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Recombination in Interpopulation Hybrids of the Copepod *Tigriopus californicus*: Release of Beneficial Variation Despite Hybrid Breakdown

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Crosses between divergent populations of the copepod *Tigriopus californicus* typically result in fitness reductions for both F_2 and backcross hybrids. Because females in this species lack chiasmatic meiosis, both recombinant and nonrecombinant backcross hybrids can be created. Recombinant hybrids were found to have significantly faster development time for both males and females in 2 pairs of crosses, indicating the creation of favorable gene combinations by disrupting parental linkage groups.

The shuffling of gene combinations by recombination and segregation can have unpredictable effects on the fitness of hybrids. On the one hand, disruption of parental gene interactions can create negative epistasis. It is the exposure of deleterious hybrid gene combinations involving recessive alleles that explains the common pattern of hybrid fitness problems being delayed until the second generation (e.g., Endler 1977; Edmands 2007) or even the third generation (Fenster and Galloway 2007).

On the other hand, hybridization may occasionally create gene combinations that are more fit than parentals. For example, in crosses between ecotypes of the annual grass *Avena barbata*, later generation hybrids have mean fitness lower than the midparent, yet some hybrid individuals outperform their parents (Johansen-Morris and Latta 2006). Hybrid superiority such as this can be due to both the creation of advantageous epistatic interactions (e.g., Erickson and Fenster 2006) and transgressive segregation in which parental alleles in repulsion phase are disrupted (e.g., Latta et al. 2007).

New hybrid gene combinations are created during meiosis through both recombination within chromosomes and segregation among chromosomes. The copepod *T. californicus* is one model in which the fitness effects of recombination and segregation in hybrids can be distinguished because recombination has not been observed in females (Ar-rushdi 1963; Burton et al. 1981) and both male and female interpopulation hybrids retain viability and fertility (e.g., Burton et al. 1981). We can therefore contrast nonrecombinant backcross hybrids (F_1 female × parental male) with recombinant backcross hybrids (F_1 male × parental female).

Crosses between divergent populations of this species have been shown to result in reduced fitness in both recombinant and nonrecombinant backcross hybrids relative to parentals, indicating that segregation among chromosomes contributed to the breakup of coadaptation (Edmands 1999). No differences in mean fitness components were detected between the 2 backcross types, but sample sizes for each cross were small. This study uses a much larger sample size for a single pair of populations and focuses specifically on development time, a trait that has been shown to be particularly prone to hybrid breakdown (e.g., Burton 1990) and is tightly correlated with fitness in continuously breeding species such as *T. californicus* (Lewontin 1974).

Materials and Methods

Populations were sampled from Laguna Beach, CA ("L," 33°33′N, 117°47′W), and Royal Palms, Palos Verdes, CA ("R," 33°42′N, 118°19′W). These 2 populations have been found to be approximately 17% divergent in mitochondrial DNA (cytochrome oxidase I; Edmands 2001; Peterson D, unpublished data). All cultures were kept in a 20 °C incubator with a 12:12 h light:dark cycle. Stock cultures were maintained in 400-ml beakers in natural seawater supplemented with commercial flake-type fish food and Spirulina algae.

Tigriopus californicus females mate only once and use stored sperm to fertilize multiple broods of offspring (Egloff 1966; Vittor 1971; Burton 1985). Inbred lines can therefore be created by isolating a single gravid female and allowing full siblings and their subsequent progeny to mate freely. Isofemale lines from the L and R populations were created by placing a single gravid female in a petri dish with

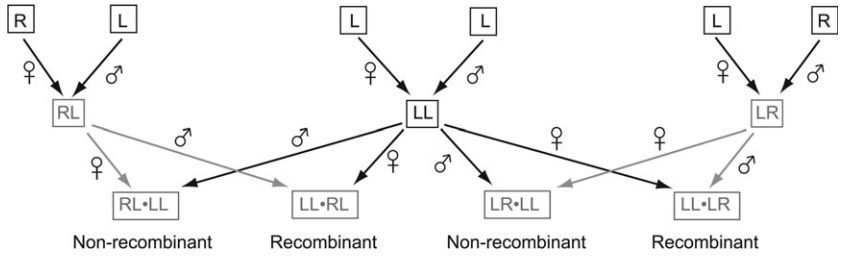

Figure 1. Design of crossing experiments using inbred lines from Royal Palms, CA (R) and Laguna Beach, CA (L). Because female *Tigriopus californicus* lack recombination, backcrossing F_1 hybrid females to parental males is a nonrecombinant cross. Parental cohorts are in black, and hybrid cohorts are in gray. Cohort names are listed as female × male.

filtered seawater (37 μm) containing 0.2 mg finely ground Spirulina per milliliter. Isofemale lines were maintained for 2–4 months before experimental crosses were begun (minimum generation time is approximately 23 days at 20 °C; Burton 1987).

In *T. californicus*, mature males use their antennae to clasp virgin females and mate guard them until the females reach reproductive maturity (Egloff 1966; Vittor 1971; Burton 1985). Virgin females can therefore be obtained by placing a clasped pair on a piece of filter paper and teasing the male and female apart under a dissecting microscope using a fine probe. Experimental backcrosses were set up by combining virgin females from one population with mature males from the other population. Both reciprocals (R female × L male and L female × R male) were set up in sets of 5 females combined with 5 males. In addition, a within-population cross (L female × L male) was set up to provide appropriate material for the backcross. Algal rations were the same as for the isofemale lines. A total of approximately 50 pairs were set up for each of the 3 crosses.

Dishes were checked 7 days a week. When females formed egg sacs, they were transferred to a new petri dish containing new seawater/Spirulina. When F_1 larvae hatched, parental females were again transferred to a new dish. When F_1 offspring formed clasped pairs, the pairs were dissected apart and F_1 females were transferred to a new dish containing appropriate males. Four types of backcrosses were established (Figure 1): 2 recombinant crosses (LL female × LR male and LL female × RL male) and 2 nonrecombinant crosses (LR female × LL male and RL female × LL male). In all, 100–300 pairs were established for each cross, in sets of 5 females and 5 males per dish. When F_1 females formed egg sacs, they were transferred to a new dish with 1 female per dish. Up to 3 clutches were assayed for each female. When backcross offspring hatched, parental females were again transferred to a new dish. Offspring dishes were monitored daily until the first male and the first female reached maturity. Maturity for females was defined as extruding an egg sac, and maturity for males was defined by the acquisition of clasping antennae. Minimum development times (birth to maturity) in the 4 crosses were compared by analysis of variance (ANOVA) using Statistica 7.1 (StatSoft, Tulsa, OK). Male and female development times were analyzed separately.

Results and Discussion

Minimum development time was quantified in an average of 183.4 clutches for each of the 8 cohorts (Figure 2). ANOVA showed significant differences in development time among

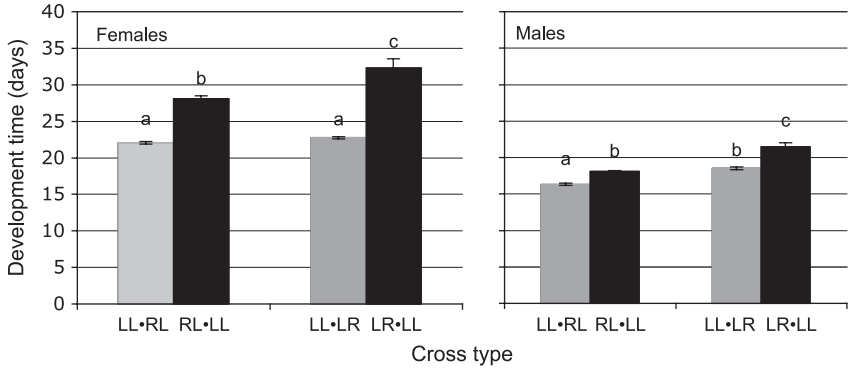

Figure 2. Development time (birth to maturity) in females and males for recombinant crosses (in gray) and nonrecombinant crosses (in black). Crosses are illustrated in Figure 1. Error bars are ±1 standard error. Mean sample size is 183.4 clutches per cohort. Letters indicate cohorts that are significantly different by ANOVA post hoc Sheffé tests, showing that development is faster in the recombinant cross than in the respective nonrecombinant cross in all 4 comparisons.

the 4 cohorts within each sex, with post hoc Scheffé tests showing faster development time in all recombinant crosses compared with the respective nonrecombinant cross. For females, mean minimum development time in the recombinant cross ranged from 6.0 days faster (LL × RL vs. RL × LL) to 9.57 days faster (LL × RL vs. LR × LL). For males, mean minimum development time in the recombinant cross ranged from 1.76 days faster (LL × RL vs. RL × LL) to 2.96 days faster (LL × LR vs. LR × LL). Recombinant crosses might be expected to show greater variance in fitness. However, a 1-tailed, paired *t*-test comparing recombinant and nonrecombinant crosses showed no difference in the coefficient of variation for development time.

One explanation for delayed development in the nonrecombinant crosses could be deleterious maternal effects stemming from F_1 hybrid mothers. This seems an unlikely explanation. Although this study did not measure fitness in the F_1 generation, studies of a large number of interpopulation crosses in this species show that F_1 hybrids are either similar to or superior than their parents for a range of fitness components including development time (e.g., Burton 1987, Edmands 1999, Edmands et al. 2005). Furthermore, our recent results show that F_1 hybrid females do not have lower viability than F_1 hybrid males (Pritchard V, Edmands S, unpublished data). A more attractive explanation than the maternal effects hypothesis is that recombinant hybrids have faster minimum development time because advantageous recombinant genotypes speed the development of some siblings, even while other siblings with detrimental recombinant genotypes develop slowly or may not even hatch successfully.

Because this study looked only at the fastest developing male and female in each clutch, it cannot reveal the likely trade-offs caused by disrupting linked genes. Despite rapid development in a subset of the surviving offspring, recombination may well have led to decreased hatching success, decreased survival to adulthood, and increased maximum development times. Future work using a more complete suite of fitness components will give a more comprehensive view of both the costs and benefits of recombination in hybrids.

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