doi:10.1111/j.1558-5646.2009.00689.x

MALADAPTED GENE COMPLEXES WITHIN POPULATIONS OF THE INTERTIDAL COPEPOD *TIGRIOPUS CALIFORNICUS*?

Suzanne Edmands,[1,2] Sara L. Northrup,[1,3,4] and AnnMarie S. Hwang[1,5]

[1]*Department of Biological Sciences, University of Southern California, Los Angeles, California 90089*

[2]*E-mail: sedmands@usc.edu*

[4]*E-mail: snorthru@zoology.ubc.ca*

[5]*E-mail: achinen@usc.edu*

Received May 28, 2008
Accepted February 25, 2009

The prevalence of F2 hybrid breakdown in interpopulation crosses of the marine copepod *Tigriopus californicus* can be explained by disruption of coadapted gene complexes. This study further dissects the nature of hybrid gene interactions, revealing that parental populations may also harbor maladapted gene complexes. Diagnostic molecular markers (14) were assayed in reciprocal F2 hybrids to test for gene interactions affecting viability. Results showed some evidence of nuclear–nuclear coadaptation. Although there were no significant examples of pairwise linkage disequilibrium between physically unlinked loci, one of the two reciprocal crosses did show an overall excess of parental double homozygotes and an overall dearth of nonparental double homozygotes. In contrast, the nuclear–cytoplasmic data showed a stronger tendency toward maladaptation within the specific inbred lines used in this study. For three out of four loci with significant frequency differences between reciprocal F2, homozygotes were favored on the wrong cytoplasmic background. A separate study of reciprocal backcross hybrids between the same two populations (but different inbred lines) revealed faster development time when the full haploid nuclear genome did not match the cytoplasm. The occurrence of such suboptimal gene complexes may be attributable to effects of genetic drift in small, isolated populations.

KEY WORDS: Coadapted gene complexes, hybrid breakdown, interpopulation hybridization, nuclear–cytoplasmic interactions, nuclear–nuclear interactions, reciprocal cross.

New alleles arising in a lineage through mutation or migration must be tested against the background of alleles already present. Selection for harmonious interactions may therefore promote coadapted gene complexes whose joint action increases the fitness of the whole organism (Wright 1969; Mayr 1970; Wallace 1981). The prevalence of different coadapted gene complexes within species has been much debated (see Wallace 1991), due largely to the vulnerability of all but the most tightly linked gene combinations to breakdown by recombination.

The threat of disruption by recombination is minimized for conspecific populations connected by low levels of gene flow. In this case, both local selection and genetic drift may promote different sets of beneficial epistatic interactions in each population. Evidence for different sets of harmoniously interacting genes in geographically isolated populations comes from numerous examples of reduced fitness (and increased variance) in second-generation interpopulation hybrids (see reviews in Endler 1977 and Edmands 2007). It is in these second generation hybrids that genomic coadaptation is first disrupted by meiotic recombination, leading to the phenomenon of hybrid breakdown.

Investigations into the evolution of beneficial gene interactions have been central to our understanding of postzygotic

[3]Present address: Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

reproductive isolation, including the mechanisms underlying Haldane's Rule and the Large X Effect (e.g., Coyne and Orr 2004). Beneficial epistasis and its breakdown by hybridization are also critical issues for conservation as populations and species are increasingly being hybridized through both accidental introductions and intentional translocations (Allendorf et al. 2001; Edmands 2007). Predictions of the severity and duration of hybrid breakdown depend on a better understanding of the numbers of loci involved and linkage relationships among them.

The tidepool copepod *Tigriopus californicus* is becoming an established model for the genetic basis of hybrid breakdown. Despite a seemingly high potential for dispersal, populations are genetically differentiated over short geographic distances, with mitochondrial DNA differences exceeding 20% (Burton and Lee 1994; Edmands 2001). Interpopulation crosses typically result in F1 hybrids with similar or higher fitness than their parents, and F2 and backcross hybrids with reduced fitness (e.g., Burton 1986; Burton 1990a,b; Edmands 1999). Although work on hybrid breakdown in other systems has focused largely on nuclear–nuclear coadaptation, much work in *Tigriopus* has focused on nuclear–mitochondrial coadaptation, particularly those interactions necessary for mitochondrial energy production (e.g., Burton et al. 2006). The likelihood of different nuclear–mitochondrial gene complexes in different populations is enhanced by the particularly rapid rate of mitochondrial DNA evolution in *T. californicus* (protein-coding mitochondrial genes evolve approximately 25- to 40-fold faster than protein-coding nuclear genes, Willett and Burton 2004), and the prospect for detailed study of these nuclear–mitochondrial complexes is enhanced by the availability of full mitochondrial genome sequences for several populations (Burton et al. 2007).

This study uses reciprocal F2 and backcross hybrids to assess both nuclear–nuclear and nuclear–cytoplasmic interactions. Fourteen diagnostic molecular markers distributed throughout the genome are used to look at gene interactions affecting viability. The simple prediction is that coadaptation within natural populations should promote parental gene combinations which have higher fitness than hybrid gene combinations.

Materials and Methods

STUDY SPECIES

Tigriopus californicus is a harpacticoid copepod whose reproductive biology has been well studied (e.g., Egloff 1967; Vittor 1971; Burton 1985). Sexes are separate, outcrossing is obligatory, and even the most divergent populations have not shown evidence of prezygotic isolation (Ganz and Burton 1995; Palmer and Edmands 2000). Adult males use their geniculate first antennae to clasp immature females and guard them until the female reaches sexual maturity. Males then mate with the females before releasing them. Virgin females can therefore be obtained by placing a clasped pair on a piece of filter paper and teasing the copepods apart under a dissecting microscope using a fine probe. Females mate only once and use stored sperm to fertilize multiple broods of offspring. Inbred lines are easily created by isolating a single gravid female and allowing full siblings and their subsequent progeny to mate freely. Development involves six naupliar (larval) stages and five copepodid (juvenile) stages before reaching adulthood. Adult males can be distinguished by the hooks at the end of their first antennae. Recombination in this species is restricted to males (Ar-rushdi 1963; Burton et al. 1981) and mitochondria are inherited maternally (Lee 1993).

F2 CROSSES

Breeding design

Tigriopus californicus were collected from intertidal rock pools at two sites in southern California: Laguna Beach (LB, 33°33′N, 117°47′W, November 2001) and Royal Palms (RP, 33°42′N, 118°19′W, October 2000). These two populations have been found to be ∼17% divergent in mitochondrial DNA (COI; Edmands 2001 and D. Peterson, unpubl. data). All cultures were kept in a 20°C incubator with a 12:12 light:dark cycle. Stock cultures were maintained in 400 mL beakers in natural seawater supplemented with commercial flake-type fish food and Spirulina algae.

Isofemale lines from the two populations were created by placing a single gravid female in a petri dish with filtered seawater (37 μm) containing 0.2 mg finely ground Spirulina per milliliter. Lines were maintained for 6–8 months before reciprocal F2 crosses (RP female × LB male and LB female × RP male) were initiated in October 2002. Given that the species has overlapping generations and a minimum generation time of 23 days at 20°C (Burton 1987), this 6–8 months of inbred line maintenance corresponds to a maximum of ∼10 generations of inbreeding. Crosses were begun by uniting five virgin females from the first population with five adult males from the second population in each petri dish. A total of approximately 25 pairs were established for each of the two crosses. Dishes were checked three times per week. When females formed egg sacs they were transferred to a new dish with new filtered seawater and Spirulina. After the female laid several clutches she was again transferred to a new dish. When F1 offspring formed clasped pairs the pairs were dissected apart and reunited with partners descending from a different original dish to avoid additional inbreeding. Five F1 virgin females and five adult F1 males were placed in each dish. These dishes were monitored seven days a week. When females formed egg sacs they were transferred to a new dish.

F2 assays utilized the fastest developing male from each brood. Mature males are easier to recognize than mature females, and minimum male development time was easily determined by

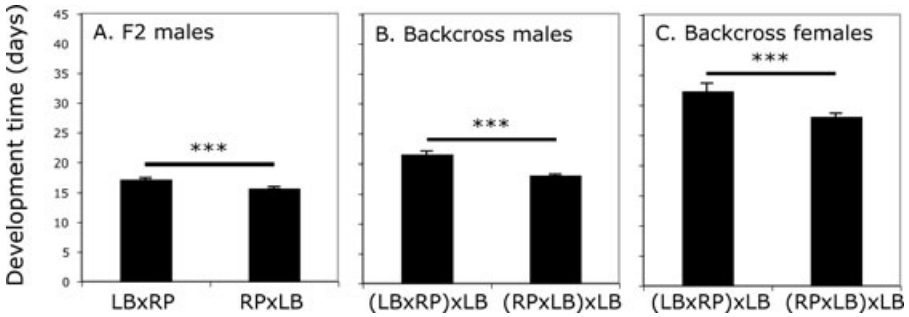

Figure 1. **Mean minimum development time (± 1 SE) in reciprocal hybrids, with significance tested by unpaired, 2-tailed *t*-tests (***$P < 0.001$). (A) F2 males LBf × RPm ($N = 178$) vs. RPf × LBm ($N = 335$). (B) Backcross males (LBf × RPm) F1f × LBm ($N = 125$) vs. (RPf × LBm) F1f × LBm ($N = 154$). (C) Backcross females (LBf × RPm) F1f × LBm ($N = 22$) vs. (RPf × LBm) F1f × LBm ($N = 166$).**

recording the date that F2 larvae hatched and the date that the first male matured (formed clasping antennae) for the first clutch of eggs for each F1 female. Development time is expected to be tightly correlated with fitness for continuously breeding species like *Tigriopus* (Lewontin 1974). Individual males were rinsed briefly with diH_2O, blotted dry on filter paper and frozen at −70°C for subsequent molecular analysis.

Molecular assays

DNA was extracted by placing individual frozen copepods in 50 μl lysis buffer (10 mM Tris pH 8.3, 50 mM KCl, 0.5% Tween 20 and 200 μg/mL Proteinase K) and incubating at 65°C for 1 h followed by 100°C for 15 min. Individuals were scored for 13 diagnostic microsatellite loci using methods previously described (Harrison et al. 2004; Edmands et al. 2005). These 13 loci were previously mapped to chromosome using a nonrecombinant backcross (Harrison et al. 2004; Edmands et al. 2005). Individuals were also scored for population-specific fragments of the cytochrome-c oxidase gene using methods developed for this study. Cytochrome c was mapped to chromosome in the same nonrecombinant backcross panel used in previous studies (Harrison et al. 2004; Edmands et al. 2005). Methods for screening cytochrome c are detailed in Appendix S1. Primers and assay conditions for all markers are listed in Table S1. For all PCR the forward primers were fluorescently labeled and run on a CEQ 8000 capillary sequencer (Beckman Coulter, Fullerton, CA).

Data analyses

Analysis of development times within and between reciprocal crosses were done using Statistica 7.1 (StatSoft, Tulsa, OK). Map Manager QTX version b20 (Manly et al. 2001) was used to determine linkage associations for each of the two crosses separately, using the Kosambi mapping function and a linkage criterion of $P = 0.05$. Calculation of allele and genotype frequencies, two-locus contingency tables and linkage disequilibrium was performed by Genepop 4.0 (Raymond and Rousset 1995). Chi-square tests were used to compare single locus genotype numbers to Mendelian expectations for each cross, and to compare genotype numbers between reciprocal crosses. To test for epistatic interactions affecting survival, observed two-locus genotype numbers were compared to expected numbers determined by multiplying single-locus ratios.

BACKCROSSES

Reciprocal backcrosses between RP and LB were previously analyzed for the effects of recombination (Edmands 2008). Here, we reanalyze the nonrecombinant crosses only to test the effects of cytoplasmic background. Experimental details are given in Edmands 2008. In brief, isofemale lines for the RP and LB populations (not the same lines used for F2 crosses) were maintained for 2–4 months before crosses began. Reciprocal F1 hybrids (RP female × LB male and LB female × RP male) were backcrossed to LB. Each clutch of backcross offspring was maintained in a separate petri dish and monitored daily. Minimum male development time was defined as described above. Minimum female development time was defined as the time from hatching until the first female in the clutch extruded an egg sac. Because we focused on only the fastest developing individuals from each brood, both the F2 and backcross studies targeted the "best" genotypic combinations (at least in terms of development time), whether they were parental or nonparental combinations. In this way, we did not sample highly dysfunctional genotypes that are least likely to contribute to future evolution.

Results

F2 HYBRIDS

Development time was significantly faster in F2 hybrid males with RP cytoplasm (15.8 ± 0.2 d) than in those with LB cytoplasm (17.3 ± 0.2 d) (Fig. 1A). Fourteen diagnostic markers were scored in an average of 140.8 F2 hybrid individuals per locus per cross. Linkage analyses show that 4 of these markers are unlinked. Loci 1_30 (chromosome_locus) and 1_558 were found to be between 22.7 cM apart (cross with LB cytoplasm) and 25.1 cM apart (cross

Table 1. Single-locus genotype data for F2 hybrid males in two reciprocal crosses (LB cytoplasm and RP cytoplasm). Loci are listed by chromosome number_locus number. Only LB homozygote frequencies are available for dominant locus 1555B as RP homozygotes are indistinguishable from LB-RP heterozygotes. χ^2 tests compared genotype numbers to expectations within crosses (3:1 for dominant locus 1555B and 1:2:1 for the remaining codominant loci) and between reciprocal crosses. Loci with significant differences between reciprocals are shown in bold. For all loci, cases in which the LB homozygote is favored on the foreign cytoplasmic background are marked by superscript a. For codominant loci, cases in which the homozygote class with higher viability does not match the cytoplasmic background are marked by superscript b. Mean sample size = 140.8 individuals per locus per cross. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, NS $P \geq 0.05$; NA = not available.

Locus	Cross	Genotype frequencies			Significance of χ^2	
		LB-LB	LB-RP	RP-RP	Within crosses	Between crosses
1_30	LB cytoplasm	0.12[a]	0.66	0.22[b]	***	NS
	RP cytoplasm	0.16[ab]	0.69	0.15	***	
1_558	LB cytoplasm	0.16[a]	0.64	0.20[b]	**	NS
	RP cytoplasm	0.26[ab]	0.56	0.18	NS	
2_228	LB cytoplasm	0.37	0.56	0.07	***	NS
	RP cytoplasm	0.33[b]	0.56	0.11	***	
5_1203	**LB cytoplasm**	**0.35**	**0.41**	**0.24**	**NS**	*
	RP cytoplasm	**0.17**	**0.55**	**0.28**	**NS**	
5_62J8	LB cytoplasm	0.17[a]	0.66	0.17	***	NS
	RP cytoplasm	0.19[ab]	0.63	0.18	**	
6_1555B	**LB cytoplasm**	**0.24**[a]	**NA**	**NA**	**NS**	*
	RP cytoplasm	**0.35**[a]	**NA**	**NA**	***	
6_cytC	LB cytoplasm	0.36[a]	0.35	0.29	NS	NS
	RP cytoplasm	0.40[ab]	0.28	0.32	**	
7_56J2	LB cytoplasm	0.24[a]	0.56	0.20	NS	NS
	RP cytoplasm	0.30[ab]	0.46	0.24	NS	
8_61	LB cytoplasm	0.11[a]	0.25	0.64[b]	***	NS
	RP cytoplasm	0.13[a]	0.20	0.67	***	
8_480	**LB cytoplasm**	**0.48**[a]	**0.45**	**0.07**	***	*
	RP cytoplasm	**0.62**[ab]	**0.26**	**0.12**	***	
9_1814	LB cytoplasm	0.22[a]	0.56	0.22	NS	NS
	RP cytoplasm	0.33[ab]	0.47	0.20	NS	
9_197	LB cytoplasm	0.29[a]	0.55	0.16	*	NS
	RP cytoplasm	0.41[ab]	0.45	0.14	***	
10_1555	LB cytoplasm	0.36	0.42	0.22	**	NS
	RP cytoplasm	0.29[b]	0.46	0.25	NS	
11_1202	**LB cytoplasm**	**0.21**[a]	**0.61**	**0.18**	*	***
	RP cytoplasm	**0.40**[ab]	**0.46**	**0.14**	***	

with RP cytoplasm). Loci 5_62J8 and 5_1203 were found to be between 24.6 cM apart (cross with LB cytoplasm) and 25.1 cM apart (cross with RP cytoplasm). Loci 9_197 and 9_1814 were found to be between 12.6 cM apart (cross with LB cytoplasm) and 19.1 cM apart (cross with RP cytoplasm). Loci 6_1555B and 6_cytC, as well as loci 8_61 and 8_480, were not significantly linked in the current F2 dataset, but are known to reside on the same chromosome based on data for nonrecombinant backcross hybrids.

Single-locus genotype data (Table 1) showed distorted ratios at 9 of 14 loci in each of the reciprocal crosses. For the 13 codominant loci there were seven cases of significant distortion within both reciprocals. In four of these cases LB homozygotes had higher viability than RP homozygotes on both cytoplasmic backgrounds and in one case RP homozygotes had higher viability than LB homozygotes on both backgrounds, indicating additive superiority of alleles linked to these markers. In one case, the homozygote with higher viability did not match the cytoplasm in both reciprocals and in another case the favored homozygote did not match the background in one reciprocal and the two homozygotes were equal in the other reciprocal. In no case did the favored homozygote class match the cytoplasmic background in both reciprocals.

For all 14 loci (including the dominant locus), there were four cases in which genotypic ratios were significantly different between reciprocal crosses. In three of these four cases, surviving

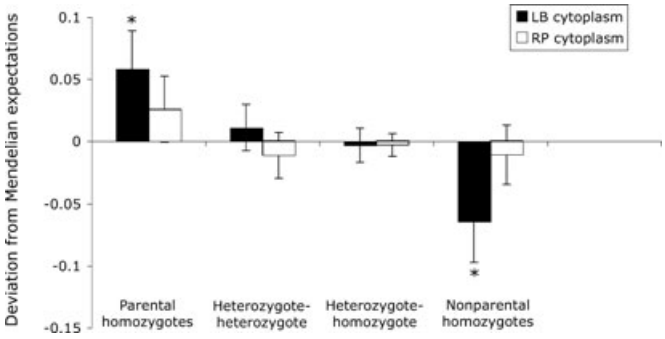

Figure 2. Mean and standard error for proportional deviation from expected two-locus genotype frequencies ((Obs-Exp)/Exp) for four classes of F2 hybrids in each of two reciprocal crosses (LB cytoplasm and RP cytoplasm). Only physically, unlinked loci are included. Asterisks denote the significance of paired, one-tailed *t*-tests of observed versus expected genotype numbers (*$P < 0.05$). $N = 74$ two-locus combinations for each cross.

LB homozygotes were more frequent on the foreign cytoplasmic background than on their native background. Allele frequencies also showed no advantage to alleles matching the cytoplasm. For the 13 codominant loci, mean LB allele frequencies were 0.52 (0.03 SE) for the cross with LB cytoplasm and 0.54 (0.03 SE) for the cross with RP cytoplasm. Significant heterozygote excesses (8) were more frequent than significant heterozygote deficits (5), but the overall heterozygote frequency for the two crosses (50.3%) was very close to the expected frequency of 50%.

To test for potential nuclear–nuclear interactions affecting survival, two-locus genotypic combinations were compared to expected numbers determined by multiplying single-locus ratios. After Bonferroni correction, linkage disequilibrium was significant only between physically linked loci. However, across all physically unlinked loci when genotypes were pooled into four different categories (parental double homozygote, homozygote–heterozygote, heterozygote–heterozygote, and nonparental double homozygote), the cross with LB cytoplasm showed a significant overall excess of parental homozygotes and a significant overall deficit of nonparental homozygotes (Fig. 2).

BACKCROSS HYBRIDS

Both male and female backcross hybrids developed significantly faster when the full haploid nuclear genome did not match the cytoplasmic background (Fig. 1B,C). When F1 hybrid females were backcrossed to paternal males ((RPf × LBm)F1f × LBm)) minimum male development time was 18.1 ± 0.2 days, as compared to 21. 5 ± 0.5 days in the maternal backcross ((LBf × RPm)F1f × LBm)). Similarly, minimum female development time was 28.1 ± 0.4 days in the paternal backcross, and 32.3 ± 1.2 days in the paternal backcross.

Discussion

TRANSMISSION RATIO DISTORTION

Distorted ratios were found at 64% of loci tested in each of the two reciprocal F2 hybrids. Previous work on interpopulation hybrids in this species shows skewed genotypic ratios in adults (Burton 1987; Willett and Burton 2001, 2003; Harrison and Edmands 2006; Willett 2006; Willett and Berkowitz 2007), but not in newly hatched larvae (Willett and Burton 2001; Willett 2006; Willett and Berkowitz 2007), suggesting that these distortions are due to differential zygote viability rather than meiotic drive.

The current study includes the same two populations (but different inbred lines) and the same microsatellite markers as an earlier study (Harrison and Edmands 2006) in which the lack of recombination in females (Ar-rushdi 1963; Burton et al. 1981) was used to create backcross hybrids with intact parental chromosomes. For the 12 microsatellite loci common to both sets of crosses, heterozygote frequencies in male LBf × RPm backcross hybrids were highly correlated with heterozygote frequencies in both the LBf × RPm F2 hybrids ($r = 0.61$, $P = 0.034$) and the RPf × LBm F2 hybrids ($r = 0.79$, $P = 0.002$). This demonstrates that relative heterozygote viabilities for a small number of loci marking pieces of recombinant chromosomes are a reasonable proxy for relative heterozygote viabilities of those same loci marking intact chromosomes, suggesting that at least some of these markers must lie near genes of large effect. The two studies differ, however, in that the nonrecombinant backcross hybrids showed heterozygote excess across most loci (mean heterozygosity 59.4%) whereas mean heterozygosity in the two F2 crosses (50.3%) was much closer to the expected frequency of 50%. The reduced viability of completely homozygous chromosomes in nonrecombinant backcross hybrids could be caused by both beneficial dominance, and detrimental homozygote–heterozygote interactions.

NUCLEAR–NUCLEAR INTERACTIONS

The current study showed some evidence for nuclear–nuclear coadaptation. There were no examples of linkage disequilibrium between pairs of unlinked loci. However, when all two-locus genotypes were combined, both reciprocal crosses showed an excess of parental double homozygotes and a deficit of nonparental double homozygotes, and both deviations were significant for the LBf × RPm cross. Frequencies of homozygote–heterozygote and heterozygote–heterozygote genotypes were close to expectations. This small number of largely nonfunctional markers therefore reveals the general pattern of nuclear–nuclear coadaptation expected under conditions of dominance (e.g., Turelli and Orr 2000).

A study by Willett (2006) revealed a very different pattern of nuclear–nuclear interactions in a different pair of *T. californicus* populations (AB and SD). In this study, F2 hybrids were screened

for three unlinked nuclear genes involved in the electron transport system. In almost all cases, double parental homozygotes, double heterozygotes, and double nonparental homozygotes were all favored whereas homozygote–heterozygote combinations were disfavored. This is certainly not consistent with simple nuclear–nuclear coadaptation, and suggests complex dominance/epistatic relationships between these three specific functional genes, or loci closely linked to them.

These complicated patterns of nuclear–nuclear interaction contrast with the more expected patterns typically reported in other taxa. For example, numerous introgressions between *Drosophila* species show nonparental double homozygotes to be more deleterious than homozygote–heterozygote combinations (Turelli and Orr 2000). Similarly, crosses between closely related marine bivalve species show that the least fit genotype was always one or the other nonparental double homozygote (Bierne et al. 2006). Such patterns fit with expectations of the dominance theory of postzygotic isolation (Turelli and Orr 2000).

NUCLEAR–CYTOPLASMIC INTERACTIONS

Results show little evidence of nuclear–cytoplasmic coadaptation, and indeed there are several instances of maladaptation, where nuclear markers fare significantly worse on their own cytoplasmic background than they do on a highly differentiated, foreign cytoplasmic background. It should be noted that both the F2 and backcross studies used a single isofemale line per population, and thus sample only a single cytoplasmic type. However, the level of intrapopulation mitochondrial variation is so low in this species (F_{ST} for mitochondrial COI is 0.98, Edmands 2001) that a single cytoplasmic type is likely to be a good representative of the whole population. There is still the possibility that the sampled cytoplasmic types contained deleterious mitochondrial mutations that altered nuclear–cytoplasmic interactions, but this is mitigated by the fact that the F2 and backcross studies used different isofemale lines.

For the F2 crosses, codominant loci that were significantly distorted in both reciprocals showed patterns consistent with additive effects in five cases, and maladaptive interactions in one case. For three out of the four loci showing significant differences between reciprocal crosses, LB homozygotes had higher viability on the wrong cytoplasmic background. Development time in the backcross hybrids also provided no evidence for nuclear–cytoplasmic coadaptation. Offspring of reciprocal F1 hybrid females backcrossed to the same parental male are expected to have identical nuclear composition but different cytoplasmic backgrounds. In this study, higher fitness (faster development time) for both males and females was found in the cross in which the full haploid nuclear genome did not match the cytoplasmic background ((RPf × LBm)F1f × LBm), in direct contrast to expectations under cytonuclear coevolution (e.g., Rand et al. 2004). Note that both the F2 and backcross studies were done under a single set of environmental conditions (20°C, normal oceanic salinity, 12 h light: 12 h dark), and that previous work has shown fitness of cytonuclear genotypes to be highly dependent on environmental, particularly temperature, conditions (Willett and Burton 2003). Still, the higher fitness of mismatched cytonuclear genotypes found in the current study under environmental parameters well within those experienced by both tested populations is not an expected result of cytonuclear coevolution.

There are at least three potential explanations for faster development in the mismatched ((RPf × LBm) F1f × LBm) backcross hybrids, given that RPf × LBm F2 hybrids also develop faster than LBf × RPm F2 hybrids. One explanation is that the RP cytoplasm itself confers faster development time. A second explanation is that RP grandmothers are phenotypically superior to LB grandmothers, perhaps due to differences in age or nutritional status, and that these differences extend across generations (e.g., Hercus and Hoffmann 2000; Magiafoglou and Hoffmann 2003). A third explanation is that LB nuclear alleles have greater negative interactions with LB cytoplasm than RP cytoplasm (i.e., maladaptation). There is also the possibility that RP nuclear alleles have greater negative interactions with LB cytoplasm than RP cytoplasm (i.e., coadaptation), but such effects should be reduced in the backcross hybrids which are expected to have only 25% RP nuclear alleles, and yet the difference between reciprocals is even greater than in the F2 hybrids expected to have 50% RP nuclear alleles. Clean distinction among potential explanations will require additional crosses, but the current data on development time certainly do not provide strong evidence for nuclear–cytoplasmic coadaptation.

Evidence for nuclear–cytoplasmic coadaptation in previous studies of this species is somewhat equivocal. Much of the work on coadaptation in *T. californicus* has focused on nuclear and mitochondrial components of the electron transport system (ETS), with particular emphasis on interactions between cytochrome-c (CYC, encoded in the nucleus) and cytochrome-c oxidase (COX, contains both nuclear and mitochondrial encoded subunits) in ETS complex IV. Cytonuclear hybrids created by repeated backcrossing exhibit COX activity levels consistent with nuclear–mitochondrial coadaptation in only a subset of interpopulation crosses (Edmands and Burton 1999). In vitro studies show higher COX activity when CYC and COX are from the same population (Rawson and Burton 2002; Harrison and Burton 2006), but the highest COX activity occurs with CYC variants (generated by site-directed mutagenesis) that are a mosaic of amino acids from populations that do and do not match the cytoplasmic background (Harrison and Burton 2006). In addition to ETS complex IV, Ellison and Burton (2006) also showed that nuclear–mitochondrial mismatch reduces activity of ETS complexes I and III, as well as ATP production when a series of interpopulation crosses are pooled together. Nevertheless, segregation ratios in

hybrids frequently show that nuclear alleles are favored on the wrong cytoplasmic background (Willett and Burton 2001, 2003; Willett 2006). Similarly, a recent study found that in two out of six interpopulation crosses, mismatched hybrids (mitochondrial DNA from one population, nuclear–encoded mitochondrial RNA polymerase from a different population) had significantly greater capacity to up-regulate mitochondrial genes in response to osmotic stress (Ellison and Burton 2008a). Finally, strong evidence for nuclear–cytoplasmic coadaptation in *Tigriopus* comes from a recent study (Ellison and Burton 2008b) in which the low fitness of F3 hybrids is restored in maternal backcrosses, which have a full haploid nuclear genome matching the mitochondrial genome, but not in paternal backcrosses, which have greater nuclear–mitochondrial mismatch. This is quite different from patterns found in the present study, and more work is needed to determine if these differences are due to the fitness components measured (minimum male development time vs. mean fecundity and survivorship), the specific populations or isofemale lines used, and/or the contrast between F1 and F3 backcrosses. The effect of the chosen fitness component is a particularly interesting subject for future study. It may be that the "best" (fastest developing) mismatched genotype could beat the "best" matched genotype, even when the mean mismatched genotype is not superior.

CAUSES AND CONSEQUENCES OF MALADAPTED GENE COMPLEXES

Interpopulation hybrids in *T. californicus* exhibit F2 hybrid breakdown for a broad range of fitness and physiological components (e.g., Burton 1986, 1990a,b; Edmands 1999; Burton et al. 2006), implying that coadaptation predominates. However, further dissection of gene interactions in the present study reveals that parental populations also harbor a surprising number of maladapted gene complexes, particularly nuclear–cytoplasmic complexes. Coyne and Orr (2004) noted that ". . .it is a hard to imagine that (a gene) would often work better (on a related genetic background) than on its own background." Although this is true, it is somewhat less difficult to imagine in *Tigriopus* where isolated populations experience repeated bottlenecks (Dybdahl 1994; Burton 1997) likely to impede the efficiency of selection and leave populations stranded on suboptimal adaptive peaks (sensu Wright 1932). The predominance of drift over selection in this species is consistent with the limited evidence for local adaptation to salinity and temperature (Edmands and Deimler 2004; but see also Willett and Burton 2003) as well as the unusually high F_{ST}/Q_{ST} ratio (Edmands and Harrison 2003). Accumulation of maladaptive mitochondrial alleles may be more likely than nuclear, due to the fourfold lower effective population size for mitochondrial loci (Wright 1969).

The accumulation of maladaptive gene combinations in small populations prone to genetic drift may create an epistatic load, in addition to the better-understood genetic load of deleterious recessives. Hybridization in such situations may simultaneously create gene combinations that are both better and worse than parental gene combinations. The creation of superior hybrid gene combinations in *Tigriopus* is evidenced by the beneficial effects of recombination on F2 hybrids (Edmands 2008), and by replicated long-term hybrid swarms showing an increase in molecular hybridity (Edmands et al. 2005) and fitness levels surpassing parental controls (Hwang et al., unpubl. data). For small populations suffering from inbreeding depression a current management dilemma is whether translocation from a genetically and demographically healthier population should be used to cure the inbreeding depression, or whether this will incur outbreeding depression (e.g., Tallmon et al. 2004; Edmands 2007). The existence of both beneficial and detrimental epistasis in small, inbred populations further complicates this issue, but introduces the hopeful scenario that translocation could in some cases create beneficial new gene combinations that aid in population recovery.

ACKNOWLEDGMENTS

This work was funded by grants to SE from the U.S. National Science Foundation (DEB-0077940 and DEB-0316807) and the USC Women in Science & Engineering Program. We gratefully acknowledge J. Curole and M. Voigt for advice on the ZipCode protocol. The manuscript also benefited from the comments of two anonymous reviewers and Associate Editor W. Owen McMillan.

LITERATURE CITED

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problem with hybrids: setting conservation guidelines. Trends Ecol. Evol. 16:613–622.

Ar-Rushdi, A. H. 1963. The cytology of achiasmatic meiosis in the female *Tigriopus* (Copepoda). Chromosoma 13:1754–1763.

Bierne, N., F. Bonhomme, P. Boudry, M. Szulkin, and P. David. 2006. Fitness landscapes support the dominance theory of post-zygotic isolation in the mussels *Mytilus edulis* and *M. galloprovincialis*. Proc. R. Soc. B 273:1253–1260.

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 development time in the copepod *Tigriopus californicus*. Evolution 44:1814–1822,

———. 1997. Genetic evidence for long-term persistence of marine invertebrate populations. Evolution 51:993–998.

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. Nat. Acad. Sci. USA 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., C. K. Ellison, and J. S. Harrison. 2006. The sorry state of F-2 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. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Dybdahl, M. F. 1994. Extinction, recolonization, and the genetic structure of tidepool copepod populations. Evol. Ecol. 8:113–124.

Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757–1765.

———. 2001. Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. Mol. Ecol. 10:1743–1750.

———. 2007. Between a rock and hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Mol. Ecol. 16:463–475.

———. 2008. Recombination in interpopulation hybrids of *Tigriopus californicus:* release of beneficial variation despite hybrid breakdown. J. Hered. 99:316–318.

Edmands, S., and R. S. Burton. 1999. Cytochrome-c oxidase activity in interpopulation hybrids of the marine copepod *Tigriopus californicus*: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. Evolution 53:1972–1978.

Edmands, S., and J. K. Deimler. 2004. Local adaptation, intrinsic coadaptation and the effects of environmental stress on interpopulation hybrids in the copepod *Tigriopus californicus*. J. Exp. Mar. Biol. Ecol. 303:183–196.

Edmands, S., and J. S. Harrison. 2003. Molecular and quantitative trait variation within and among populations of the intertidal copepod *Tigriopus californicus*. Evolution 57:2277–2285.

Edmands, S., H. V. Feaman, J. S. Harrison, and C. C. Timmerman. 2005. Genetic consequences of many generations of hybridization between divergent copepod populations. J. Hered. 96:114–123.

Egloff, D. A. 1967. Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod *Tigriopus californicus*. Ph.D. diss., Stanford University, Palo Alto, CA.

Ellison, C. K., and R. S. Burton. 2006. Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. Evolution 60:1382–1391.

———. 2008a. Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. Proc. Nat. Acad. Sci. USA 105:15831–15836.

———. 2008b. Interpopulation hybrid breakdown maps to the mitochondrial genome. Evolution 62:631–638.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Princeton Univ. Press, Princeton, NJ.

Ganz, H. H., and R. S. Burton. 1995. Genetic differentiation and reproductive incompatibility among Baja California populations of the copepod *Tigriopus californicus*. Mar. Biol. 123:821–827.

Harrison, J. S., and R. S. Burton. 2006. Tracing coadapted gene complexes to single amino acid substitutions. Mol. Biol. Evol. 23:559–564.

Harrison, J. S., and S. Edmands. 2006. Chromosomal basis of viability differences in *Tigriopus californicus* interpopulation hybrids. J. Evol. Biol. 19:2040-2051.

Harrison, J. S., D. L. Peterson, J. R. Swain, and S. Edmands. 2004. Microsatellite DNA markers for the intertidal copepod *Tigriopus californicus*. Mol. Ecol. Notes 4:736–738.

Hercus, M. J., and A. A. Hoffmann. 2000. Maternal and grandmaternal age influence offspring fitness in *Drosophila*. Proc. R. Soc. Lond. B 267:2105–2010.

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

Lewontin, R. C. 1974. The genetic basis of evolutionary change. Columbia Univ. Press, NY.

Magiafoglou, A., and A. A. Hoffmann. 2003. Cross-generation effects due to cold exposure in *Drosophila serrata*. Funct. Ecol. 16:664–672.

Manly, K. F., R. H. Cudmore, and J. M. Meer. 2001. Map manager QTX, cross-platform software for genetic mapping. Mammalian Genome 12:930–932.

Mayr, E. 1970. Populations, species, and evolution. Harvard Univ. Press, Cambridge, MA.

Palmer, C. A., and S. Edmands. 2000. Mate choice in the face of both inbreeding and outbreeding in the intertidal copepod *Tigriopus californicus*. Mar. Biol. 136:693–698.

Rand, D. M., R. A. Haney, and A. J. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. 19:645–653.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

Rawson, P. D., and R. S. Burton. 2002. Functional coadaptation between cytochrome c and cytochrome c oxidase within allopatric populations of a marine copepod. Proc. Nat. Acad. Sci. USA 99:12955–12958.

Tallmon, D. A., G. Luikart, and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends Ecol. Evol. 19:489–496.

Turelli, M., and H. A. Orr. 2000. Dominance, epistasis and the genetics of postzygotic isolation. Genetics 154:1663–1679.

Vittor, B. A. 1971. Effects of the environment on fitness-related life history characters in *Tigriopus californicus*. Ph.D. Diss., University of Oregon, Eugene, OR.

Wallace, B. 1981. Basic population genetics. Columbia Univ. Press, NY.

———. 1991. Coadaptation revisited. J. Heredity 82:89–95.

Willett, C. S. 2006. Deleterious epistatic interactions between electron transport system protein-coding loci in the copepod *Tigriopus californicus*. Genetics 173:1465–1477.

Willett, C. S., and J. N. Berkowitz. 2007. Viability effects and not meiotic drive cause dramatic departures from Mendelian inheritance for malic enzyme in hybrids of *Tigriopus californicus* populations. J. Evol. Biol. 20:1196–1205.

Willett, C. S., and R. S. Burton. 2001. Viability of cytochrome c genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. Evolution 55:1592–1599.

———. 2003. Environmental influences on epistatic interactions: viabilities of cytochrome c genotypes in interpopulation crosses. Evolution 57:2286–2293.

———. 2004. Evolution of interacting proteins in the mitochondrial electron transport system in a marine copepod. Mol. Biol. Evol. 21:443–453.

Wright, S. 1932. The role of mutation, inbreeding, crossbreeding and selection in evolution. Proc. Sixth Intl. Congr. Genet. 1:356–366.

———.1969. Evolution and the genetics of populations, Volume 2. The theory of gene frequencies. Univ. of Chicago Press, Chicago.

Associate Editor: O. McMillan

Supporting Information

The following supporting information is available for this article:

Appendix S1. Methods for screening cytochrome C.

Table S1. Marker information including locus, code, marker type, forward and reverse primers, and annealing temperature.

Supporting Information may be found in the online version of this article.
(This link will take you to the article abstract).

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.
Additional results and discussion can be found in a document at http://www.repository.naturalis.nl/record/289893.