line 2 F_1 hybrid, etc.) was the main effect, and the covariates were sex, protein content, and the interaction between sample and microtitre plate number.

Models of Gene Action

Each generation of backcrossing should, on average, replace half of the maternal nuclear genome with the paternal nuclear genome (Fig. 1). Therefore, after F_1 hybridization and three generations of backcrossing, individuals are expected to carry 100% maternal cytoplasm (including mitochondria) and 93.75% paternal nuclear DNA. These numbers are based on three assumptions (Grun 1976): (1) no selection on maternal versus paternal genes; (2) no change in the cytoplasmic factors during the introgression process; and (3) no contribution of cytoplasm by sperm. Further, because there is no recombination in *T. californicus* females (Ar-rushdi 1963; Burton et al. 1981), introgression of paternal nuclear DNA will involve intact chromosomes. Therefore, the expected percent introgression refers to the average of all individuals in the line.

With no maternal effects and no gene interactions, the F_1 phenotype should equal the midparent value, P, where P is the average of the sire (S) and the dam (D) phenotypes. In the experimental lines, each generation of backcrossing to the paternal line should bring the phenotype halfway closer to the paternal phenotype: $B_x = P + (1 - [1/2]^x)(S - P)$, where x is the backcross generation (F_1 = backcross generation 0). In the control lines, each generation of backcrossing to the maternal line should bring the phenotype halfway closer to the maternal phenotype: $B_x = P + (1 - [1/2]^x)(D - P)$. Deviations from this simple model due to nuclear-cytoplasmic interactions should scale to the proportion of paternal nuclear genes: $1 - (1/2)^{x+1}$ in the experimental lines and $(1/2)^{x+1}$ in the control lines. In contrast, deviations due to nuclear-nuclear interactions should be maximized in the F_1 and should be reduced by half with each generation of backcrossing in both the experimental and control lines: $(1/2)^{x+1}$. We tested the fit to these gene interaction models by regressing deviations from the simple noninteraction model (COX activity − B_x) against either $1 - (1/2)^{x+1}$ or $(1/2)^{x+1}$.

Results

Effects of backcrossing on COX activity varied markedly in crosses between outbred populations (Fig. 2). In the SC female × AB male cross (Fig. 2A), the experimental backcross line had lower COX activity than expected under the additive model, whereas the control cross showed no such deviations. In the RP female × SD male cross (Fig. 2B), there was an initial increase in COX activity followed by a return to expected levels, with no directional change in the control line. In the reciprocal cross (Fig. 2C), there was also little change in the control line, but here there was an erratic decline in COX activity in the experimental line. Reciprocal crosses between LB and SD (Fig. 2D,E) and between SC and SD (Fig. 2F,G) showed no clear trend in COX activity changes over time. In the backcrosses between AB isofemale lines and males from population SC (Fig. 3), all five experimental crosses (each a different mtDNA isolate from the AB population) showed progressive declines in COX activity relative to expectations based on the additive model.

Table 1 shows the extent to which these deviations from expectations under the additive model are explained by nuclear-nuclear or nuclear-cytoplasmic interactions. Deviations from expected levels in the experimental crosses should show a positive correlation with the proportion paternal nuclear genome if nuclear-cytoplasmic interactions predominate and a negative correlation if nuclear-nuclear interactions predom-

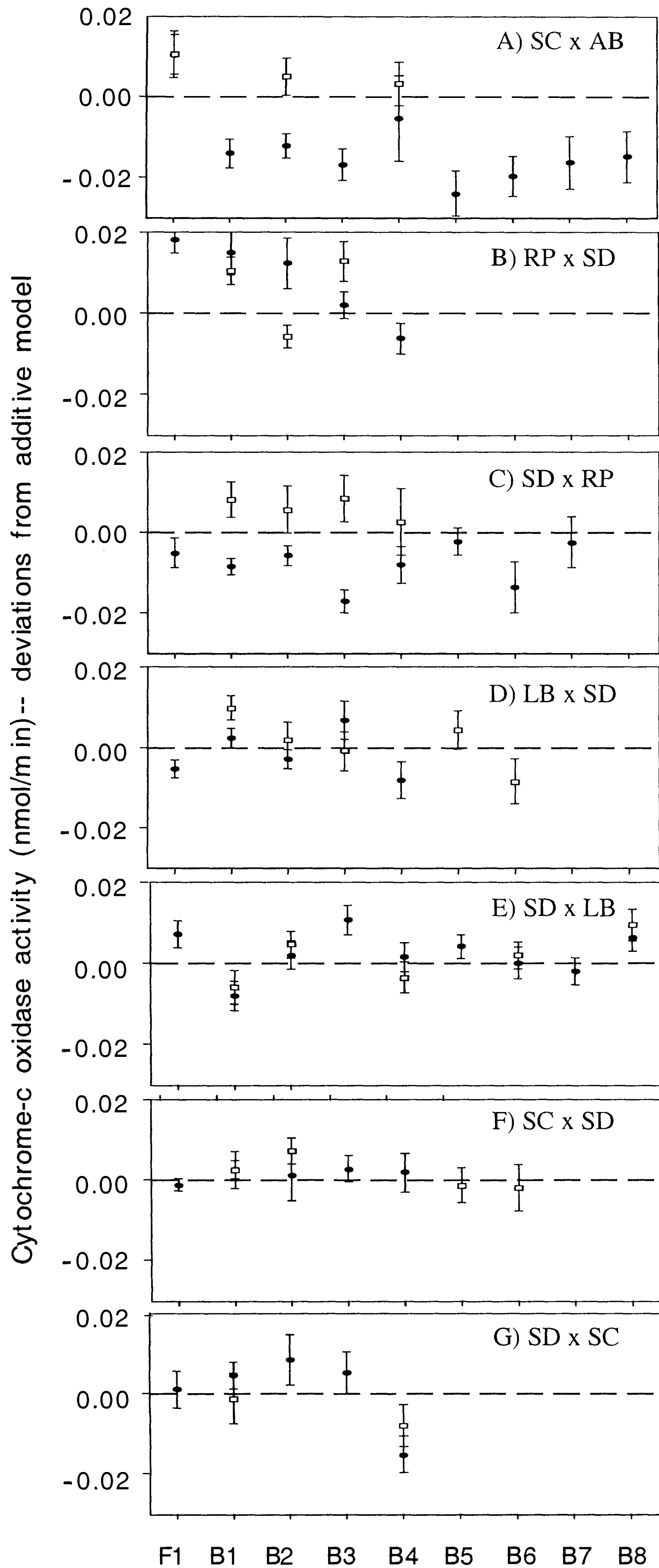

FIG. 2. Deviations from expected mean cytochrome *c* oxidase activity in seven backcrosses between outbred populations. Under the simple additive model, expected activity levels in F_1 hybrids are intermediate between the two parents. Each generation of backcrossing then brings expected activity levels halfway closer to the paternal parent in the experimental lines (black ovals) and halfway ← closer to the maternal parent in the control lines (white rectangles). All crosses are listed as female (dam) × male (sire). Breeding design is shown in Figure 1. Each point represents the mean activity (± SE) for a minimum of three copepods, with ANOVA adjustments for total protein content, gender, and sample × plate interaction.

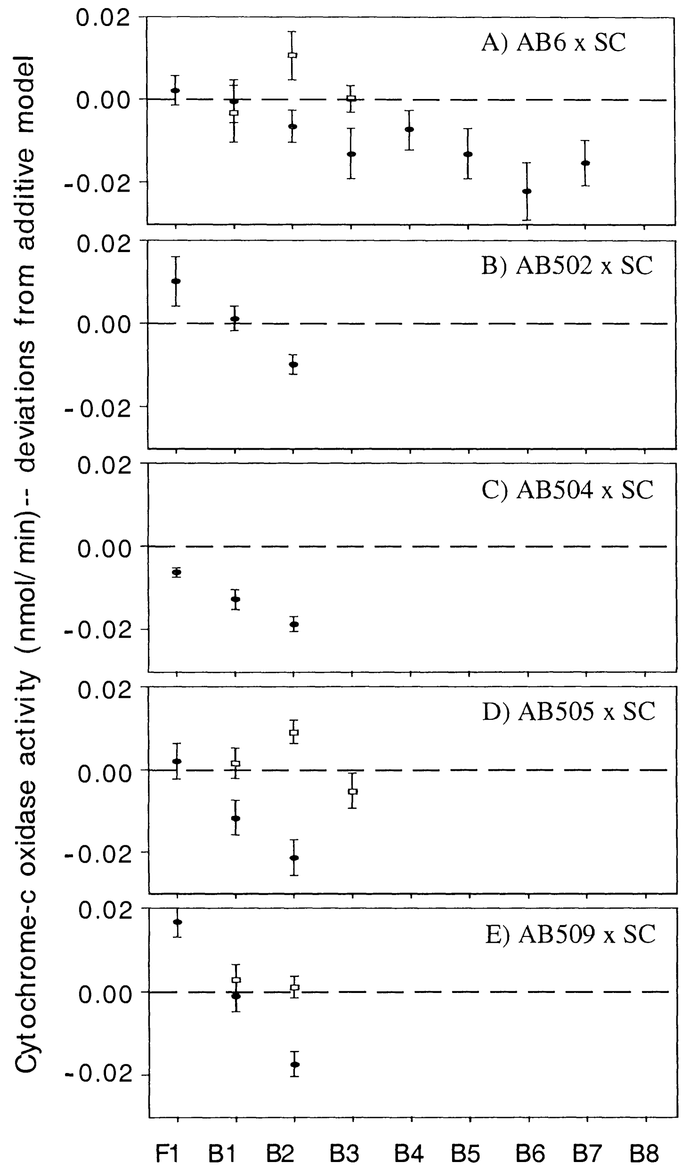

FIG. 3. Deviations from expected mean cytochrome *c* oxidase activity in five isofemale lines backcrossed to the paternal population. See caption to Figure 2 for details.

inate. Deviations in the control crosses should show a negative correlation with the proportion paternal nuclear genome with either nuclear-cytoplasmic or nuclear-nuclear interactions. All six crosses between SC and AB (cross SC × AB in Fig. 2 plus all five crosses in Fig. 3) showed a positive correlation to the model, a pattern consistent with nuclear-cytoplasmic interactions (nonparametric sign test, P = 0.016). However, the fit to the model was significant in only

TABLE 1. Linear regressions of deviation in COX activity from expected additive effects against proportion paternal nuclear genome in hybrid. See text for details. *n*, number of generations assayed; *r*, correlation coefficient; ND, no data.

Cross	Experimental lines			Control lines		
	n	*r*	*P*	*n*	*r*	*P*
SC × AB	9	0.378	0.316	3	−0.999	0.025*
RP × SD	5	−0.860	0.062	4	−0.726	0.274
SD × RP	8	0.147	0.729	5	−0.065	0.918
LB × SD	5	0.341	0.574	6	−0.168	0.751
SD × LB	9	−0.406	0.278	6	−0.298	0.566
SC × SD	5	0.372	0.538	5	0.211	0.733
SD × SC	5	0.775	0.124	3	0.869	0.330
AB6 × SC	8	0.740	0.036*	4	0.218	0.782
AB502 × SC	3	0.227	0.854	ND	ND	ND
AB504 × SC	3	0.987	0.102	ND	ND	ND
AB505 × SC	3	0.981	0.123	4	0.644	0.356
AB509 × SC	3	0.152	0.903	3	−0.974	0.146

* $P < 0.05$.

one of these six cases (AB6 × SC). Other than the AB/SC experimental crosses, the strongest fit to the model was found in cross RP × SD ($r = -0.860$). This negative correlation is consistent with nuclear-nuclear interactions, but was marginally nonsignificant ($P = 0.062$). A negative correlation in cross SC × AB was the only significant fit to the model in the control lines. This negative correlation is consistent with either nuclear-nuclear or nuclear-cytoplasmic interactions.

With all crosses combined, results of the total fitness assays (Table 2) show similar total offspring production in parents and F_1 hybrids, with a trend toward fewer offspring in the first and sixth backcross generations. In contrast, total offspring production in the control backcrosses was slightly higher than in the parentals. However, analysis of variance shows that the effect of offspring type was not significant. COX activities in these same lines (Table 2) show no clear trend among offspring types and no significant effect of offspring type when analyzed by analysis of covariance. Total offspring production shows no correlation with COX activity ($n = 57$ lines, $R^2 = 0.015$, $P = 0.446$).

DISCUSSION

Over the past two decades, we have witnessed explosive growth in the use of mtDNA for evolutionary genetic analyses (e.g., Avise 1994). Much of this work implicitly assumes that mtDNA sequence variation is selectively neutral. Yet the compact nature of the mitochondrial genome and the known functional importance of its gene products would seem to make such a sweeping assumption tenuous at best. Experimental evidence for nonneutral mtDNA variation has been obtained from a few studies (Clark and Lyckegaard 1988; Rand et al. 1994; Kilpatrick and Rand 1995) that have focused on population-level analyses of cytonuclear hybrids. Here we attempt to directly assess the functional significance of mtDNA variation by monitoring the activity of an enzyme that is partly encoded in both nuclear and mitochondrial genomes. The work itself is made possible by the remarkable genetic structure of the target species *T. californicus*, in which reproductively compatible populations show mtDNA sequence variation exceeding 20% (Burton 1998) and even some highly conserved genes, such as cytochrome *c*, are differentiated at the amino acid level among natural populations (Burton et al. 1999).

The results presented here strongly support the hypothesis of nuclear-mitochondrial coadaptation for a specific pair of populations, SC and AB. Five independent sets of crosses introgressing SC nuclear genes onto an AB cytoplasm (using five different AB isofemale lines for the initial hybridization) yielded nearly identical patterns of progressive decline in COX activity that were not apparent in the control backcrosses. The one set of reciprocal crosses (AB nuclear genes introgressed onto SC cytoplasm) also showed a pronounced decline in COX activity that was not observed in the control backcrosses. These results are unique in demonstrating that a high degree of *intergenomic* coadaptation exists among natural populations of *T. californicus*. Although this coadaptation is evident in the function of COX, we do not yet have evidence that fitness components are affected either by altered COX activity or by other unstudied physiological consequences of nuclear-mitochondrial interactions in the backcrossed lines. The only fitness measure attempted here, productivity of females over a 30-day period, showed an interesting trend of reduced fitness in experimental versus control backcrosses in both generations tested (first and sixth); however, the variance of this measure was too high to make it a reliable fitness estimator. Future work will focus of other types of fitness estimates.

The effects of hybridization on COX activity varied greatly among crosses. In contrast with the consistent pattern presented in the AB × SC crosses, several crosses showed only slight deviations from the additive model and no consistent

TABLE 2. Mean number of offspring produced per female in 57 lines of *Tigriopus californicus* and mean COX activity in those same lines. In B_{1C} and B_{6C} (first and sixth generation backcross controls), hybrids were backcrossed to the maternal line, effectively restoring the original maternal cytonuclear environment. Standard errors are in parentheses.

Offspring type	Number of lines	Mean number of offspring produced by a female over 30 days					Mean COX activity	
		Assays/line	Nauplii	Copepodids	Adults	Total offspring	Assays/line	COX activity
P	5	18.60 (2.23)	16.04 (6.19)	7.71 (2.18)	0.28 (0.10)	24.02 (7.43)	99.0 (28.9)	0.029 (0.003)
F_1	18	5.11 (0.95)	8.85 (2.96)	14.16 (3.98)	0.93 (0.40)	24.00 (6.09)	8.1 (1.5)	0.028 (0.002)
B_1	18	6.56 (1.29)	9.91 (2.78)	8.00 (2.10)	0.54 (0.17)	16.51 (4.19)	7.2 (1.7)	0.029 (0.003)
B_6	4	10.00 (2.04)	3.85 (1.29)	3.79 (2.03)	0.41 (0.28)	8.05 (3.06)	4.5 (0.6)	0.022 (0.004)
B_{1C}	9	11.78 (1.60)	16.43 (4.30)	11.98 (3.88)	2.36 (0.90)	30.77 (8.47)	4.9 (0.6)	0.025 (0.002)
B_{6C}	3	2.67 (1.20)	12.93 (12.93)	18.83 (17.60)	0.33 (0.33)	32.10 (30.86)	5.0 (1.4)	0.024 (0.003)

pattern with increasing hybridization. One cross showed a trend toward stronger deviations in the earliest generations of backcrossing, a pattern consistent with nuclear-nuclear interactions. This range of results is entirely consistent with the fact that coadaptation, to whatever extent it may occur, takes place independently within each allopatric population. Thus, one cannot predict the extent of genic interactions that may arise when such independent lineages are hybridized.

Similarly, the magnitude of hybridization effects on COX activity was only partially consistent with expectations based on levels of genetic differentiation previously documented between populations. Burton and Lee (1994) showed substantial DNA sequence differentiation in a 500-base segment of the COX mitochondrial subunit I gene (including three inferred amino acid substitutions diagnostic for central California [including SC] vs. southern California populations [including AB and SD]). A nuclear gene encoding histone H1 showed a similar pattern of differentiation between central and southern *T. californicus* populations (Burton and Lee 1994). More recently, DNA sequence data for the nuclear gene cytochrome *c* (Burton et al. 1999) also shows three amino acid substitutions between these two regions (SC vs. [AB and SD]). Based on these data we might expect the strongest gene interactions in crosses between central and southern California populations (AB/SC and SC/SD). Although strong deviations were found in crosses between AB and SC, the SC × SD cross did not show significant deviations from expectations. However, it must be noted that, to date, sequence data on only a portion of one of the three mitochondrial encoded COX subunits are available; the full extent of regional differentiation in COX subunit amino acid sequences is currently under study (Burton et al. 1999). Interestingly, one cross between southern California populations (RP × SD) showed a trend consistent with nuclear-nuclear interactions that *increased* COX activity in hybrids. Just as negative effects of hybridizing different populations are expected to be unpredictable, occasional positive interactions can be expected (e.g., Charlesworth 1995; Rieseberg 1997).

Although various crosses showed trends consistent with both nuclear-nuclear and nuclear-cytoplasmic interactions, the only significant fit to our models of gene interaction was consistent with nuclear-cytoplasmic interactions. Little is currently known about what gene interactions may be involved. Our measure of COX function, enzyme activity standardized to total protein, depends on both the amount and the activity of the enzyme. Gene interactions affecting COX function may therefore do so via a variety of mechanisms, including changes in mitochondrial number, transcription rate, transcript processing, message stability, translation efficiency, protein stability, or intrinsic catalytic efficiency (Clark and Wang 1994). Furthermore, it should be noted that our ability to detect genomic coadaptation in COX function may have been limited by the COX activity assay itself, in which commercially available cytochrome *c* from horse heart was used as a substrate. This assay therefore neglects any coadaptation between COX and cytochrome *c*, a possibility that seems especially likely in light of recent work on patterns of population divergence of COXI, COXII and cytochrome *c* (Burton and Lee 1994; Burton et al. 1999). Efforts are therefore currently underway to produce useable quantities of cytochrome *c* from *T. californicus* populations.

The molecular mechanism underlying the nuclear-mitochondrial coadaptation observed between SC and AB populations remains to be determined. Although the catalytic functions of cytochrome *c* oxidase (electron transfer and proton pumping) are attributed to the mitochondrial subunits, nuclear genes may play a variety of other roles (see Capaldi 1996; Tsukihara et al. 1996). For example, nuclear subunits have been implicated in assembly of the enzyme complex (Capaldi 1990) and they may also play a role in regulation of COX activity (Tsukihara et al. 1996). Intergenomic coadaptation likely involves nuclear genes other than those encoding COX subunits; for example, nuclear genes have been shown to affect COX expression in yeast (Pel et al., 1992). The role of cytochrome *c* (nuclear encoded) interaction with COX in interspecific studies (Osheroff et al. 1983) suggests that this is another potentially important site for coadaptation. The observed population differentiation of the cytochrome *c* gene in *T. californicus* (Burton et al. 1999) raises intriguing questions about the extent of enzyme-substrate coadaptation within this species.

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Literature Cited

Ar-rushdi, A. H. 1963. The cytology of achiasmatic meiosis in the female Tigriopus (Copepoda). Chromosoma 13:526.

Attardi, G., and G. Schatz. 1988. Biogenesis of mitochondria. Annu. Rev. Cell. Biol. 4:289–333.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Breeuwer, J. A., and J. H. Werren. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. Evolution 49:705–717.

Burton, R. S. 1985. Mating system of the intertidal copepod *Tigriopus californicus*. Mar. Biol. 86:247–252.

———. 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod *Tigriopus californicus*. Bull. Mar. Sci. 39:526–535.

———. 1987. Differentiation and integration of the genome in populations of the marine copepod *Tigriopus californicus*. Evolution 41:504–513.

———. 1990a. Hybrid breakdown in physiological response: a mechanistic approach. Evolution 44:1806–1813.

———. 1990b. Hybrid breakdown in developmental time in the copepod *Tigriopus californicus*. Evolution 44:1814–1822.

———. 1997. Genetic evidence for persistence of marine invertebrate populations in an ephemeral environment. Evolution 51: 993–998.

———. 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52:734–745.

Burton, R. S., and M. W. Feldman. 1981. Population genetics of *Tigriopus californicus*. II. Differentiation among neighboring populations. Evolution 35:1192–1205.

Burton, R. S., and B.-N. Lee. 1994. Nuclear and mitochondrial

gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. Proc. Natl. Acad. Sci. U.S.A. 91:5197–5201.

Burton, R. S., M. W. Feldman, and S. G. Swisher. 1981. Linkage relationships among five enzyme-coding gene loci in the copepod *Tigriopus californicus*: a genetic confirmation of achiasmatic meiosis. Biochem. Genet. 19:1237–1245.

Burton, R. S., P. D. Rawson, and S. Edmands. 1999. Genetic architecture of physiological phenotypes: empirical evidence for coadapted gene complexes. Am. Zool. 39:451–462.

Cann, R. L., W. M. Brown, and A. C. Wilson. 1984. Polymorphic sites and the mechanism of evolution in human mitochondrial DNA. Genetics 106:479–499.

Capaldi, R. A. 1990. Structure and function of cytochrome c oxidase. Annu. Rev. Biochem. 59:569–596.

———. 1996. The complexity of a respiratory complex. Nature Struct. Biol. 3:570–574.

Charlesworth, D. 1995. Hybrid speciation—evolution under the microscope. Curr. Biol. 5:835–836.

Clark, A. G., and E. M. S. Lyckegaard. 1988. Natural selection with nuclear and cytoplasmic transmission. III. Joint analysis of segregation and mtDNA in *Drosophila melenogaster*. Genetics 118:471–482.

Clark, A. G., and L. Wang. 1994. Comparative evolutionary analysis of metabolism in nine *Drosophila* species. Evolution 48: 1230–1243.

Edmands, S., and R. S. Burton. 1998. Variation in cytochrome-c oxidase activity is not maternally inherited in the copepod *Tigriopus californicus*. Heredity 80:668–674.

Grun, P. 1976. Cytoplasmic genetics and evolution. Columbia Univ. Press, New York.

Kilpatrick, S. T., and D. M. Rand. 1995. Conditional hitchhiking of mitochondrial DNA: frequency shifts of *Drosophila melanogaster* mtDNA variants depend on nuclear genetic background. Genetics 141:1113–1124.

King, M. P., and G. Attardi. 1989. Human cells lacking mtDNA: repopulation of exogenous mitochondria by complementation. Science 246:500–503.

Lee, B.-N. 1993. Genetic structure of *Tigriopus californicus* populations inferred from mitochondrial cytochrome oxidase I DNA sequences. Ph.D. diss., University of Houston, Houston, TX.

Liepens, A., and S. Hennen. 1977. Cytochrome oxidase deficiency during development of amphibian nucleocytoplasmic hybrids. Dev. Biol. 57:284–292.

Osheroff, N., S. H. Speck, E. Margoliash, E. C. L. Veerman, J. Wilms, B. W. Konig, and A. O. Muijsers. 1983. The reaction of primate cytochromes *c* with cytochrome *c* oxidase. J. Biol. Chem. 258:5731–5745.

Pel, H. J., A. Tzagoloff, and L. A. Grivell. 1992. The identification of 18 nuclear genes required for the expression of the yeast mitochondrial gene encoding cytochrome *c* oxidase subunit 1. Curr. Genet. 21:139–146.

Rand, D. M., M. Dorfsman, and L. M. Kann. 1994. Neutral and non-neutral evolution of *Drosophila* mitochondrial DNA. Genetics 138:741–756.

Rieseberg, L. H. 1997. Hybrid origins of plant species. Annu. Rev. Ecol. Syst. 28:359–389.

Simon, L. M., and E. D. Robin. 1971. Relationship of cytochrome oxidase activity to vertebrate total and organ oxygen consumption. Int. J. Biochem. 2:569–573.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenck. 1985. Measurement of protein using bicinchoninic acid. Analyt. Biochem. 150:76–85.

Tsukihara, T., H. Aoyama, E. Yamashita, T. Tomizaki, H. Yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono, and S. Yoshikawa. 1996. The whole structure of the 13-subunit oxidized cytochrome *c* oxidase at 2.8 Å. Science 272:1136–1144.

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