Journal of Heredity 2005:96(2):114–123
doi:10.1093/jhered/esi014
Advance Access publication December 23, 2004

Genetic Consequences of Many Generations of Hybridization Between Divergent Copepod Populations

S. EDMANDS, H. V. FEAMAN, J. S. HARRISON, AND C. C. TIMMERMAN

From the Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371. H. V. Feaman is currently at the School of Criminal Justice and Criminalistics, California State University, Los Angeles, CA 90032. J. S. Harrison is currently at the Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202. C. C. Timmerman is currently at Methinks.com, 971 W. 30th St., Los Angeles, CA 90007.

Address correspondence to Suzanne Edmands at the address above, or e-mail: sedmands@usc.edu.

Abstract

Crosses between populations of the copepod *Tigriopus californicus* typically result in outbreeding depression. In this study, replicate hybrid populations were initiated with first generation backcross hybrids between two genetically distinct populations from California: Royal Palms (RP) and San Diego (SD). Reciprocal F_1 were backcrossed to SD, resulting in expected starting frequencies of 25% RP/75% SD nuclear genes on either a pure RP cytoplasmic or a pure SD cytoplasmic background. After 1 year of hybridization (up to 15 generations), seven microsatellite loci were scored in two replicates on each cytoplasmic background. Frequencies of the rarer RP alleles increased significantly in all four replicates, regardless of cytoplasmic source, producing a mean hybridity of 0.97 (maximum = 1), instead of the expected 0.50. Explicit tests for heterozygote excess across loci and replicates showed significant deviations. Only the two physically linked markers showed linkage disequilibrium in all replicates. Subsequent fitness assays in parental populations and early generation hybrids revealed lower fitness in RP than SD, and significant F_2 breakdown. Computer simulations showed that selection must be invoked to explain the shift in allele frequencies. Together, these results suggest that hybrid inferiority in early generations gave way to hybrid superiority in later generations.

The consequences of hybridization between divergent populations or species is a subject of rising concern as anthropogenic disturbance continues to increase the chances of mixing between formerly isolated gene pools (Johnson 2000; Allendorf et al. 2001). High-profile examples include accidental introgression between farmed salmon and wild salmon (Garant et al. 2003) and between domesticated crop plants and their wild relatives (Ellstrand 2003). Intentional translocation between populations is also increasingly proposed as a management tool to increase population size, prevent inbreeding depression, and enhance evolutionary potential. A controversial example is the augmentation of an ailing population of Florida panthers with Texas panthers of a different subspecies (Maehr and Lacy 2002; Mansfield and Land 2002). In addition to their importance for conservation, consequences of hybridization are of considerable import to evolutionary biology. Due in part to opportunities made possible by new molecular and statistical methods, a resurgence of interest has focused on what hybridization can tell us about the nature of reproductive isolation and the role of hybridization in creating new species (Arnold 1997; Barton 2001; Coyne and Orr 2004; Welch and Rieseberg 2002).

Hybrid fitness is difficult to predict because the mixing of gene pools can simultaneously create both beneficial and detrimental gene interactions, and these interactions can change substantially between generations due to recombination. In some cases, crosses result in an increase in fitness (i.e., heterosis or hybrid vigor), which is generally attributed to overdominance or the masking of deleterious recessives, although epistasis can also be involved (Lynch 1991). In other cases, crosses result in a decrease in fitness known as outbreeding depression or hybrid breakdown. This can occur in first generation (F_1) hybrids where it can be attributed to disruption of local adaptation (i.e., gene by environment interactions), underdominance or epistatic interactions (heterozygote × heterozygote interactions or interactions involving sex chromosomes). Frequently, however, fitness declines are delayed until the second (F_2) or backcross generations (e.g., Adkins et al. 1991; Burton 1986; Endler

1977; Marr et al. 2002). Here, the original parental gene combinations are disrupted by recombination allowing the possibility of deleterious heterozygote × homozygote or homozygote × homozygote interactions (Lynch 1991; Turelli and Orr 2000). Beyond the second generation of hybridization, fitness could decline further as recombination continues to disrupt tight linkages (Fenster and Galloway 2000), or it could increase and even surpass parental fitness as selection promotes extreme hybrid phenotypes with superior fitness (i.e., transgressive hybridization, Rieseberg et al. 1999). The likelihood of these alternative scenarios is related to the number and linkage relationships of genes underlying hybrid incompatibility and to the strength of selection on these genes.

The tidepool copepod *Tigriopus californicus* is well suited to laboratory studies of hybridization due to its ease of husbandry and short generation time. Despite a seemingly high potential for dispersal, gene flow is restricted, facilitating the development of numerous population-specific markers (e.g., Burton et al. 1979; Burton and Lee 1994; Burton 1997; Edmands and Harrison 2003). The species inhabits a semi-continuous linear strip of seemingly similar habitat extending from Alaska to central Baja California, Mexico. Within this range, populations that are at least partially reproductively compatible have mitochondrial DNA differences (cytochrome oxidase I) that vary from 0.2 to 23% (Burton and Lee 1994; Burton 1998; Edmands 2001).

Allopatric populations show no evidence of prezygotic isolation (Ganz and Burton 1995; Palmer and Edmands 2000). However partial hybrid breakdown is found in the F_2 and backcross generations for a variety of measures including response to osmotic stress (Burton 1990a), cytochrome c oxidase activity (Edmands and Burton 1998, 1999; Burton et al. 1999) and various reproductive components (Burton 1986, 1987, 1990b; Edmands 1999). Deleterious hybrid gene interactions have been detected involving blocks of nuclear genes marked by allozyme loci (Burton 1987) as well as between nuclear and mitochondrial components of the cytochrome c oxidase enzyme complex (Rawson and Burton 2002; Willett and Burton 2001, 2003, 2004). Natural hybrid zones have not been reported for this species.

In this study we assessed genetic composition of replicated experimental hybrid populations after 1 year (maximum of 15 generations) of free mating. Hybrid populations began with first-generation backcross hybrids on alternative cytoplasmic backgrounds to test whether nuclear alleles were favored on their native cytoplasmic background. Replicates of each type of population were compared to assess the repeatability of evolutionary changes.

Materials and Methods

T. californicus Biology

The reproductive biology of *T. californicus* has been studied by a number of authors, including Egloff (1967), Vittor (1971), and Burton (1986, 1987). Reproduction is strictly sexual and mating occurs year-round. Males use their antennae to clasp virgin females and guard them until females reach maturity. Females mate only once, whereas males mate repeatedly. Females produce up to 15 clutches of up to 140 eggs each, with 2–5 days between clutches. Minimum generation time is ~ 23 days at 20°C. Mitochondria are inherited maternally (Lee 1993), and recombination is restricted to males (Ar-rushdi 1963; Burton et al. 1981).

Long-Term Hybridizations

Populations were sampled from two southern California locations in October 2000: San Diego (SD, 32° 45′ N, 117° 15′ W) and Royal Palms, Palos Verdes, California (RP, 33° 42′ N, 118° 19′ W). These two populations are ~18% divergent for the mitochondrial gene cytochrome oxidase I (Edmands 2001). Crosses were set up by teasing clasped pairs apart using a fine probe and uniting virgin females with males from the alternate population in a petri dish. Approximately 100 pairs were established for each of the two reciprocal crosses (RP female × SD male and SD female × RP male). All crosses were maintained in filtered sea water (37 μm) containing 0.2 mg finely ground *Spirulina* algae per ml and housed in a 20°C incubator with a 12 h light:12 h dark cycle. Crosses were monitored three times per week.

When females formed egg sacs they were transferred to 400-ml beakers (~50 parental females per beaker) for the production of F_1. After a maximum of 2 weeks, parental females were transferred to a new beaker to produce additional F_1. When F_1 offspring formed clasped pairs, they were dissected apart, and virgin F_1 females were backcrossed to SD males in a petri dish. When females formed egg sacs they were transferred to beakers (~50 F_1 females per beaker) to produce backcross offspring. After a maximum of 2 weeks, F_1 females were moved to a new beaker. In this way, beakers began with an expected nuclear gene ratio of 25% RP/75% SD with a pure RP cytoplasmic background in one reciprocal and a pure SD cytoplasmic background in the other reciprocal. Backcross individuals were then left to mate freely with each other for the duration of the experiment. Five beakers were established for each of the two reciprocal crosses.

Every 2 weeks beakers were rehydrated and partial sea water changes were conducted by pouring half of the water through a nitex sieve and replacing it with fresh sea water plus *Spirulina*. Four beakers (one SD cytoplasm and three RP cytoplasm) died out completely over the course of the experiment due in part to an outbreak of nematodes. Population sizes in the surviving beakers over the course of the experiment were unknown, but are estimated to have ranged from a minimum of 20 to a maximum of 200. Twelve months after the first backcross offspring were born, 40–50 individuals (half male, half female) from each of the surviving beakers were frozen at –70°C for subsequent molecular analyses.

Microsatellite Assays

An enriched DNA library from the RP population was used for microsatellite isolation and primer development (Harrison et al. in press). The seven loci chosen for the study

Table 1. F_{ST} between each pair of experimental hybridizations for each of seven microsatellite loci (following Weir and Cockerham 1984)

	Replicates compared						
Loci	RP1/RP2	SD1/SD2	RP1/SD1	RP1/SD2	RP2/SD1	RP2/SD2	All 4
1 (TCS197)	0.1048***	0.0055*	0.0000^{ns}	0.0458***	0.0473**	0.0117^{ns}	0.0381***
2 (TC1202)	0.0408***	0.1613***	-0.0067^{ns}	0.2016***	0.0373***	0.1441***	0.1019***
3 (TCS228)	0.0628*	0.3679***	0.0639*	0.5836***	-0.0135^{ns}	0.3665***	0.2898***
4 (TCS030)	0.1779***	0.0050^{ns}	0.0034^{ns}	-0.0163^{ns}	0.2753***	0.1606***	0.1076***
5 (TC1555)	-0.0035^{ns}	0.3772***	0.0341^{ns}	0.2079***	0.0756**	0.1415**	0.1464***
6 (TC1203)	0.0062*	-0.0076^{ns}	-0.0077^{ns}	-0.0099^{ns}	-0.0045^{ns}	0.0092**	−0.0020*
7 (TC62J8)	0.0006*	0.0085^{ns}	-0.0036^{ns}	0.0331^{ns}	-0.0090^{ns}	0.0149*	0.0075*
All 7	0.0548	0.1454	0.0090	0.1767	0.0596	0.1269	0.0931

Asterisks show the significance of exact tests of genic differentiation at each locus (following Raymond and Rousset 1995b). ***$P < .001$; **$P < .01$; *$P < 0.05$; ns, $P \geq .05$.

(Table 1) are diagnostic for populations RP versus SD and easily scored on manual gels. Primers for six of these loci are described in Harrison et al. (in press). The seventh locus (TC62J8) used the following primers (5′–3′): F-ACGGTCATCTCAATGCTGAA and R-GGTGAAAAATCGGAAAACCA. A linkage mapping study shows that loci TC1203 and TC62J8 are on the same chromosome, and the remaining five loci are on different chromosomes (Harrison et al. in press; Harrison and Edmands unpublished data). Total fragment sizes ranged from 62 to 213 bp.

DNA for microsatellite assays was obtained 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. Polymerase chain reaction (PCR) was performed in 10 μl reactions containing 1 μl template and 2.5 mM $MgCl_2$. Thermal cycles were composed of a 5 min denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, finally followed by 5 min extension at 72°C. PCR products (2–3 μl + 0.4 μl loading dye) were run on small (15 cm × 17 cm × 0.8 mm) nondenaturing TAE polyacrylamide gels, with the lower portion of the gel supplemented with the polymer Spreadex (Elchrom Scientific, Switzerland). Acrylamide concentrations ranged from 6 to 11%, depending on fragment size. Gels were run for 2 h at 250 V and were then stained with ethidium bromide and visualized on a UV light box.

Arlequin ver. 2.0 (Schneider et al. 2000) was used to test for deviations from linkage equilibrium and deviations from Hardy Weinberg equilibrium (following Guo and Thompson 1992). Because we were specifically interested in the hypothesis of heterozygote excess, we also used more powerful U-tests (Rousset and Raymond 1995) for heterozygote excess across all loci and across all replicates (implemented by GENEPOP Web version 3.1c, Raymond and Rousset 1995a). GENEPOP was also used to calculate pairwise F_{ST} among replicates (following Weir and Cockerham 1984) and to test the significance of genic differentiation among replicates using a Fisher exact test (as described in Raymond and Rousset 1995b). Hybrid indices for each individual were calculated by scoring 0 for each RP allele and 1 for each SD allele. Scores for all seven loci thus ranged from 0 (all RP alleles) to 14 (all SD alleles). Hybrid indices were also expressed in terms of "hybridity" (*sensu* Carney et al. 2000) wherein the most intermediate genotype (hybrid index of 7) is scored as 1 and the pure parental genotypes (hybrid indices 0 and 14) are scored as 0.

Fitness Assays

Fitness was assayed in parental populations to determine if they were differentially adapted to laboratory conditions. Fitness was also assessed in first- and second-generation interpopulation hybrids to determine if this particular pair of populations exhibits the F_2 breakdown commonly found in other population pairs (e.g., Burton 1990b; Edmands 1999). Populations RP and SD were collected in March 2003, and crosses were initiated the following day. Four types of crosses were established (listed as female × male): (1) SD × SD, (2) RP × RP, (3) SD × RP, and (4) RP × SD. For each cross, 10 petri dishes were set up with 5 virgin females and 5 males, for a total of 50 pairs per cross. Culture conditions were the same as for the long-term crosses (37 μm filtered sea water, 0.2 mg *Spirulina* per ml, 20°C, 12 h light:12 h dark). Dishes were monitored daily. When females formed egg sacs they were transferred to their own new dish. When eggs hatched, the larvae were pipetted one by one into a new dish to determine hatching number. Seven days later, offspring in each dish were recounted to determine survivorship.

After the first clutch of eggs, the maternal parent was transferred to a 400-ml group beaker to produce subsequent clutches. Parents from dishes 2, 4, 6, 8, and 10 were combined in "even" beakers and parents from dishes 1, 3, 5, 7, and 9 were combined in "odd" beakers. Group beakers were monitored for the formation of F_1 pairs. Because these pairs are potentially full siblings, and because we want to distinguish outbreeding effects from inbreeding effects, F_1 pairs were dissected apart and reunited with members of the opposite sex descended from a different dish. Therefore SD × SD odd females were united with SD × SD even males; SD × RP even females were united with SD × RP odd males, and so on. Five females and five males were again

placed in each dish. When females formed egg sacs they were placed in their own new dish. When eggs hatched larvae were counted by pipetting them individually to a new dish. Seven days later, surviving offspring were recounted. In this way, values for hatching number and survival number were obtained for the first and second generation of each of the four crosses. Values for RP × SD F_2 and SD × RP F_2 were corrected for possible time effects by subtracting from each F_2 value the midparent mean in generation two minus the midparent mean in generation one.

Computer Simulations

A computer model (Edmands and Timmerman 2003; available from the authors on request) was used to assess whether the observed distribution of hybrid indices in the long-term hybridizations could be attributed to chance. Simulations modeled 15 generations of random mating among diploid individuals following Mendelian rules. Both individuals and gametes were unisexual, and self-fertilization was allowed. In each generation, individuals produced a set number of gametes depending on the fitness function and these gametes were paired randomly to form zygotes. For baseline simulations the population size was set at 100—if 100 or fewer zygotes were formed then all survived to reproduce; if greater than 100 zygotes were formed then 100 were selected randomly. Individuals had one locus on each of five chromosomes, and two loci on the sixth chromosome. There were two possible alleles at each locus: RP (0) and SD (1). To begin the simulation F_1 individuals heterozygous (0,1) for all seven loci were backcrossed to SD individuals homozygous (1,1) for all seven loci to produce generation 0 backcross individuals. These backcross individuals mated randomly for an additional 15 generations, at which point hybrid indices were calculated based on the number of 0 and 1 alleles. Simulations were run for 15 generations based on 1 year of hybridization in the empirical replicates and a minimum generation time of 22.4–23.3 days at 20°C (Burton 1987). Fifteen generations is intended as an upper limit for the period of time over which evolutionary change could have occurred. The effective number of generations (e.g., Crow and Chung 1967) in the empirical replicates is unknown but is certainly lower than 15, because there are overlapping generations and mean generation time is estimated as 36.46 at 15°C and 22.71 at 23°C (Egloff 1967).

For baseline simulations of the long-term hybridizations there was a constant population size of 100 with no mutation, no recombination, and no selection (i.e., each individual produced 40 gametes regardless of genotype). Subsequent simulations explored the effects of altering each of these parameters, with 1000 replications for each parameter set. Selection was simulated by varying the number of gametes produced by each genotype. For example, a 2% selective advantage for each 0 allele would mean that an F_1 hybrid individual would produce 45.6 gametes [40 + (40)(7)(0.02)]. Noninteger gamete values such as this one were treated in a probabilistic fashion. That is, the individual would produce 45 gametes with a 60% probability of producing the 46th gamete.

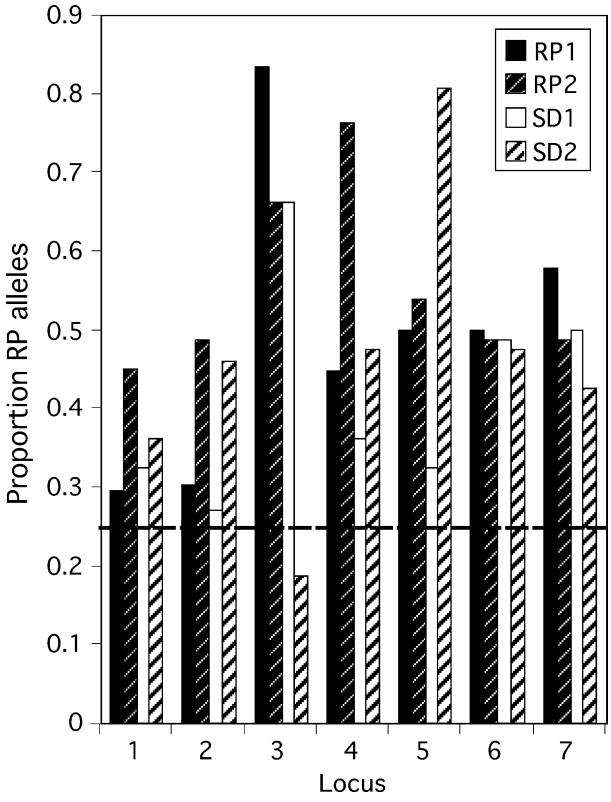

Figure 1. Proportion RP alleles at seven microsatellite loci for each of the four replicates. Horizontal dashed line shows the expected frequency of 0.25. Locus 1 = TCS197; locus 2 = TC1202; locus 3 = TCS228; locus 4 = TCS030; locus 5 = TC1555; locus 6 = TC1203; locus 7 = TC62J8.

Results

After 1 year of hybridization, an average of 38.7 (SE = .2) individuals were scored for each of the seven microsatellite loci in each of the four replicates. RP alleles increased from the expected starting frequency of .25 in 27 out of 28 cases, with no loci going to fixation (Figure 1). There were several instances of extreme allele frequency differences between replicates on the same cytoplasmic background (e.g., SD1 and SD2 for loci 3 and 5). Overall, there was a tendency toward higher RP allele frequencies on the native cytoplasmic background (mean RP allele frequency in the two replicates with RP cytoplasm = 0.524; mean RP allele frequency in the two replicates with SD cytoplasm = .427). However, exact tests of genic differentiation (Table 1) show no loci where both replicates with RP cytoplasm have significantly higher RP allele frequencies than both replicates with SD cytoplasm, providing no clear evidence for cytonuclear coadaptation. Similarly, estimates of pairwise F_{ST} (Table 1) show that differentiation between replicates on the same cytoplasmic background (.0548 and .1454) is similar to that between replicates on different backgrounds (.0090–.1767).

Figure 2 shows the distribution of hybrid indices in the four replicates, with each RP allele counting as 0 and each SD allele

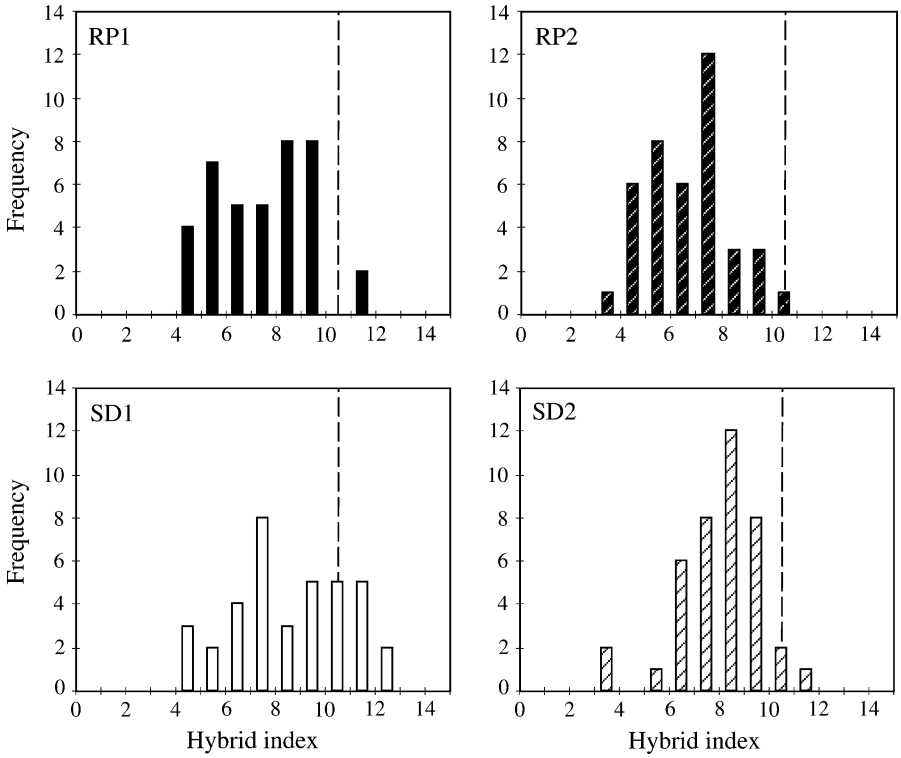

Figure 2. Frequency of individual hybrid indices in each of the four replicates. Vertical dashed lines indicate expected mean hybrid index of 10.5.

counting as 1. Instead of the expected mean of 10.5, mean indices were 7.0 for RP1, 6.2 for RP2, 8.1 for SD1 and 7.6 for SD2. Converting these same measurements to hybridity, the mean of the four replicates was .97 instead of the expected .50.

Tests of Hardy Weinberg equilibrium are shown in Table 2. There were 17 cases of heterozygote excess and 11 cases of heterozygote deficit, but probability tests (Guo and Thompson 1992) showed only two of these deviations to

Table 2. Observed and expected heterozygosities for seven microsatellite loci in four experimental hybridizations between populations RP and SD

	Locus							
	1 (TC S197)	2 (TC 1202)	3 (TC S228)	4 (TC S030)	5 (TC 1555)	6 (TC 1203)	7 (TC 62J8)	Het. excess across loci
Replicate RP1								
Observed	0.44	0.79	0.33	0.49	0.64	0.64	0.42	
Expected	0.47	0.72	0.30	0.51	0.52	0.51	0.50	
Replicate RP2								
Observed	**0.73***	0.72	0.43	0.48	0.46	0.68	0.65	*
Expected	0.54	0.75	0.48	0.37	0.50	0.60	0.58	
Replicate SD1								
Observed	0.54	0.86	0.46	0.56	0.38	0.65	0.68	*
Expected	0.50	0.76	0.45	0.47	0.46	0.55	0.54	
Replicate SD2								
Observed	0.53	0.79	0.28	**0.23***	0.38	0.50	0.50	
Expected	0.47	0.71	0.33	0.50	0.31	0.52	0.52	
Het. excess across replicates	*		*					**

Replicates RP1 and RP2 have a RP cytoplasmic background and replicates SD1 and SD2 have a SD cytoplasmic background. Significant deviations from Hardy Weinberg proportions are shown in boldface. Significance of explicit tests for heterozygote (het.) excess across all loci and all replicates are shown on the margins. $^{**}P < .01$; $^{*}P < .05$.

Table 3. Pairwise tests of linkage equilibrium between seven microsatellite loci in four experimental hybrid populations

	Replicate			
Pair of loci	RP1	RP2	SD1	SD2
(1,2)	ns	ns	ns	ns
(1,3)	ns	*	ns	ns
(1,4)	ns	ns	ns	ns
(1,5)	ns	ns	ns	ns
(1,6)	ns	**	*	ns
(1,7)	ns	***	ns	ns
(2,3)	**	ns	ns	*
(2,4)	ns	ns	ns	ns
(2,5)	ns	ns	ns	ns
(2,6)	ns	ns	**	ns
(2,7)	ns	ns	***	ns
(3,4)	ns	ns	ns	*
(3,5)	*	ns	ns	ns
(3,6)	ns	ns	ns	ns
(3,7)	*	ns	ns	ns
(4,5)	ns	ns	ns	ns
(4,6)	ns	ns	ns	ns
(4,7)	ns	ns	ns	ns
(5,6)	*	ns	ns	ns
(5,7)	ns	ns	ns	*
(6,7)	*	***	***	***

Significant deviations are shown in boldface. Locus 1 = TCS197; locus 2 = TC1202; locus 3 = TCS228; locus 4 = TCS030; locus 5 = TC1555; locus 6 = TC1203; locus 7 = TC62J8. ***$P < .001$; **$P < .01$; *$P < .05$; ns, $P \geq .05$.

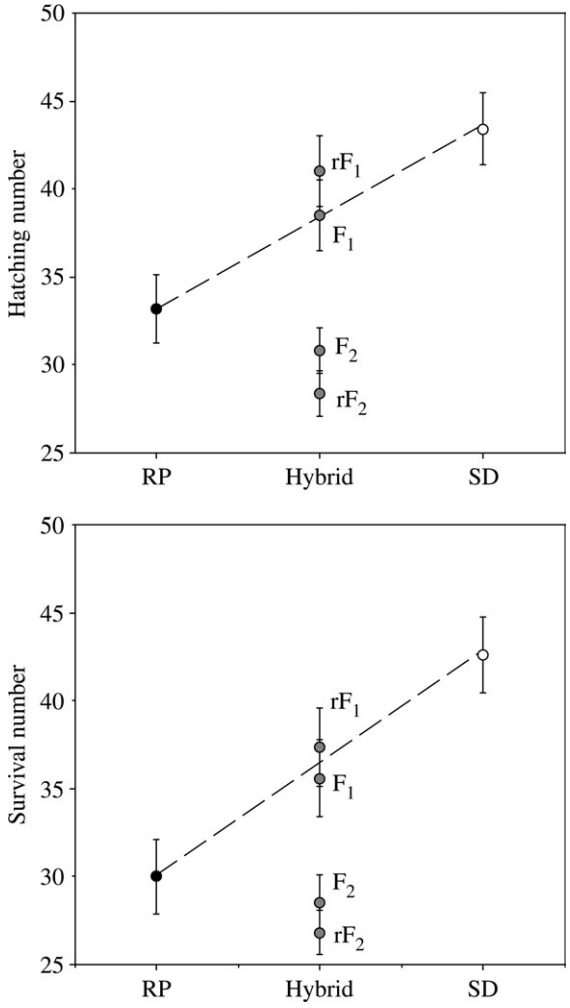

Figure 3. Mean and SE for hatching number and survival number in population RP, population SD, F_1 hybrids (RP female × SD male), reverse F_1 (rF_1) hybrids (SD female × RP male), F_2 hybrids ($F_1 \times F_1$) and reverse F_2 (rF_2) hybrids ($rF_1 \times rF_1$). Dashed line shows the expected hybrid fitness with purely additive gene action.

be significant (one excess and one deficit). Because we were interested in the hypothesis of heterozygote advantage to explain the increases in hybridity, we also used more powerful U-tests (Rousset and Raymond 1995) to explicitly test for heterozygote excess across loci and across replicates. Significant excesses were found for two of the seven loci (TCS197 and TCS228) and two of the four replicates (RP2 and SD1). A global test across all loci and replicates found very significant heterozygote excess. Table 3 shows tests of linkage disequilibrium for each pair of loci. Out of 84 tests there were 17 significant deviations. Only physically linked loci six and seven showed significant linkage disequilibrium across all four replicates.

Fitness assays were performed on an average of 36.4 broods (range 29–40) for each of the four cross types in each of the two generations (Figure 3). Both hatching number and survival number were significantly lower in population RP than in population SD (unpaired, two-tailed *t* tests; $P < .001$). Mean F_1 fitness was similar to the midparent for both fitness components in both reciprocals. Mean F_2 fitness was more than two standard errors below the midparent for both fitness components in both reciprocals.

Computer simulations (Table 4, with selected simulations illustrated in Figure 4) explored the effects of various parameters on the distribution of hybrid indices. Using baseline settings, the mean hybrid index in the 15th generation was 10.525 ± .024 (SE), with zero probability of generating mean hybrid indices as low or lower than the four that were observed (6.2, 7.0, 7.6, and 8.1). Adding mutation or recombination had little effect on the mean or the variance. Genetic drift did increase the variance. Going from the baseline population size of 100 to either a constant population size of 20 or a single generation bottleneck of 2 approximately doubled the standard error but still cannot explain the empirical data. A 30% SD advantage was simulated based on the results of the fitness assays (30% higher survival number in SD relative to RP), and this increased the mean hybrid index to 11.227. A series of simulations was run to determine the form of selection most likely to explain the observed data. A mean hybrid index approximating the observed mean (7.225) could be achieved through a 119% RP advantage or a 200% hybridity advantage (maximal fitness when hybrid index = 7). Heterozygote advantage (maximal fitness when heterozygous at all loci) was much less effective in explaining the results,

Table 4. Examples of the effects of altering baseline settings for simulated hybridizations.

Parameters	Mean hybrid index (SE)	*P*
Baseline (population size = 100, no mutation, no recombination, no selection)	10.525 (0.024)	0
Mutation = 0.0001 per locus per generation	10.525 (0.022)	0
Recombination = 0.05 per locus per generation	10.484 (0.022)	0
Drift		
Population size = 20	10.454 (0.047)	8.8×10^{-7}
Population bottleneck ($N = 100$ for 7 gen., $N = 2$ for 1 gen., $N = 100$ for 7 gen.)	10.478 (0.044)	1.3×10^{-7}
Selection		
30% SD advantage (2.14% for each 1 allele)	11.227 (0.019)	0
119% RP advantage (8.5% for each 0 allele)	7.169 (0.025)	.030
200% hybridity advantage (maximal fitness when hybrid index = 7)	7.239 (0.011)	6.0×10^{-4}
200% heterozygote advantage (maximal fitness when heterozygous at all loci)	8.086 (0.016)	0
500% heterozygote advantage (maximal fitness when heterozygous at all loci)	7.623 (0.014)	9.0×10^{-5}
Selection + drift		
119% RP advantage (8.5% for each 0 allele) and population size = 20	7.635 (0.053)	.024
200% hybridity advantage (maximal fitness when hybrid index = 7) and population size = 20	7.406 (0.026)	.010
200% heterozygote advantage (maximal fitness when heterozygous at all loci) and population size = 20	8.466 (0.039)	3.7×10^{-4}
500% heterozygote advantage (maximal fitness when heterozygous at all loci) and population size = 20	7.992 (0.033)	5.5×10^{-4}

Mean hybrid index (SE in parentheses) is shown for the 15th generation (1,000 replicates for each parameter set). *P* is the probability of generating mean hybrid indices as low as those in the four empirical replicates.

as a 200% heterozygote advantage brought the hybrid index only to 8.086. Even a 500% heterozygote advantage brought the hybrid index only to 7.623. None of the simulated forms of selection could explain the disparate hybrid indices in the four empirical replicates, even when selection was combined with genetic drift ($P < .05$). Together, these simulations suggest that selection is required to explain the overall reduction in hybrid indices, with varying magnitudes of selection being necessary to explain the dissimilarity among the four replicates.

Discussion

A primary motive for the crossing design was to test whether nuclear alleles were favored on their own cytoplasmic background, as might be predicted from studies showing nuclear-cytoplasmic coadaptation in this system (Burton et al. 1999; Edmands and Burton 1998, 1999; Rawson and Burton 2002; Willett and Burton 2001, 2003, 2004). Little evidence of this type of coevolution was found, as none of the seven loci showed a consistent pattern of higher allele frequencies on the native cytoplasmic background. However, recent linkage mapping shows that cytochrome c, a nuclear gene that is a prime candidate for interactions with mitochondrially encoded proteins, is not on the same chromosome as any of the seven loci that were surveyed (Chinen and Edmands, unpublished data).

In 27 out of 28 cases, RP alleles increased from their expected frequencies of 25%, resulting in a mean hybridity of .97 instead of .50. Computer simulations showed that selection must be invoked to explain this change in gene frequencies in all four replicates. One form of selection that might explain this pattern is an RP fitness advantage under laboratory conditions. However, the breeding study showed that population RP had ~ 30% lower fitness than population SD under conditions similar to those used for the long-term hybridizations. Still, it could be that RP has higher competitive fitness despite having significantly lower fitness in isolation. Or it could be that the superiority of SD is highly dependent on beneficial epistasis, making it vulnerable to assimilation by RP alleles even though the pure RP population is inferior.

Alternative explanations for the replicated increases in hybridity involve some sort of hybrid superiority. A possible explanation is heterozygote advantage across all loci. Probability tests (Guo and Thompson 1992) found only one significant excess of heterozygotes at the end of the experiment. However, such tests are notoriously weak, and more powerful tests of the explicit hypothesis of heterozygote excess (Rousset and Raymond 1995) did find a very significant deviation across all loci and replicates. Computer simulations, however, show that even a 500% heterozygote advantage could not explain the mean empirical hybrid index, apparently because of the high load generated by overdominance. A much lower level of hybridity advantage, meaning maximal fitness for the most intermediate genotypes with a hybrid index of 7, was more effective at explaining the observed results. This hybridity advantage was intended to mimic a situation of beneficial hybrid epistasis where highest fitness is achieved through the interaction between different parental genes at different loci. Such epistatic interactions might be expected to result in consistent patterns of linkage

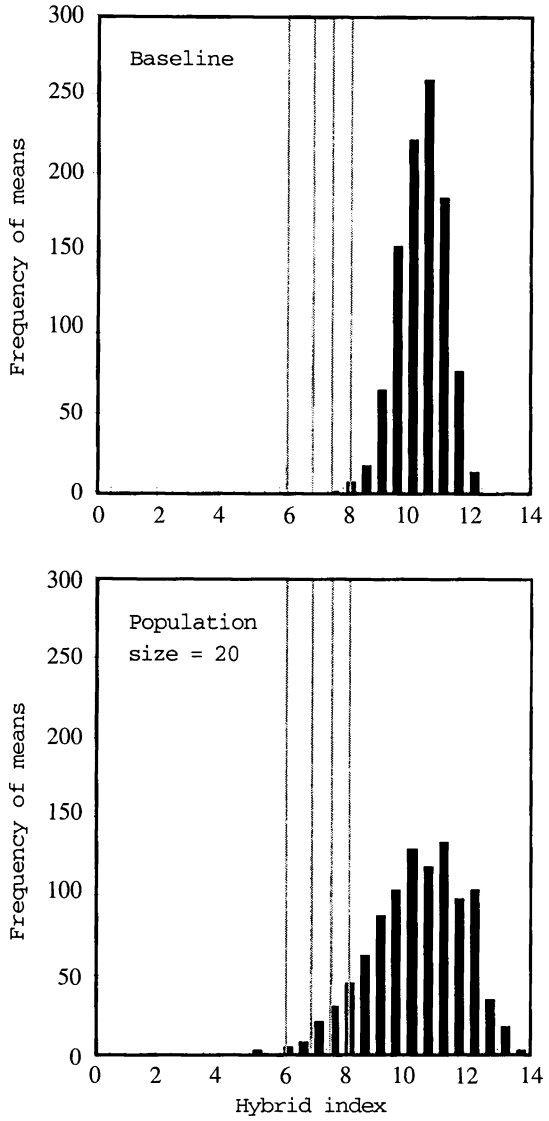

Figure 4. Distribution of mean hybrid indices in simulated hybridizations for baseline parameters (above) and for a constant population size of 20 (below). Vertical gray lines are the observed means in the four empirical replicates.

disequilibrium, yet the only pair of loci showing significant linkage equilibrium across all four replicates were physically linked loci six and seven. This could be because marker loci were too distant from the loci under selection to reveal disequilibrium after many generations of recombination. Alternatively, different hybrid combinations could have been favored in each of the different replicates.

There has been considerable interest recently in the extent to which the outcome of hybridization is repeatable. For example, there is increasing evidence that many allopolyploid species have multiple independent origins (Soltis and Soltis 1993). Even diploid species are sometimes thought to stem from recurrent hybridization, although this cannot always be documented unambigously in natural hybrids (Brochman et al. 2000; Schwarzbach and Rieseberg 2002). A particularly compelling example of repeatability comes from sunflowers in which the genetic composition of three artificial hybrids was compared to that of an ancient hybrid species (Rieseberg et al. 1996). Significant concordance was found among the four lineages, and the conservation of large linkage blocks suggested the effects of strong epistatic interactions. Examples such as this imply that consequences of hybridization may in some cases be influenced by strong deterministic forces. The present study showed only partial concordance among replicates. All four populations had an increase in RP allele frequencies at (nearly) all loci, but the populations achieved this result via different evolutionary pathways as evidenced by the different patterns of linkage disequilibrium and the significant genic differentiation at all seven loci tested. Furthermore, computer simulations found no single set of parameters that could explain the disparate hybrid indices at the end of the experiment.

The replicated increases in hybridity are surprising given the evidence for significant fitness declines in F_2 hybrids under similar laboratory conditions. Such a result lends support to the prediction that outbreeding depression is likely to be a temporary phenomenon, with incompatible gene interactions being rapidly purged by natural selection (Templeton 1986). Empirical data on the duration of outbreeding depression are scant, although there have been a few reports suggesting rapid recovery despite much reduced fitness in the early generations of hybridization (Heiser 1947; Rieseberg et al. 1996; Templeton 1986).

A rapid recovery from outbreeding depression might have been expected to be especially unlikely in this particular experimental system. First, rapid purging of hybrid incompatibilities has been predicted to occur in crosses between closely related groups where there are only a few interacting loci underlying the fitness problems (Templeton 1986). Instead, the present study used a very wide cross between populations with 18% mitochondrial COI divergence, a distance which translates into ~ 13 million years using standard calibrations (Knowlton and Weigt 1998). Second, computer simulations suggest outbreeding depression caused by disruption of intrinsic coadaptation may last longer than that caused by disruption of local adaptation, apparently because selection acts more efficient on purely additive gene action (Edmands and Timmerman 2003). In *T. californicus*, outbreeding depression appears largely driven by disruption in intrinsic coadaptation, as laboratory studies of varying temperature and salinity conditions revealed no significant gene × environment interactions (Edmands and Deimler 2004, but see also Willett and Burton 2003), and studies of molecular versus quantitative genetic variation suggest that quantitative trait divergence among local environments is restrained (Edmands and Harrison 2003). Third, hybrid advantage might be expected to be particularly likely in highly novel or stressful conditions, yet conditions for the current experiment were specifically intended to be benign (salinity of 35 ppt, ample food, and 20°C, a temperature well within the range normally experienced by these populations). Hybrids are often restricted to marginal habitat (Arnold 1997; Barton and Hewitt 1985), and numerous studies have shown

increased heterosis under conditions of environmental stress (Armbruster et al. 1997; Barlow 1981; Hoffmann and Parsons 1991; Pederson 1968). Indeed, previous work on *T. californicus* has shown that under conditions of moderate thermal stress (25°C), F_2 hybrid breakdown still occurs but is less pronounced than that found at 15°C (Edmands and Deimler 2004).

In sum, even in this extremely wide cross where there are reasons to expect outbreeding depression to be severe and long-lasting, hybridity increased in all four replicates assayed. Importantly, we do not know how fitness changed over the time course of the experiment, and we do not know the genetic composition of the replicates that went extinct. Still, results suggest that fitness problems in early generation hybrids may in some cases be a weak barrier to hybridization.

Acknowledgments

Funding for this study was provided by the U.S. National Science Foundation (grant no. DEB0077940 to S.E.) and the University of Southern California's Women in Science and Engineering Program (grants to S.E. and H.V.F).

References

Adkins RM, McBee K, Porter CA, and Baker RJ, 1991. Hybrid breakdown in *Peromyscus leucopus* and examination of the recombinational-breakdown model. J Mammal 72:535–541.

Allendorf FW, Leary RF, Spruell P, and Wenburg JK, 2001. The problem with hybrids: setting conservation guidelines. Trends Ecol Evol 16:613–622.

Armbruster P, Bradshaw WE, and Holzapfel CM, 1997. Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. Evolution 51:451–458.

Ar-rushdi AH, 1963. The cytology of achiasmatic meiosis in the female *Tigriopus* (Copepoda). Chromosoma 13:526–539.

Arnold M, 1997. Natural hybridization and evolution. Oxford: Oxford University Press.

Barlow R, 1981. Experimental evidence for interaction between heterosis and environment in animals. Anim Breed Abstr 49:715–737.

Barton NH, 2001. The role of hybridization in evolution. Mol Ecol 10: 551–568.

Barton NH and Hewitt GM, 1985. Analysis of hybrid zones. Annu Rev Ecol Syst 16:113–148.

Brochmann C, Borgen L, and Stabbetorp OE, 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundungii* (Asteraceae). Plant Syst Evol 220:77–92.

Burton RS, 1986. Evolutionary consequences of restricted gene flow in the copepod *Tigriopus californicus*. Bull Mar Sci 39:526–535.

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

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

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

Burton RS, 1997. Genetic evidence for long-term persistence of marine invertebrate populations in an ephemeral environment. Evolution 51:993–998.

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

Burton RS and Lee B-N, 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 RS, Rawson PD, and Edmands S, 1999. Genetic architecture of physiological phenotypes: empirical evidence for coadapted gene complexes. Am Zool 39:451–462.

Burton RS, Feldman MW, and Curtsinger JW, 1979. Population genetics of *Tigriopus californicus* (Copepoda: Harpacticoida): I. Population structure along the central California coast. Mar Ecol Prog Ser 1:29–39.

Burton RS, Feldman MW, and Swisher SG, 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.

Carney SE, Gardener KA, and Rieseberg LH, 2000. Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (*Helianthus*). Evolution 54:462–474.

Coyne JA and Orr HA, 2004. Speciation. Sunderland, MA: Sinauer.

Crow JF and Chung YJ, 1967. Measurement of effective generation length in *Drosophila* population cages. Genetics 57:951–955.

Edmands S, 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757–1768.

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

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

Edmands S and Burton RS, 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.

Edmands S and Deimler JK, 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(2):183–196.

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

Edmands S and Timmerman CC, 2003. Modeling factors affecting the severity of outbreeding depression. Cons Biol 17:883–892.

Egloff DA, 1967. Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod *Tigriopus californicus* (PhD diss.). Palo Alto, CA: Stanford University.

Ellstrand NC, 2003. Dangerous liaisons? When cultivated plants mate with their wild relatives. Baltimore, MD: John Hopkins University Press.

Endler JA, 1977. Geographic variation, speciation and clines. Princeton, NJ: Princeton University Press.

Fenster CB and Galloway LF, 2000. Population differentiation in an annual legume: genetic architecture. Evolution 54:1157–1172.

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

Garant D, Fleming IA, Einum S, and Bernatchez L, 2003. Alternative male life-history tactics as potential vehicles for speeding introgression of farm salmon traits into wild populations. Ecol Lett 6:541–549.

Guo S and Thompson E, 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Harrison JS, Peterson JL, Swain JR, and Edmands S, in press. Microsatellite DNA markers for the intertidal copepod *Tigriopus californicus*. Mol Ecol Notes.

Heiser CB, 1947. Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. Evolution 1:249–262.

Hoffmann AA and Parsons PA, 1991. Evolutionary genetics and environmental stress. Oxford: Oxford University Press.

Johnson MS, 2000. Measuring and interpreting genetic structure to minimize the genetic risks of translocations. Aquacult Res 31:133–143.

Knowlton N and Weigt LA, 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc R Soc Lond B 265:2257–2263.

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

Lynch M, 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 43:622–629.

Maehr DS and Lacy RC, 2002. Avoiding the lurking pitfalls in Florida panther recovery. Wild Soc Bull 30:971–978.

Mansfield KG and Land ED, 2002. Cryptorchidism in Florida panthers: prevalence, features, and influence of genetic restoration. J Wildlife Dis 38:693–698.

Marr AB, Keller LF, and Arcese P, 2002. Heterosis and outbreeding depression in descendants of natural immigrants to an inbred population of song sparrows (*Melospiza melodia*). Evolution 56:131–142.

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

Pederson DG, 1968. Environmental stress, heterozygote advantage and genotype-environment interaction in *Arabidopsis*. Heredity 23:127–138.

Rawson PD and Burton RS, 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.

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

Raymond M and Rousset F, 1995b. An exact test for population differentiation. Evolution 49:1280–1283.

Rieseberg LH, Sinervo B, Linder CR, Ungerer MC, and Arias DM, 1996. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. Science 272:741–745.

Rieseberg LH, Archer MA, and Wayne RK, 1999. Transgressive segregation, adaptation and speciation. Heredity 83:363–372.

Rousset F and Raymond M, 1995. Testing heterozygote excess and deficiency. Genetics 140:1413–1419.

Schneider S, Roessli D, and Excoffier L, 2000. Arlequin: a software for population genetics data analysis: Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, Univ. of Geneva.

Schwarzbach AE and Rieseberg LH, 2002. Likely multiple origins of a diploid hybrid sunflower species. Mol Ecol 11:1703–1715.

Soltis DE and Soltis PS, 1993. Molecular data and the dynamic nature of polyploidy. Crit Rev Plant Sci 12:243–275.

Templeton AR, 1986. Coadaptation and outbreeding depression. In: Conservation biology: the science of scarcity and diversity (Soulé M, ed). Sunderland, MA: Sinauer; 105–116.

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

Vittor BA, 1971. Effects of the environment on fitness-related life history characters in *Tigriopus californicus* (PhD diss.). Eugene: University of Oregon.

Weir BS and Cockerham CC, 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Welch ME and Rieseberg LH, 2002. Patterns of genetic variation suggest a single, ancient origin for the diploid hybrid species *Helianthus paradoxus*. Evolution 56:2126–2137.

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

Willett CS and Burton RS, 2003. Environmental influences on epistatic interactions: Viabilities of cytochrome c genotypes in interpopulation crosses. Evolution 57:2286–2292.

Willett CS and Burton RS, 2004. Evolution of interacting proteins in the mitochondrial electron transport system in a marine copepod. Mol Biol Evol 21(3):443–453.

Received January 12, 2004
Accepted July 31, 2004

Corresponding Editor: Brian Bowen