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HETEROSIS AND OUTBREEDING DEPRESSION IN INTERPOPULATION CROSSES SPANNING A WIDE RANGE OF DIVERGENCE

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Abstract.—The intertidal copepod *Tigriopus californicus* was used as a model organism to look at effects of crossing distance on fitness and to investigate the genetic mechanisms responsible. Crosses were conducted between 12 pairs of populations spanning a broad range of both geographic distance (5 m to 2007 km) and genetic distance (0.2% to 22.3% sequence divergence for a 606-bp segment of the mitochondrial COI gene). For each pair of populations, three fitness components (hatching number, survivorship number, and metamorphosis number) were measured in up to 16 cohorts including parentals, reciprocal F₁, F₂, F₃, and first-generation backcross hybrids. Comparisons of each set of cohorts allowed estimation of within- and between-locus gene interaction. Relative to parentals, F₁ hybrids showed a trend toward increased fitness, with no correspondence with population divergence, and a decrease in variance, which in some cases correlated with population divergence. In sharp contrast, F₂ hybrids had a decrease in fitness and an increase in variance that both corresponded to population divergence. Genetic interpretation of these patterns suggests that both the beneficial effects of dominance and the detrimental effects of breaking up coadaptation are magnified by increasing evolutionary distance between populations. Because there is no recombination in *T. californicus* females, effects of recombination can be assessed by comparing F₁ hybrid males and females backcrossed to parentals. Both recombinant and nonrecombinant backcross hybrids showed a decline in fitness correlated with population divergence, indicating that segregation among chromosomes contributes to the breakup of coadaptation. Although there was no difference in mean fitness between the two backcross types, recombinational backcrosses showed greater variance for fitness than nonrecombinational backcrosses, suggesting that the breakup of parental gene combinations within chromosomes has both beneficial and detrimental effects.

Key words.—Copepod, fitness, quantitative genetics, speciation, *Tigriopus californicus*.

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The fitness consequences of outbreeding between divergent populations or species are difficult to predict. Many hybrids are more fit than their parents, and the pursuit of this hybrid vigor or “heterosis” has been important in agriculture and animal breeding for over a century (Darwin 1876; Gowen 1952). This fitness increase is generally attributed to either overdominance (heterozygote advantage) or the masking of deleterious recessive alleles, although epistatic interactions may also be involved (Lynch 1991). However, some interpopulation or interspecific crosses result in fitness reductions known as “outbreeding depression” or “hybrid breakdown.” Such fitness declines can be apparent in F₁ hybrids, which have a complete haploid set of genes from each parent. Reduced fitness in these hybrids may be due to disruptions in local adaptation (i.e., gene × environment interactions; Templeton 1986), underdominance (Schierup and Christiansen 1995), or epistatic interactions (Waser 1993). Frequently, however, outbreeding depression is not manifested until the F₂ (Dobzhansky 1948; Templeton 1986), in which the original parental gene combinations are broken up by recombination, thus resulting in some offspring that are homozygous for one parent’s genes at one locus and the other parent’s genes at another locus. These genome rearrangements may disrupt epistatic interactions that confer fitness in specific environments (local adaptation), as well as gene interactions that are independent of the environment (intrinsic coadaptation).

The fitness effects of hybridization are a topic of renewed concern to a variety of fields in biology. Evolutionary biology

has experienced a resurgence of interest in the role of fitness effects in hybrid zone evolution (e.g., Barton and Hewitt 1985; Arnold et al. 1999) and hybrid speciation (e.g., Rieseberg et al. 1995; Rieseberg and Carney 1998). Plant and animal breeders are becoming increasingly interested in whether consequences of hybridization are predictable or whether they will continue to be a “hit-or-miss proposition” (Tave 1993, p. 198). Conservation biologists are also becoming progressively more concerned with hybridization effects. This concern is fueled by the increased frequency of gene exchange among field populations caused by accidental introductions, introgression between cultivated populations and wild populations (Linder et al. 1998), and translocation efforts aimed at bolstering population size and combating genetic erosion (Griffith et al. 1989; Mills and Allendorf 1996). All of these disparate fields would benefit from a better understanding of factors that might predict the consequences of hybridization.

A number of studies have looked at the relationship between outbreeding effects and the genetic or geographic divergence between populations or species. Some investigations of prezygotic isolation have reported a positive relationship between reproductive isolation and divergence both among populations (Lonsdale et al. 1988; Tilley et al. 1990) and among closely related species (Knowlton et al. 1993; Coyne and Orr 1997). However, others have found no apparent pattern either within species (Fenster and Sork 1988) or between species (Lessios and Cunningham 1990). Studies of postzygotic isolation have shown a positive relationship between reproductive isolation and divergence in a variety of taxa, including both within- and between-species crosses (Grosberg 1987; Lonsdale et al. 1988; Coyne and Orr 1997; Sasa et al. 1998). Investigations of plant populations have

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frequently shown "optimal outcrossing" at intermediate distances in which fitness is apparently maximized by avoiding the hazards of both inbreeding and outbreeding depression (reviewed in Waser 1993). All of these studies are limited to F_1 hybrids. Because gene action changes between the first and second generations of hybridization, F_1 fitness may not be a good predictor of fitness in the F_2 and later generations.

This paper focuses on postzygotic isolation up to the third generation of hybridization in a series of crosses between allopatric populations of the tidepool copepod *Tigriopus californicus*. These copepods have many advantages for breeding studies. They have a short generation time (approximately 23 days at 20°C; Burton 1987), breed continuously, and are easily maintained in captivity for many generations. The species is abundant in supralittoral pools ranging from Alaska to Baja California and population samples on the order of thousands of individuals can be collected routinely (pers. obs.). Population genetic structure has been well studied and has revealed extensive differentiation even between neighboring populations (e.g., Burton and Feldman 1981; Burton and Lee 1994; Burton 1997, 1998). The species has 12 pairs of chromosomes (Ar-rushdi 1963), with no recombination in females (Ar-rushdi 1963; Burton et al. 1981). Crosses between populations typically result in F_1 hybrid vigor and F_2 hybrid breakdown for a number of measures including development time (Burton 1987, 1990b), response to osmotic stress (Burton 1990a), and cytochrome *c* oxidase activity (Edmands and Burton 1998; Burton et al. 1999). However, outbreeding depression has also been observed in the F_1 (Brown 1991). The present study expands on these works by examining the correspondence between fitness and crossing distance and the genetic mechanisms underlying these effects. *Tigriopus californicus* is an ideal model organism for such a study because some reproductively compatible populations exceed 18% divergence in mitochondrial DNA (Burton 1998), thus allowing a continuum ranging from crosses between closely related populations to crosses between taxa that are more divergent than most species or even orders.

Gene actions were estimated by means of a line cross analysis. Although most genetic explanations of outbreeding effects focus on favorable within-locus interactions (dominance) driving heterosis and unfavorable between-locus interactions (epistasis) driving outbreeding depression, the situation may actually be more complex, with beneficial epistasis contributing to hybrid vigor and underdominance contributing to outbreeding depression (Lynch 1991). By comparing the phenotypic mean and variance for various cohorts (F_1 , F_2 , B_1 , etc.) within a cross, the composite effects of within- and between-locus gene interactions can be estimated (see reviews in Lynch and Walsh 1998). The lack of recombination in *T. californicus* females allowed further dissection of gene action because the role of recombination could be assessed by comparing fitness in hybrid males and females backcrossed to parentals.

MATERIALS AND METHODS

Reproductive Biology of the Study Organism

Tigriopus californicus is an obligately sexual, harpacticoid copepod whose reproductive biology has been well studied

(Egloff 1966; Vittor 1971; Burton 1985). Mature males use their first antennae to clasp immature females for up to seven days until the female undergoes her final molt. The female is then inseminated and released. Whereas males mate multiply, females mate only once in their lifetime and use stored sperm to fertilize multiple broods of eggs. Females that are not fertilized may still produce egg sacs, but the eggs invariably fail to develop. The mating system of *T. californicus* facilitates collection of virgin females: Males and immature females that have formed clasped pairs are simply dissected apart using a fine probe. At 15°C, fertilized females typically develop an egg sac within two to four days. New egg sacs are green to black and become orange or red as they mature, with hatching occurring after three to six days.

Population Sampling and Culture Maintenance

Copepods were collected from high intertidal pools at nine locations ranging from southeastern Alaska to Baja California, Mexico (Fig. 1). Because previous work showed high gene flow among different tidepools on the same rock outcrop (Burton and Swisher 1984), pools within outcrops were combined for all but one of the locations (CA). All cultures were maintained at 15°C with a 12:12 L:D photoperiod. Stock cultures were kept in natural seawater in 150-ml beakers with the addition of small amounts of commercial flake-type fish food to promote an algal bloom. Replicate beakers from the same population were periodically mixed together to promote panmixia.

Animals used for fitness assays were maintained in natural seawater filtered twice through Whatmann #1 filter paper, with the addition of 0.02 g nutritional yeast per 100 ml seawater; 35 ml of this mixture was used in petri dishes and 50–100 ml was used in beakers. Newly hatched larvae were transferred to new seawater-yeast after 14 days. In all containers not containing new larvae, the seawater-yeast mixture was changed weekly. This protocol appeared to provide excess food at all times. All animals used for the fitness assays were maintained in the same walk-in incubator.

Mating Design

Twelve pairs of populations were hybridized, with inter-population geographic distances ranging from 0 km to 2007 km (Table 1). Populations were maintained in the laboratory for two to eight months (approximately two to eight generations) before crosses were begun. For each cross (line A × line B), up to 16 cohorts were evaluated (listed as male × female): P_1 (A), P_2 (B), F_1 (A × B), rF_1 (B × A), F_2 (AB × AB), rF_2 (BA × BA), B_{1-1} (A × AB), B_{1-2} (AB × A), B_{1-3} (A × BA), B_{1-4} (BA × A), B_{2-1} (B × AB), B_{2-2} (AB × B), B_{2-3} (B × BA), B_{2-4} (BA × B), F_3 (AB × AB)(AB × AB), and rF_3 (BA × BA)(BA × BA). As a control, the fitness of the outbred parental populations (P_1 and P_2) for each cross was assessed in each generation of the experiment (maximum of three generations). No inbreeding was done during the course of the experiment. Therefore, F_3 hybrids (and generation 3 controls) descended from four different sets of great-grandparents (Fig. 2).

Crosses were done in three blocks. In the first block (three crosses), copepods were mated individually. One male and

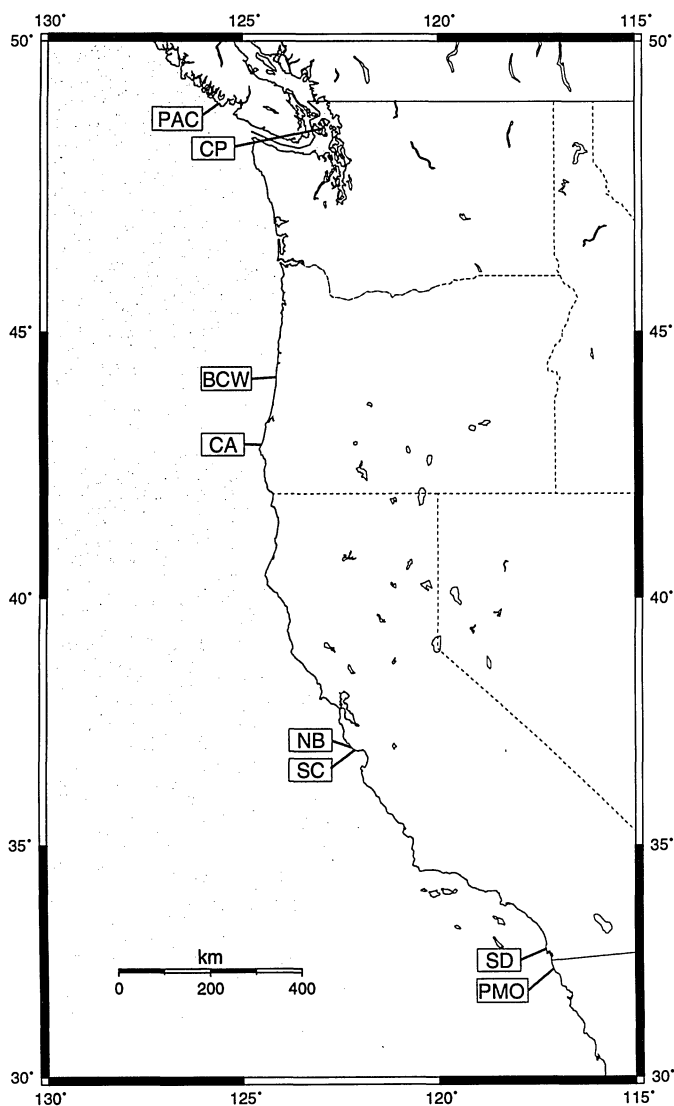


FIG. 1. Localities sampled for *Tigriopus californicus*. Site names, geographic coordinates and collecting dates are: PAC (Pachena Bay, Vancouver Island, BC, 48°46'N, 125°08'W, 7/96); CP (Cattle Point, San Juan Island, WA, 48°27'N, 122°58'W, 7/96 and 4/97); BCW (Bob Creek Wayside, OR, 44°15'N, 124°07'W, 8/96, 1/97, and 6/97); CA (CA1, tidepool 1; CA3, tidepool 3, Cape Arago, OR, 43°18'N, 124°24'W, 1/97); NB (Natural Bridges State Park, CA, 36°57'N, 122°04'W, 7/96 and 12/96); SC (Santa Cruz, CA, end of Swift Strait, 36°57'N, 122°03'W, 12/96); SD (San Diego, CA, south of Ocean Beach Pier, 32°45'N, 117°15'W, 7/96 and 6/97); PMO (Punta Morro, Baja California, Mexico, 31°52'N, 116°40'W, 7/96).

one virgin female were united in each petri dish. When females formed egg sacs they were removed to a new dish. On the day that larvae emerged, hatching number was determined by using a pasteur pipette to transfer each larva into a new dish. Occasionally, females dropped their egg sacs, but no larvae could be found. In these cases, the females were monitored to see if offspring were produced in the second or third clutches. If no larvae were found, the zero for the first clutch was not included in the dataset because the female may never have been fertilized. To determine survivorship number and metamorphosis number, the first clutch of offspring was re-

TABLE 1. Geographic distance, mean cytochrome oxidase I (COI) nucleotide divergence (proportion), and mean COI amino acid divergence (proportion) between the 12 pairs of populations used for breeding studies. See Figure 1 for population abbreviations.

Populations	Geographic distance (km)	Mean nucleotide divergence	Mean amino acid divergence
CA1, CA3	0.005	0.00165	0.00000
NB, SC	1	0.00165	0.00000
CA3, BCW	121	0.02310	0.01485
CP, PAC	164	0.00660	0.00495
CP, BCW	475	0.04950	0.01980
NB, SD	641	0.22277	0.03465
NB, PMO	751	0.20297	0.01980
BCW, NB	829	0.16832	0.03465
SD, BCW	1409	0.19885	0.03960
CA1, PMO	1440	0.19967	0.00990
BCW, PMO	1520	0.19142	0.02475
PAC, PMO	2007	0.19142	0.00990

counted after 14 days by transferring individuals (larvae plus metamorphosed copepodids) to a new dish. To identify mature males and virgin females, dishes were monitored until clasped pairs formed between siblings. Males and females were then dissected apart and reunited with unrelated mates (one male and one female per dish). Each clutch was divided approximately evenly between cohorts (i.e., F_1 s were split between matings to produce F_2 s, B_1 s, and B_2 s). Generation 2 offspring were monitored up until the 14-day recount. Control crosses followed exactly the same procedures, and the same outbred stocks were used for controls and backcrosses (see Fig. 2).

Procedures in the second block (six crosses) and third block (three crosses) were very similar to the first block, except that copepods were mass-mated to increase sample size and a larger number of cohorts were followed (all reciprocal F_1 s, F_2 s, B_1 s, B_2 s and, in some cases, F_3 s). Crosses were initiated by uniting either 10 males plus 10 females or 20 males plus 20 females in a petri dish. When females formed egg sacs they were removed to individual petri dishes and monitored as in the first block. Once offspring were recounted they were transferred to a beaker, with multiple clutches of the same cohort united in the same beaker to increase sample size. When clasped pairs were formed, males and females were dissected apart and placed in an appropriate mating dish (i.e., F_1 offspring went to F_2 , B_1 , or B_2 dishes; F_2 offspring went to F_3 dishes). Up to 20 males and 20 females were included in each dish. When females formed egg sacs, they were removed to individual petri dishes and monitored as previously described.

Fitness Assays

Three fitness components were measured in the first clutch of offspring: (1) hatching number: the number of live nauplii (larvae) on the day of hatching; (2) survivorship number: the number of larvae + copepodites (metamorphosed juveniles) alive 14 days after hatching; and (3) metamorphosis number: the number of copepodites alive 14 days after hatching. All experimental cultures were checked daily throughout the course of the experiment (524 days). Larvae and juveniles were counted by using a dissecting microscope to slowly scan

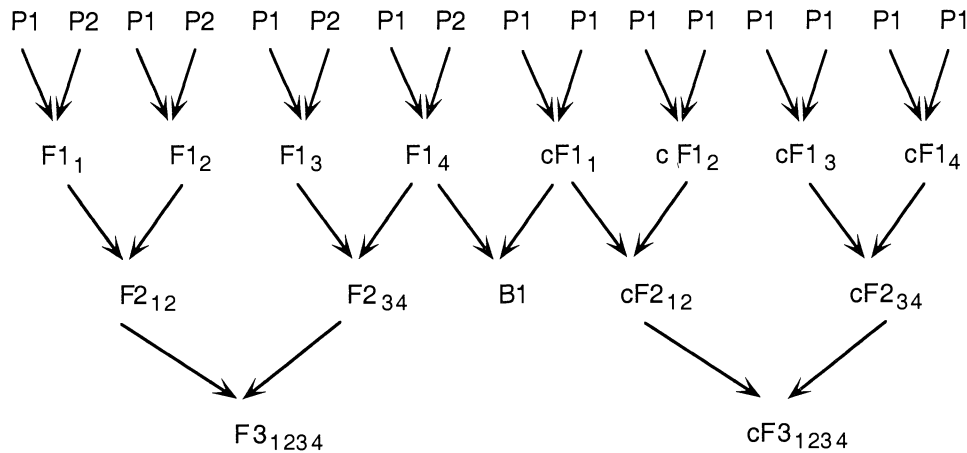


FIG. 2. Mating design to prevent inbreeding in experimental and control (c) crosses. Each P₁ and P₂ represents a different outbred individual or group of individuals. Not all matings are shown. For example, cohort B₁ could include matings between any F₁ male (F_{1,1-4}) and any control F₁ female (cF_{1,1-4}).

each petri dish, one segment at a time, and individually pipetting each animal into a new dish. Searches were considered complete when two thorough scans recovered no additional animals. For each generation of each cross, fitness estimates were standardized to have midparent means (the unweighted average of the two replicated parental means) equal to one.

Estimates of Population Divergence

Genetic divergence between populations was based on proportion sequence divergence for a 606-bp region of the mitochondrial gene cytochrome oxidase I (COI). It seems reasonable to expect this single mitochondrial locus to accurately reflect the true phylogeny because previous work on other *T. californicus* populations showed strong concordance between nuclear and mitochondrial gene genealogies (Burton and Lee 1994; Burton 1998) and there is no evidence for sex-biased dispersal in these organisms. As part of a larger phylogenetic study (S. Edmands, unpubl. ms.), the polymerase chain reaction (PCR) was used to amplify a 710-bp fragment of COI from one to three individual copepods per population using primers LCO1490 and HCO2198 in Folmer et al. (1994). DNA was extracted by boiling single copepods for 8 min in 50 μ l of 10% chelating resin (Walsh et al. 1991). The small sample size per population was justified based on the extremely low levels of within-population variation (0–0.3%). Amplification followed standard procedures (Saiki et al. 1988), using a reaction volume of 50 μ l and a magnesium chloride concentration of 2 mM. The following thermocycle profile was used: 3 min at 94°C; followed by 35 cycles of 60 sec at 94°C, 60 sec at 45°C, and 90 sec at 72°C; followed by 5 min at 72°C. All samples were sequenced using primer HCO2198 and ambiguous sites were resolved by also sequencing the opposite strand using primer LCO1490. PCR products were electrophoresed on a low-melting-point agarose gel, excised from the gel using sterile razor blades, and purified using either QIAquick spin columns (Qiagen, Valencia, CA) or GeneClean II silica matrix (Bio 101, Carlsbad, CA). Purified products were sequenced using Taq-DyeDeoxy Terminator Cycle sequencing (Applied Biosystems, Inc., Fos-

ter City, CA) and electrophoresed on an ABI Prism 377 DNA sequencer. DNA sequences were deposited in GenBank (accession numbers AF096936, AF096937, AF096940, AF096941, AF096943, AF096955, AF096956, AF096960, AF096961, AF09964–AF096968). DNA sequences were aligned using Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI) and percent sequence divergence was determined using the distance option in PAUP* version 4.0.0d60 (Sinauer Associates, Sunderland, MA).

Geographic divergence between populations was measured as the shortest straight-line distance between localities. Latitude and longitude for each locality were determined from 24,000:1 or 60,000:1 scale maps. The distance between each pair of latitude/longitude coordinates was calculated by a program provided by the Xerox PARC Map Server (<http://www.hetherman.com/links/distance/html>).

Line Cross Analyses

Gene action in each cross was partitioned by two different methods. Both methods assume free recombination ($c \sim 0.5$) and will therefore be biased if the genes underlying a particular trait are in close proximity. Assuming that the loci in question are distributed evenly across chromosomes, the equations could be modified if the average recombination fraction were known. However, this depends on the total map length, which is unknown in *Tigriopus*, as well as the number of chromosomes ($n = 12$ in *Tigriopus*; effectively $n = 6$ in females, in which there is no recombination within chromosomes). For species in which $n > 6$, c is typically greater than 0.45, and linkage is unlikely to have much effect on the interpretation of line cross means (Lynch and Walsh 1998).

In the first method, the same simplified genetic model was used to estimate gene action in each cross. Mean fitness in the midparent ($z[P]$), F₁ ($z[F_1]$) and F₂ ($z[F_2]$) were compared using a genetic model incorporating composite dominance (δ_1), additive \times additive epistasis (a_2), and dominance \times dominance epistasis (δ_2) effects using the following equations (Lynch and Walsh 1998):

$$z(F_1) - z(F_2) \sim \delta_1 + \delta_2 \quad (1)$$

and

$$z(F_1) - 2z(F_2) + z(P) \sim \alpha_2 + 2\delta_2. \quad (2)$$

In the second method, a joint scaling technique (e.g., Cockerham 1954; Hayman 1958; Hill 1982; Mather and Jinks 1982; Lynch 1991; Lynch and Walsh 1998) was used to determine the most appropriate genetic model for each cross and to further partition gene actions into maternal and digenic epistatic effects by comparing the mean and variance of fitness components for all cohorts within each cross. For crosses in which both reciprocals were assayed, this included 14 cohorts: P_1 , P_2 , F_1 , rF_1 , F_2 , rF_2 , B_{1-1} , B_{1-2} , B_{1-3} , B_{1-4} , B_{2-1} , B_{2-2} , B_{2-3} , and B_{2-4} . Composite effects were estimated using a Mathcad 4.0 program developed by Lair et al. (1997). This program uses a weighted least-squares model (Hayman 1958; Mather and Jinks 1982; Lynch and Walsh 1998) in which:

$$\mathbf{a} = (\mathbf{M}^T\mathbf{V}^{-1}\mathbf{M})^{-1}\mathbf{M}^T\mathbf{V}^{-1}\mathbf{z}, \quad (3)$$

$$\mathbf{C} = (\mathbf{M}^T\mathbf{V}^{-1}\mathbf{M})^{-1}, \quad (4)$$

and

$$\mathbf{y} = \mathbf{M}\mathbf{a}, \quad (5)$$

where \mathbf{a} is the vector of mean (μ_0), additive (α_1), dominance (δ_1), additive-maternal (α_{m1}), dominance-maternal (δ_{m1}), additive \times additive epistasis (α_2), additive \times dominance epistasis ($\alpha_1\delta_1$), and dominance \times dominance epistasis (δ_2); \mathbf{M} is the coefficient matrix for these parameters based on expected generation means using an additive-dominance-digeneic epistasis model with maternal effects (see table 2 in Lair et al. 1997); \mathbf{M}^T is the transpose of \mathbf{M} ; \mathbf{V} is a diagonal matrix of squared phenotypic standard errors of the line means; \mathbf{z} is a vector of observed line means; \mathbf{C} is a matrix with sampling variances of the respective elements in \mathbf{a} on the diagonal; and \mathbf{y} is a vector of fitted mean values for P_1 , P_2 , F_1 , rF_1 , F_2 , rF_2 , B_1 (B_{1-1} combined with B_{1-3}), rB_1 (B_{1-2} combined with B_{1-4}), B_2 (B_{2-1} combined with B_{2-3}), and rB_2 (B_{2-2} combined with B_{2-4}).

This full model (the ADME model) thus uses nine cohort means and variances to estimate eight parameters (μ_0 – δ_2) involving additive, dominance, maternal, and epistatic effects. The model can be condensed to a number of simpler forms. The nine cohort means can also be used to test an additive-dominance-maternal effects model (the ADM model) involving five parameters (μ_0 , α_1 , δ_1 , α_{m1} , and δ_{m1}). Alternatively, reciprocals can be combined to form six cohorts (P_1 , P_2 , F_1 , F_2 , B_1 , and B_2) which can be used in an additive model (the A model, which estimates μ_0 and α_1), an additive-dominance model (the AD model which estimates μ_0 , α_1 , and δ_1), and an additive-dominance-epistasis model (the ADE model, which estimates μ_0 , α_1 , δ_1 , α_2 , $\alpha_1\delta_1$, and δ_2).

The goodness-of-fit for each model was tested by the chi-square statistic:

$$\chi^2 = \mathbf{z}^T\mathbf{V}^{-1}\mathbf{z} - \mathbf{z}^T\mathbf{E}^{-1}\mathbf{y}, \quad (6)$$

with degrees of freedom equal to the number of cohorts minus the number of parameters. For each cross the A model was tested first. If this model was rejected then progressively more complex models were tested to see if they provided a significantly improved fit using the likelihood ratio test statistic, which is the difference in χ^2 -values between the full model and the reduced model with degrees of freedom equal

to the difference in degrees of freedom for the full and reduced models (Lynch and Walsh 1998). For crosses in which reciprocals were assayed (blocks 2 and 3), the models were tested in the order: A, AD, ADM, ADME. For crosses in which all reciprocals were not assayed (block 1), model A was tested first followed by model AD. If AD was rejected, then ADE was automatically accepted because the number of means exactly equals the number of parameters to be estimated (degrees of freedom = 0) and thus the adequacy of ADE cannot be tested. After the appropriate genetic model was fitted, the composite effects were obtained from vector \mathbf{a} . The significance of each of these effects was tested by:

$$t_i = a_i/(\text{Var } \sqrt{a_i}). \quad (7)$$

RESULTS

Geographic divergence between the 12 pairs of populations used for breeding studies ranged from 5 m to 2007 km (Table 1). Genetic divergence for these same populations based on a 606 bp segment of the mitochondrial COI gene ranged from 0.2% to 22.3% nucleotide divergence and from 0% to 3.5% amino acid divergence (Table 1). Pairs of populations to be hybridized were chosen in an attempt to span a broad spectrum of geographic distances. Subsequent sequencing of these nine populations plus 17 others (S. Edmands, unpubl. ms.) produced a COI genealogy showing a simple south-to-north trend, with northern populations being derived and showing reduced interpopulation divergence. Genetic distance in the population pairs used for the present study fell into two fairly tight clusters: 0.2–5.0% and 16.8–22.3% nucleotide divergence. Geographic divergence was highly correlated with nucleotide divergence ($R = 0.81$, $P = 0.001$), but not with amino acid divergence ($R = 0.39$, $P = 0.213$).

A total of 149 cohorts were assayed with a mean sample size of 13.4 ± 0.6 (SE). The complete dataset is available from the author. For each generation of each cross, fitness estimates were standardized to have midparent means (the unweighted average of the two replicated parental means) equal to one.

StatViewII (SAS Institute, San Francisco, CA) was used for simple linear regressions of hybrid fitness on both the geographic distance and nucleotide divergence between populations (Table 2). Regressions of fitness on amino acid divergence between populations were also tested but no significant relationships were found. The three fitness components (hatching number, survivorship number, and metamorphosis number) generally showed very similar patterns, although regressions involving metamorphosis number tended to be weaker, apparently due to substantially higher variance in these estimates. F_1 hybrids and F_2 hybrids had strikingly different patterns for both mean (Table 2, Fig. 3) and variance (Table 2, Fig. 4). F_1 hybrid means were generally similar to or greater than the midparents, with no significant correspondence to either geographic distance or genetic distance for any of the three fitness components. In contrast, F_2 hybrid means for all three fitness components showed a significant decline with increasing genetic and geographic distance. Because of the large difference in mean between generations, changes in variance were compared

TABLE 2. Regression coefficients for the relationship between geographic or genetic population divergence (X) and mean or coefficient of variation of fitness components (Y). H, hatching number; S, survivorship number; M, metamorphosis number; NRB, nonrecombinant backcross; RB, recombinant backcross; CV, coefficient of variation.

Cross type (# crosses)	Fitness component	Geographic divergence		COI nucleotide divergence	
		Regression equation	R ²	Regression equation	R ²
F ₁ (21)	mean H	Y = 1.049 + 2.981 × 10 ⁻⁵ X	0.009 ^{ns}	Y = 1.040 + 0.300X	0.016 ^{ns}
	mean S	Y = 1.266 + 1.582 × 10 ⁻⁴ X	0.064 ^{ns}	Y = 1.280 + 1.000X	0.045 ^{ns}
	mean M	Y = 1.202 + 4.826 × 10 ⁻⁴ X	0.185 ^{ns}	Y = 1.285 + 2.672X	0.098 ^{ns}
	CV H	Y = 1.049 - 1.518 × 10 ⁻⁴ X	0.107 ^{ns}	Y = 1.126 - 1.773X	0.254*
	CV S	Y = 1.103 - 3.519 × 10 ⁻⁴ X	0.164 ^{ns}	Y = 1.165 - 3.059X	0.215*
	CV M	Y = 0.826 - 4.866 × 10 ⁻⁵ X	0.020 ^{ns}	Y = 0.817 - 0.268X	0.011 ^{ns}
F ₂ (21)	mean H	Y = 0.893 - 2.377 × 10 ⁻⁴ X	0.366**	Y = 0.949 - 2.196X	0.541***
	mean S	Y = 1.044 - 3.834 × 10 ⁻⁴ X	0.390**	Y = 1.125 - 3.457X	0.549***
	mean M	Y = 1.849 - 8.702 × 10 ⁻⁴ X	0.364**	Y = 1.753 - 5.317X	0.235*
	CV H	Y = 0.989 + 4.749 × 10 ⁻⁴ X	0.238*	Y = 0.988 + 3.241X	0.219*
	CV S	Y = 0.831 + 4.794 × 10 ⁻⁴ X	0.275*	Y = 0.808 + 3.477X	0.285*
	CV M	Y = 0.847 + 2.429 × 10 ⁻⁴ X	0.131 ^{ns}	Y = 0.733 + 2.726X	0.324**
F ₃ (5)	mean H	Y = 1.210 - 3.577 × 10 ⁻⁴ X	0.168 ^{ns}	Y = 1.218 - 2.814X	0.179 ^{ns}
	mean S	Y = 1.657 - 6.064 × 10 ⁻⁴ X	0.142 ^{ns}	Y = 1.685 - 4.886X	0.158 ^{ns}
	mean M	Y = 1.836 - 7.622 × 10 ⁻⁴ X	0.184 ^{ns}	Y = 1.884 - 6.239X	0.212 ^{ns}
NRB (36)	mean H	Y = 1.157 - 3.408 × 10 ⁻⁴ X	0.307***	Y = 1.140 - 2.639X	0.268**
	mean S	Y = 1.379 - 0.001X	0.351***	Y = 1.368 - 4.402X	0.331***
	mean M	Y = 3.282 - 0.002X	0.121*	Y = 3.239 - 14.494X	0.336*
RB (36)	mean H	Y = 0.964 - 1.337 × 10 ⁻⁴ X	0.079 ^{ns}	Y = 0.960 - 1.068X	0.073 ^{ns}
	mean S	Y = 1.390 - 3.856 × 10 ⁻⁴ X	0.137*	Y = 1.385 - 3.138X	0.132*
	mean M	Y = 3.038 - 0.001X	0.100 ^{ns}	Y = 2.984 - 11.73X	0.090 ^{ns}

^{ns} P > 0.05; * P < 0.05; ** P < 0.01; *** P < 0.001.

using coefficients of variation, which control for differences in mean when standard deviations scale with means ($P < 0.001$ for regressions of standard deviation on mean for each of the three fitness components). Coefficients of variation in F₁ hybrids tended to be lower than the midparent, and variation for both hatching number and survivorship number declined significantly with geographic distance. F₂ hybrids showed the opposite pattern: Coefficients of variation tended to be higher than the midparent, and in all but one of six cases (metamorphosis number vs. geographic divergence) variation increased significantly with geographic and genetic divergence. For five of the 21 crosses (including reciprocals), fitness measures were extended to the F₃. Mean F₃ hybrid fitness ranged from lower than the F₂ (CA3 × BCW) to higher than the midparent (CP × BCW) and no significant correspondence between fitness and population divergence was found (Table 2). Note that all these fitness estimates exclude data for females that produced zero offspring in the first three clutches because these females may never have mated. No obvious patterns were found in the number of zeroes obtained from different crosses. For example, after standardization to the control, the average proportion of zero clutches was 0.16 ± 0.02 (SE) in the F₁ and 0.13 ± 0.04 in the F₂ (two-tailed t -test for 21 crosses: $t = 0.56$, $P = 0.58$).

Because there is no recombination in *T. californicus* females, comparisons between F₁ hybrid females crossed to parentals ("non-recombinant backcross") and F₁ hybrid males crossed to parentals ("recombinant backcross") allow a test of the effects of recombination. Both nonrecombinant backcrosses (B₁₋₁, B₁₋₃, B₂₋₁, and B₂₋₃) and recombinant backcrosses (B₁₋₂, B₁₋₄, B₂₋₂, and B₂₋₄) showed significant fitness

reductions with increasing population divergence, although regressions were considerably stronger for nonrecombinant backcrosses (Table 2, Fig. 5). If breakdowns in coadaptation are driven largely by recombination within chromosomes, then recombinant backcrosses should have a lower mean and a higher variance than nonrecombinant backcrosses. For the nine crosses in which all reciprocal backcrosses were assayed, one-tailed, paired t -tests using the appropriate pairs (B₁₋₁ and B₁₋₂, B₁₋₃, and B₁₋₄, etc.) showed no difference in mean (Table 3). However, there was a trend toward higher variance in recombinants for all three fitness components and this pattern was significant for survivorship number (Table 3). Variance in recombinants is also significantly higher ($\chi^2 = 15.923$, $df = 6$, $P < 0.05$) when probabilities for the three fitness components are combined using the method described by Fisher (Sokal and Rohlf 1981, p. 780).

Gene interactions in all 12 crosses (with reciprocals combined) were inferred by applying a simplified model comparing parental, F₁, and F₂ means (eqs. 1 and 2). Results for hatching number are shown in Figure 6. All fitness components showed a similar pattern: Both $\delta_1 + \delta_2$ (the sum of composite dominance effects) and $\alpha_2 + 2\delta_2$ (the net loss in fitness due to segregation and recombination among parental gene combinations) are generally positive and increase with population divergence.

The most appropriate model for each cross was determined by means of joint scaling tests (eqs. 3-7). In each of the 12 crosses, the additive model could be rejected for at least one of the three fitness components (Table 4). In some cases, the most complex model that could be tested (the ADME model) still did not adequately explain the data, suggesting that linkage, higher order epistasis, or maternal-offspring genotype

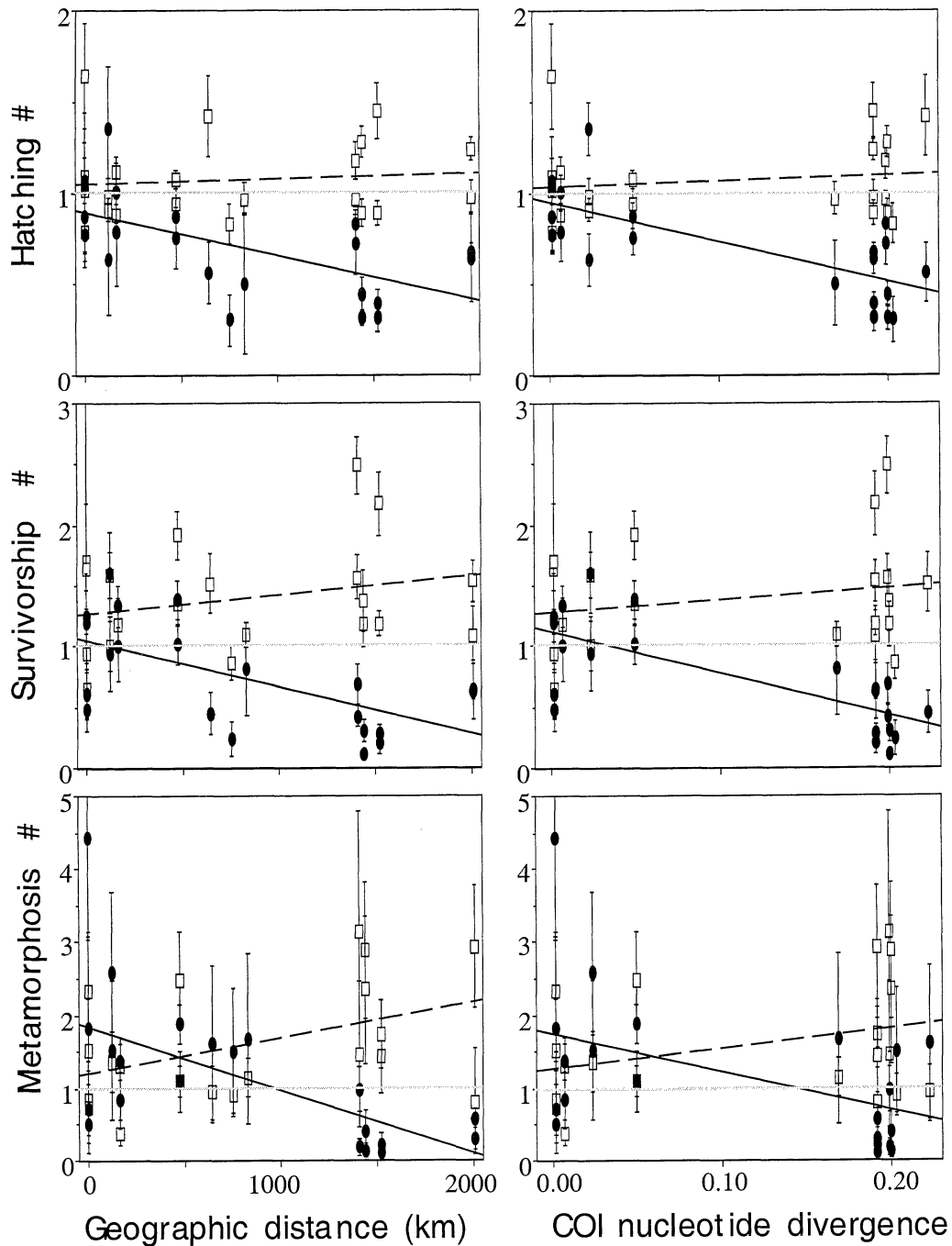


FIG. 3. Linear regressions of mean fitness components in F₁ (white rectangles, dashed lines) and F₂ (black ovals, solid lines) inter-population hybrids on the genetic and geographic divergence between populations. Data are standardized to have midparent means equal to one (stippled lines). Error bars are ± 1 SE. Regression coefficients are given in Table 3.

interaction may be involved. The pattern of gene interactions varied widely among crosses. Significant dominance interactions were always positive thus indicating a favorable effect on fitness. Significant additive \times additive epistatic interactions were also always positive, indicating a detrimental effect on fitness. Both favorable and detrimental effects were found for additive \times dominance and dominance \times dominance interactions.

DISCUSSION

F₁ Heterosis and F₂ Hybrid Breakdown

Distinctly different patterns were found for fitness in first and second generation hybrids. F₁ hybrids tended to show an increase in mean and a decrease in variance, whereas F₂ hybrids showed a decrease in mean and an increase in variance. Similar changes in mean have been shown in a variety

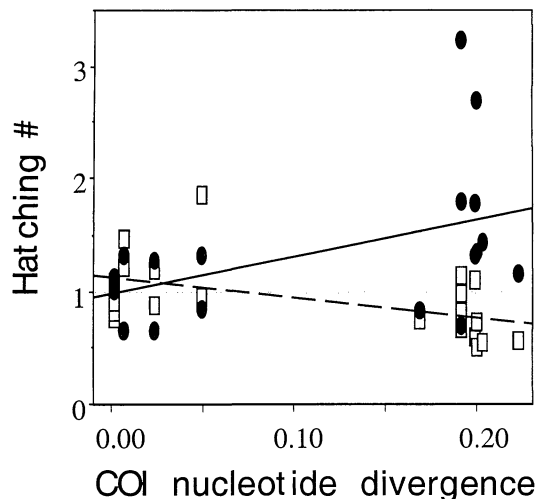


FIG. 4. Linear regressions of coefficients of variation for hatching number in F_1 (white rectangles, dashed lines) and F_2 (black ovals, solid lines) interpopulation hybrids on the genetic divergence between populations. Data are standardized to have midparent coefficients of variation equal to one (stippled lines). Regression coefficients are given in Table 3.

of taxa (reviewed in Endler 1977), including *T. californicus* (reviewed in Burton et al. 1999). This study is unusual, however, in demonstrating that the effects are magnified with increasing levels of population differentiation. Genetic interpretation of parental, F_1 , and F_2 means suggests that composite dominance effects are favorable and increase with population divergence. Although one might expect highly differentiated alleles at the same locus to show conflict (underdominance), these results show the benefits of heterozygosity to continually increase with distance, even in crosses between populations that are more than 20% divergent. In contrast to the benefits of within-locus hybridity, between-locus hybridity was interpreted as being detrimental, and these interactions were also strongly correlated with population divergence.

Variance in F_1 hybrids might be predicted to be either higher or lower than parentals. Increased F_1 variance has been reported in a number of studies (Vetukhiv 1953; Oliver 1972; Gharrett and Smoker 1991), and can be attributed to increased nonadditive genetic variation in crosses between populations that do not have fixed allelic differences. This study, like previous studies in *T. californicus* (Burton 1990b) and other organisms (Wallace 1955; Vetukhiv and Beardmore 1959), showed a decrease in F_1 variance, and this effect was magnified at higher levels of population divergence. This result

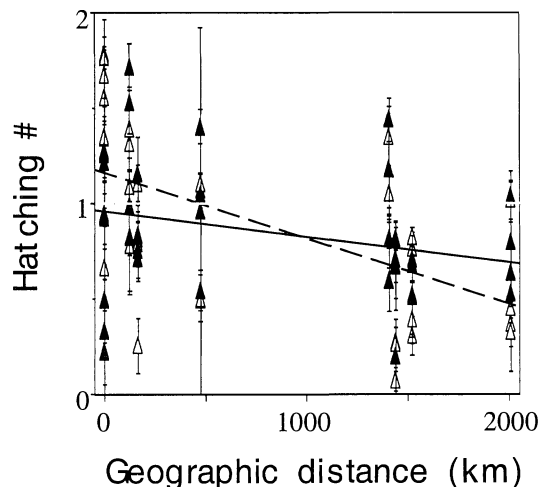


FIG. 5. Linear regressions of hatching number in nonrecombinant (white triangles, dashed lines) and recombinant (black triangles, solid lines) interpopulation backcross hybrids on geographic divergence between populations. Data are standardized to have midparent means equal to one (stippled lines). Error bars are ± 1 SE. Regression coefficients are given in Table 3.

may be attributed to the masking of deleterious recessives and the general reduction in genetic variation in crosses between populations with fixed differences (Endler 1977). Alternatively, the reduced variance may be due to a correspondence between developmental stability and heterozygosity (e.g., Lerner 1954), although evidence for such a relationship is equivocal (Mitton and Grant 1984; Palmer and Strobeck 1986) and the mechanism is unknown. In contrast to the F_1 , F_2 hybrids showed increased variance. This effect was expected and has been reported in a multitude of taxa (see Endler 1977), including *T. californicus* (Burton 1990b). The increase in variance is presumably caused by recombination and segregation among the original parental genotypes.

Geographic and Genetic Divergence

Geographic distance and nucleotide divergence for the 12 pairs of populations assayed were highly correlated, and the two distance measures were equally good predictors of hybrid fitness. However, the geographic distances were fairly evenly distributed, whereas the genetic distances formed a closely related group (0–5%) and a more distantly related group (17–22%). With one exception, crosses between populations with less than 5% sequence divergence showed little clear evidence of F_2 breakdown: Both reciprocal crosses were not significantly below the midparent for any of the three fitness

TABLE 3. One-tailed, paired *t*-tests of the mean and variance of fitness components in nonrecombinant versus recombinant first-generation backcross hybrids.

Fitness component	df	Mean			Variance		
		Nonrecomb. – recomb.	Paired <i>t</i> -value	<i>P</i>	Nonrecomb. – recomb.	Paired <i>t</i> -value	<i>P</i>
Hatching number	35	0.030	0.342	0.3672	–0.103	–1.440	0.0794
Survivorship number	35	–0.139	–1.015	0.1584	–0.403	–2.086	0.0222
Metamorphosis number	35	–0.068	–0.096	0.4621	–7.086	–0.860	0.1977

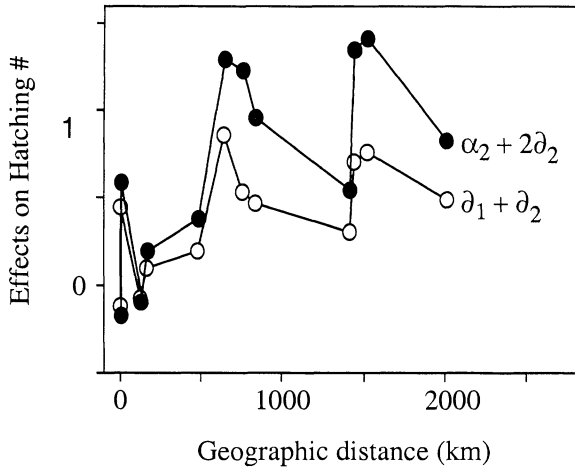


FIG. 6. Relationship between geographic divergence and genetic interactions inferred from a simple genetic model comparing mean hatching number in midparent, F_1 , and F_2 . Both $\delta_1 + \delta_2$ (the sum of composite dominance effects) and $\alpha_2 + 2\delta_2$ (the net loss in fitness due to segregation and recombination among parental gene combinations) are positive and increase with population divergence. Data for reciprocal crosses were combined and standardized to have midparent means equal to one.

components. The exception is the cross between NB and SC (1 km apart, 0.2% sequence divergence), in which a trend toward breakdown was found in all cases, with significant declines in survivorship number for both reciprocal crosses. Because these populations are only 1 km apart and live in seemingly indistinguishable habitats, it is difficult to imagine that this incompatibility is driven by localized selection, although a definitive test for local adaptation would require reciprocal transplants.

Role of Recombination

Nonrecombinant backcrosses (F_1 female \times parental male) and recombinant backcrosses (F_1 male \times parental female) showed no difference in mean fitness, and both showed significant declines with increasing population divergence. This suggests that recombination alone does not explain the breakup of coadaptation and, therefore, that harmonious gene interactions extend beyond single chromosomes. This is consistent with previous work on *T. californicus* by Burton (1987) in which analysis of differential viability of F_2 genotypes identified by allozyme markers showed evidence for interchromosomal coadaptation. Although recombinant and nonrecombinant backcrosses had similar means in the current study, recombinant backcrosses had significantly higher variance. This suggests that the breakup of parental gene combinations by recombination had both positive and negative effects. The difference in variance between backcross types is conservative because recombinants had higher variance despite having a lower sample size (comparisons of standard error show an even stronger difference). Although efforts were made to equalize the number of parents in the 36 pairs of recombinant and nonrecombinant backcrosses, significantly fewer females produced offspring in the first, second, or third clutches when the male was the hybrid parent (paired, two-tailed t -test: $t = 2.44$, $df = 35$, $P = 0.02$). This pattern

could be the result of prezygotic isolation (reduced mating success or sterility in hybrid males) or postzygotic isolation (complete inviability in the offspring of recombinant crosses).

It should be noted that differential fitness in male and female hybrids may complicate the interpretation of the backcross data. A pattern known as Haldane's rule has been found in a variety of taxa: When F_1 hybrid inviability or sterility occurs in only one of the two sexes, it is almost always the heterogametic sex (for a recent review, see Orr 1997). This pattern is generally attributed to interactions between X-linked genes and autosomal genes or cytoplasmic factors, although the exact mechanism is a subject of continued controversy (Orr 1997). If Haldane's rule extends beyond inviability or sterility to F_1 fecundity, it could have affected results in the backcrosses. In *T. californicus*, sex-determining mechanisms have yet to be deciphered (Egloff 1966) and karyotyping shows no obvious sex chromosomes (Ar-rushdi 1963) so it is not known which sex, if either, is heterogametic. If females are heterogametic, which seems likely given the correspondence between restricted recombination and heterogamy in other taxa (Bull 1983), then nonrecombinant (female) backcrosses may result in reduced fitness due to Haldane's rule. Alternatively, male *T. californicus* may be heterogametic, and this may explain the smaller sample sizes when males are the hybrid parents. In this case, hybrid breakdown in the nonrecombinant backcrosses must be attributed to segregation alone, whereas breakdown in recombinant backcrosses could involve the combined effects of recombination, segregation, and Haldane's rule. Further work on sex chromosomes in *T. californicus* is needed before these effects can be teased apart.

Duration of Outbreeding Depression

The nature of coadapted gene complexes has important implications for the duration of outbreeding depression. The extent of coadaptation is expected to be a compromise between the buildup of gene complexes by selection and the breakdown of these complexes by recombination and segregation. Some evidence suggests that these conflicting forces often limit coadapted gene complexes to very few, tightly linked genes (Templeton 1986). Such compact linkage groups are likely to be difficult to disrupt, and the full extent of outbreeding depression may not be apparent until several generations of recombination have occurred. However, once gene interactions are disrupted, selection might be expected to rapidly reestablish parental genotypes or even genotypes with superior fitness (Templeton 1981, 1986). Whereas many coadapted loci may be in close proximity, associations may also develop between loci on distal ends of the same chromosome or even on multiple chromosomes if the breakup of coadaptation is sufficiently restricted by population subdivision, inbreeding, asexual reproduction, or physical blocks to recombination. Such loose associations could easily be disrupted by hybridization, and natural selection is likely to be inefficient at recovering fitness, because recombination and segregation will repeatedly generate maladapted genotypes. In addition to the effects of linkage, the duration of outbreeding depres-

TABLE 4. Significant positive (+) and negative (–) gene interactions using the most appropriate genetic model for each fitness component (H, hatching number; S, survivorship number; M, metamorphosis number) in 12 interpopulation crosses, listed in order of increasing geographic distance between populations. Crosses BCW × NB, NB × PMO, and NB × SD were not tested for maternal effects α_{m1} and δ_{m1} . In some analyses the data deviated significantly from the most parsimonious model that could be tested. See text for details. δ_1 , dominance; α_{m1} , additive-maternal; δ_{m1} , dominance-maternal; α_2 , additive × additive epistasis; $\alpha_1\delta_1$, additive × dominance epistasis; δ_2 , dominance × dominance epistasis.

Cross	Fitness component	Model used	δ_1	α_{m1}	δ_{m1}	α_2	$\alpha_1\delta_1$	δ_2
CA1 × CA3	H	A						
	S	A						
	M	ADME	+					–
NB × SC	H	ADM	+		–			
	S	ADM			–			
	M	A						
CA3 × BCW	H	A						
	S	ADME**	+		+	+	+	–
	M	ADME***					+	
CP × PAC	H	ADME					+	+
	S	A						
	M	A						
CP × BCW	H	ADM		+	–			
	S	AD	+					
	M	A						
NB × SD	H	ADE						
	S	ADE						
	M	A						
NB × PMO	H	ADE	+			+	–	
	S	ADE	+			+	–	–
	M	A						
BCW × NB	H	ADE	+			+		–
	S	ADE	+			+		–
	M	A						
SD × BCW	H	ADM		+	–			
	S	ADME*		+		+		
	M	A						
CA1 × PMO	H	ADME***			+	+		+
	S	ADME*		+	+	+		+
	M	ADME				+		
BCW × PMO	H	ADME***	+			+		
	S	ADME***			+	+		
	M	ADM		+	–			
PAC × PMO	H	ADME		+	+			+
	S	ADME		+	+			+
	M	A						

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

sion will depend on the number of genes involved in co-adapted complexes. Outbreeding depression driven by few genes of large effect should be more easily repaired by natural selection than outbreeding depression driven by many genes of small effect.

In the present study, the five crosses that were extended to the F_3 provide a glimpse of the duration of outbreeding depression. Results were highly variable. In the two crosses with little evidence for F_2 breakdown, fitness in F_3 hybrids actually exceeded parental values in one case (CP × BCW), whereas F_3 fitness showed a significant decline in another case (CA3 × BCW). In crosses with significant F_2 breakdown, F_3 fitness returned to parental values in one case (SD × BCW), whereas F_3 fitness showed little improvement in 2 cases (reciprocal BCW × PMO).

Conservation and Management Implications

Results of hybridization in one taxon cannot be expected to provide accurate prescriptions for the conservation of other taxa. However, the consequences of outbreeding are so poorly known that studies of even a single species may contribute to conservation decisions that must be made long before a broad consensus can emerge. Perhaps the most relevant result of the present study is the striking change in fitness between the F_1 and F_2 generations. This pattern has been found in a variety of organisms (e.g., Endler 1977; Burton 1990b) and underscores the need to follow hybridizations beyond the first generation. This study expands on previous works by showing that fitness in the F_1 is of no value whatsoever in predicting fitness in the F_2 (regressions of F_2 on F_1 : hatching

number: R [correlation coefficient] = 0.10, P = 0.67; survivorship number: R = 0.03, P = 0.91; metamorphosis number: R = 0.01, P = 0.97). To take an extreme example of the change in fitness between generations, survivorship number in the PMO \times BCW cross increased to more than double parental values in the F_1 , but it then decreased to less than 30% of parental values in the F_2 . The temporary outbreeding enhancement in the first generation could exacerbate the problem of hybridizing populations, because F_1 hybrids may outcompete "pure" individuals, resulting in a disproportionate influx of unfit backcross and F_2 hybrids.

Based on studies of *Drosophila*, it has been suggested (Templeton 1986) that coadapted gene complexes often involve few tightly linked genes and that disruption of such small gene complexes can be rapidly repaired by natural selection, thus causing outbreeding depression to be a short-lived phenomenon. As discussed above, the backcross data for *Tigriopus* suggest that coadapted gene complexes may be more widespread. This may cause outbreeding depression to be more difficult to purge than has often been assumed.

It is important to note that all crosses in this study were conducted in a controlled, seemingly benign laboratory environment. Conditions in the wild are likely to be more stressful, and such adverse conditions have been shown to alter the consequences of hybridization in a variety of ways. For example, work by Vetukhiv and Beardmore (1959) on *Drosophila pseudoobscura* showed that stress reduced both the benefits and disadvantages of hybridity (reduced F_1 hybrid vigor and F_2 hybrid breakdown). In contrast, studies of the mosquito *Wyeomyia smithii* by Armbruster et al. (1997) showed that stress simply increased the benefits of hybridity (increased F_1 hybrid vigor and reduced F_2 hybrid breakdown). Further work is needed to show how environmental conditions modify the consequences of outbreeding in *T. californicus* as well as in other taxa.

In sum, this study suggests that both genetic and geographic distance are useful, although not perfect, predictors of F_2 hybrid fitness and that hybridizations are best restricted to the least divergent possible populations. The extent to which outbreeding effects in *T. californicus* are representative of other taxa will not be known until similar studies extending to the F_2 are done in other species.

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