

Long-term experimental hybrid swarms between moderately incompatible *Tigriopus californicus* populations: hybrid inferiority in early generations yields to hybrid superiority in later generations

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Abstract The deleterious effects of hybridization are a serious concern for the conservation and management of species, particularly when populations mix as a result of human activity. Outbreeding depression is the typical result observed in early-generation interpopulation hybrids of *Tigriopus californicus*. We examined both controlled crosses and long-term, freely-mating, experimental hybrid populations composed of southern California populations Royal Palms (RP) and San Diego (SD). Controlled crosses included parentals plus all reciprocal F1, F2, F3 and backcross cohorts, and only F2 cohorts showed significant declines in fitness compared to midparent values, indicating recovery in the F3. For long-term studies, four treatment groups were initiated: 100% RP, 100% SD, 50% RP: 50% SD, and 80% RP: 20% SD. Replicates were surveyed at 3-month intervals for morphometric, census and fitness measures. Fitness of hybrid treatments showed declines relative to midparent values followed by rapid recovery, with two hybrid replicates ultimately showing higher fitness than parentals at the final 15-month time-point (up to 20 generations). In contrast, both males and females in hybrid treatments were larger than the midparent for several morphometric characters at the first time-point, and smaller than the midparent at the final time-point, indicating a possible tradeoff between fitness and body size. Microsatellites for a subset of samples revealed extensive introgression in hybrid treatments. This adds to previous

evidence that hybrid breakdown in early generations may be a temporary phenomenon followed by the persistence of highly fit recombinant genotypes.

Keywords Fitness · Hybrid breakdown · Interpopulation hybridization · Morphology · Multiple generation · Outbreeding depression

Introduction

Hybridization, whether natural or anthropogenically induced, raises a variety of concerns that are relevant to species management. In addition to the reduction of the genetic integrity of any native stock, deleterious fitness effects may have several origins. Invasive species may be a threat to native populations if they hybridize and cause a reduction in the number of individuals with native genotypes (Ellstrand and Schierenbeck 2006; Bowman et al. 2007; Randi 2008). The act of introducing individuals from a divergent population to rescue endangered species from inbreeding depression can be an attractive management tool (Tallmon et al. 2004; Hedrick and Fredrickson 2010), but there is much concern over the possibility that intentional translocation could do more harm than good if outbreeding depression occurs (Allendorf et al. 2001; Edmands 2007).

Deleterious effects of hybridization are particularly likely in anthropogenically-altered environments. For commercially important species such as salmon and trout there is particular concern over impacts on both the stock abundance and evolutionary trajectory that may result from anthropogenic introductions, as in the event of farmed individuals mating with wild populations (McGinnity et al. 2003; Gilk et al. 2004; Hutchings and Fraser 2008; Roberge et al. 2008; Muhlfeld et al. 2009; Houde et al.

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2010). Also, anthropogenic impacts may lead to a loss of environmental heterogeneity which could result in the hybridizing of populations that were previous products of ecological speciation (Gow et al. 2006; Taylor et al. 2006). Biodiversity could then be lost due to extinction by introgressive hybridization (Seehausen et al. 2008). These consequences may result from the mixing of taxa on the order of species as well as ecotypes or populations. An understanding of the long-term outcome of hybrid swarm formation can enhance the success of efforts to conserve and manage threatened species.

Hybrid fitness in advanced generations is difficult to predict because the mixing of gene pools can simultaneously create both deleterious and beneficial effects. Hybrid fitness problems have largely been discussed in terms of the Dobzhansky-Muller incompatibility model in which isolated populations accumulate neutral or advantageous mutations over time. Selection may then contribute to the formation of interlocus combinations with positive epistatic interactions. Reduction in fitness of F1 hybrids may occur as a result of the disruption of local adaptation, underdominance, or negative epistasis including heterozygote-heterozygote, heterozygote-sex chromosome or heterozygote-cytoplasm interactions (Lynch 1991; Edmands 2007). Often reductions in fitness are delayed until the F2 generation or later when coadapted gene complexes are disrupted by recombination and deleterious homozygote-homozygote interactions are exposed (e.g. Burton 1987; Byrom et al. 1993; Edmands 2007).

On the other hand, hybridization may also have beneficial effects. While between-locus hybrid interactions are often detrimental, within-locus interactions are often favorable due either to dominance or overdominance (Lynch 1991). Hybrid superiority could also be due to extrinsic factors. The enhanced genetic diversity in hybrids may allow them to thrive in spatially or temporally varying environments (Dowling and Secor 1997; Arnold 2006). Similarly, hybrids with intermediate phenotypes may be favored in intermediate habitats (Grant and Grant 1992) while those with extreme phenotypes may have superior fitness in marginal habitats (Rieseberg et al. 1999).

The intertidal copepod *Tigriopus californicus* is an excellent model for laboratory studies of hybridization because of its short generation time (at least 23 days, Burton 1987) and the minimal care that cultures require. This species inhabits rocky intertidal outcrops extending from Alaska down to central Baja California, Mexico. Despite a seemingly high potential for dispersal, populations are genetically differentiated over short geographic distances, with mitochondrial DNA differences that range from 0.2 to 23% (Burton and Lee 1994; Edmands 2001). Interpopulation crosses typically result in enhanced F1 hybrid fitness compared to parents and in reduced F2

hybrid fitness (e.g. Burton 1986, 1987, 1990a, b; Edmands and Burton 1999).

Little empirical data exist on the duration of outbreeding depression and few studies go beyond the first few generations of hybridization. Some suggest that outbreeding depression may be temporary, with rapid recovery from fitness declines (Templeton 1986; Rieseberg et al. 1996; Erickson and Fenster 2006). Yet, for taxa such as *Tigriopus*, which show strong evidence of intrinsic coadaptation, there are also reasons to believe that outbreeding depression may be long lasting. For example, computer simulations show that populations take longer to recover from the disruption of intrinsic coadaptation than from the disruption of local adaptation (Edmands and Timmerman 2003).

In this study we used experimental hybrid swarm populations to assess the magnitude and duration of outbreeding depression over 15 months of free mating (a maximum of about 20 generations). This is among the first studies to monitor long-term consequences of hybridization.

Materials and methods

Population sampling

Populations were sampled from two southern California locations, Royal Palms, CA (RP, 33° 42' N, 118° 19' W) and San Diego, CA (SD, 32° 45' N, 117° 15' W) in June 2004. These two populations show approximately 18% mitochondrial cytochrome oxidase I divergence (Edmands 2001). Samples were maintained as mass cultures in 400 ml beakers with filtered seawater (37 µm) containing finely ground *Spirulina* (0.2 mg/ml) and housed in a 20°C incubator with a 12 h light: 12 h dark cycle.

Tigriopus biology

The reproductive biology of *Tigriopus californicus* has been well-documented (Egloff 1966; Vittor 1971). Mating and reproduction occur year round. Adult males use their antennae to clasp virgin females and guard them until the females are sexually mature. Males have multiple matings while females mate only once and store sperm to fertilize multiple broods of offspring (Burton 1985). Individual lifespan may be as long as 95 days and females can produce up to 20 clutches of eggs. Clutch size varies from less than 10 nauplii to over 100 (pers obs, Vittor 1971). Minimum generation time is approximately 23 days at 20°C.

Controlled crosses

Virgin females and sexually mature males were collected using fine needle probes to tease apart clasped pairs.

Reciprocal crosses were made by placing 10 virgin females with 10 mature males in a large Petri dish. Four replicate dishes (A–D) were set up for each of 4 types of crosses (all crosses listed as female \times male): two controls (RP \times RP and SD \times SD) and two reciprocal crosses (RP \times SD and SD \times RP). Each mating dish contained approximately 35 ml filtered seawater and ground *Spirulina* (0.2 mg/ml). A maximum of 40 pairs were formed for each cross. Mating dishes were observed three times per week to identify females with eggs. Females that formed an egg sac were isolated into a small Petri dish containing 15 ml of seawater and *Spirulina* mixture. Each of these birthing dishes was monitored three times a week to check for the presence of nauplii. On the day of hatching, 10 larvae were pipetted into a new small Petri dish. Survivors were counted after 14 days and mature males were counted after 28 days. If adult males were present at day 28, one was randomly selected and photographed for morphometric analysis following procedures in Edmands and Harrison (2003; see below). If no adult males were present, the dish was set aside and monitored for the presence of an adult male 1 week later.

After the first clutch of eggs hatched, the adult female was transferred into a 400 ml pair-forming beaker to produce subsequent clutches. Females from mating dish RP \times RP A were combined into pair-forming beaker RP \times RP A, females from mating dish RP \times RP B were combined into pair-forming beaker RP \times RP B, and so on. Parental females were removed after 2 weeks. Each pair-forming beaker was then monitored three times a week for pairs and, once a week, grown-offspring females with eggs were removed in order to maintain one distinct generation in the beaker. Pairs that were removed were split and placed in appropriate mating dishes to produce the second generation. Four replicates of each of the following second generation cohorts were established (female parent listed first): RP \times RP control, SD \times SD control, RP \times SD F2, SD \times RP F2, Backcross to RP and Backcross to SD. The replicates were designed to avoid inbreeding (e.g. A \times B, B \times A, C \times D and D \times C). For the backcross cohorts, the replicates were the four possible backcross types (e.g. F1 \times RP, RP \times F1, reciprocal F1 \times RP, RP \times reciprocal F1) with no inbreeding. Two replicates of each of the following third generation cohorts were established: RP \times RP control, SD \times SD control, RP \times SD F3, and SD \times RP F3. Again, replicates were designed to avoid inbreeding (e.g. AB/BA \times CD/DC and CD/DC \times AB/BA). Protocols for fitness and morphometric assays described above were repeated for the second and third generation cohorts.

To adjust for any temporal changes in the culture environment or the copepods themselves, all phenotypic values were assessed relative to the midparent (the average of the two replicated parental controls) for that same

generation. Analyses of variance including planned linear contrasts among cohorts were done using Statistica 7.1 (StatSoft, Tulsa, OK).

Long-term hybrid swarms

Four different culture treatments were set up for this experiment: 100% RP, 100% SD, 50% RP:50% SD, and 80%RP:20%SD. Five replicates per treatment were each initiated by placing 500 gravid females in 1000 ml beakers containing 800 ml live algal culture (*Platymonas* and *Monochrysis*) supplemented with 0.16 g finely ground *Spirulina* and Tetramin flakes. Beakers were housed together in one incubator at 20°C set to a 12 h light: 12 h dark cycle. Once every 2 weeks a 50% seawater change was performed. At the same time beakers were also fed and rotated within the incubator. Every 3 months a census estimate was taken for each replicate. This was performed by pouring the contents of an entire beaker into a 1L plastic bottle. The bottle was gently inverted several times to evenly distribute copepods, after which 200 ml of culture was poured into a 600 ml transparent Gladware container. A light box was used for visual assistance in sorting copepods into males, females with eggs, pairs and subadult categories using a Pasteur pipet. Each category was counted and all individuals were returned to source beakers. Every 3 months, 20 gravid females and 20 mature males were removed from each replicate beaker and were used for morphometric assays. Females were also used for fitness assays. All copepods were returned to their source beakers after assays were completed. Replicates were maintained for up to 30 months, at which point 20 males and 20 females from surviving beakers were frozen for later molecular analyses.

Fitness assays

At each 3 month interval, 20 gravid females were sampled from each replicate and isolated into individual Petri dishes containing 11 ml filtered seawater supplemented with ground *Spirulina* and Tetramin flakes. Females with red egg sacs (red eggs being more mature and therefore closer to hatching) were preferred to those whose eggs were still green in color. Each dish was monitored once daily until eggs hatched. Upon hatching, 10 larvae per clutch were pipetted into a new dish with fresh seawater culture medium. Fourteen days later individuals in each dish were counted to determine survivorship.

Morphological assays

Morphometric measurements were taken from digital images of adult copepods following procedures in Edmands and Harrison (2003). At each 3 month interval,

up to 20 females and 20 males were randomly chosen from each replicate. All measurements were done at a magnification of 32× using a Leica MZ12 dissecting microscope. Digital images were captured and morphological measurements were taken using Optimas 5.2. Absolute size was calibrated using a stage micrometer. Eight measurements were taken for males: cephalothorax length (CTL), cephalothorax width (CTW), urosome length (UL), urosome width (UW), telson width (TW), caudal seta length (CSL), antennule width (AW) and clasper width (CLW). Four of the same measurements were taken for females (CTL, CTW, UW and AW). Egg sac length and area was also measured for each female. Every 3 months up to 40 individuals were scored from each replicate.

Microsatellite assays

Eleven microsatellite loci and primers used here were developed using an enriched DNA library from the RP population (Harrison et al. 2004; Edmands et al. 2005). DNA was extracted from individual copepods using the lysis protocol previously described in Edmands et al. (2005). Individual copepods were incubated in 50 µl lysis buffer at 65°C for 1 h followed by 100°C for 15 min. Polymerase chain reactions were carried out in 12 µl volumes containing 0.5 µl template DNA, 0.25 µM fluorescently labeled forward primer, 1 µM reverse primer and 2.5 mM MgCl₂. Temperature cycling was as follows: 5 min denaturation at 94°C; 35 cycles of 30 s at 94°C, 35 s at 55°C, and 30 s at 72°C; 5 min at 72°C. This was with the exception of locus 480 which required an annealing temperature of 62°C. Fluorescently labeled PCR products were run on a Beckman-Coulter CEQ 8000 Capillary Sequencer according to commercially recommended protocols. Allele sizes were scored by eye.

Standardized hybrid indices for each individual were calculated by assigning a 0 for each RP allele and a 1 for each SD allele and then dividing by the number of loci scored. In this way hybrid indices ranged from 0 to 1, with an expected hybrid index of 0.2 based on the 80RP:20SD starting frequencies. Seven diagnostic loci were used to calculate hybrid indices for one 50:50 replicate and 9 diagnostic loci were used for one 80RP:20SD replicate.

Statistical analyses

Analyses of morphological and fitness characters within and between experimental population treatments were done using Statistica 7.1 (StatSoft, Tulsa, OK). Nested analysis of variance (ANOVA) was used to quantify differences in measures among the different treatment types. Multivariate analysis of variance (MANOVA), followed by a Wilks' test, was used to quantify combined measures. When

appropriate, Bonferroni post hoc tests were utilized to determine the statistical significance between group means. To compare each experimental replicate as well as all replicates of a population type to the midparent value, we conducted ANOVAs and contrast tests using SAS (proc GLM, SAS Institute 2006).

Calculation of allele and genotype frequencies and deviations from Hardy-Weinberg equilibrium were performed by Genepop 4.0 (Raymond and Rousset 1995). Calculations of linkage disequilibrium were performed using Genepop web version 3.4. Correlations between hybrid indices and fitness were assessed using Statistica 7.1.

Results

Controlled crosses

Survivorship and morphology

Survivorship (Table 1 and Fig. 1) showed no significant difference from the midparent in the F1 or backcross cohorts. F2 cohorts, however, showed large (35–45%) and significant declines. Both F3 cohorts recovered to survivorship values 11% below the midparent, and neither of these deviations were significant. Compared to the survivorship data, male morphometric characters (Table 1) tended to show smaller deviations from the midparent. For F1 cohorts there were significant, moderate (5–12%) increases in morphometric characters in 7 out of 16 cases, and a significant decrease (4%) in one case. For backcross cohorts there were two cases with significant increases in individual measures (8–9%). F2 and F3 cohorts tended to be smaller than the midparent in 26 out of 32 cases, but the deviation was significant in only one case (4%).

Long-term hybrid swarms

Census

Throughout the duration of the hybrid swarm experiment, census counts for individual replicate beakers showed large fluctuations between time points (Fig. 2). Midparent values were calculated using all 5 replicates for each parental line, including those with a census count of zero. The census taken for a beaker at any given point was not a good indicator of what the population size would be in the next 3 months ($r = 0.08$, $P > 0.05$). With a few exceptions, census counts from hybrid swarm beakers were lower than midparent values for the first 6 months of the experiment. By month 15, replicates remaining with living copepods were the following: two RP controls, two SD controls, four

Table 1 Means and midparent values for survival and eight male morphometric characters in three-generation controlled crosses

Cohort	Proportional survival	CTL	UL	CTW	UW	TW	CSL	AW	CLW
RP × RP control	0.881 (0.026)	0.435 (0.006)	0.289 (0.004)	0.272 (0.005)	0.139 (0.002)	0.033 (0.001)	0.654 (0.015)	0.047 (0.001)	0.064 (0.002)
SD × SD control	0.825 (0.065)	0.421 (0.004)	0.294 (0.006)	0.283 (0.002)	0.138 (0.003)	0.034 (0.000)	0.652 (0.012)	0.045 (0.001)	0.060 (0.001)
RP × SD F1	0.851 (0.023)	0.441 (0.007)	0.325 (0.003)	0.299 (0.005)	0.150 (0.004)	0.031 (0.001)	0.678 (0.010)	0.049 (0.001)	0.067 (0.001)
SD × RP F1	0.902 (0.038)	0.409 (0.010)	0.283 (0.006)	0.281 (0.003)	0.141 (0.003)	0.033 (0.001)	0.648 (0.019)	0.048 (0.002)	0.066 (0.001)
RP-backcross	0.581 (0.146)	0.442 (0.011)	0.331 (0.005)	0.322 (0.007)	0.157 (0.002)	0.035 (0.002)	0.700 (0.023)	0.055 (0.001)	0.069 (0.003)
SD-backcross	0.538 (0.136)	0.487 (0.013)	0.331 (0.007)	0.325 (0.001)	0.156 (0.001)	0.038 (0.001)	0.742 (0.033)	0.053 (0.002)	0.070 (0.002)
RP × SD F2	0.318 (0.091)	0.459 (0.009)	0.321 (0.003)	0.314 (0.007)	0.150 (0.002)	0.032 (0.001)	0.633 (0.034)	0.052 (0.001)	0.067 (0.001)
SD × RP F2	0.269 (0.136)	0.429 (0.013)	0.309 (0.007)	0.292 (0.001)	0.152 (0.001)	0.033 (0.001)	0.673 (0.033)	0.051 (0.002)	0.067 (0.002)
RP × SD F3	0.691 (0.029)	0.474 (0.009)	0.318 (0.006)	0.327 (0.000)	0.148 (0.000)	0.040 (0.004)	0.663 (0.015)	0.054 (0.001)	0.068 (0.001)
SD × RP F3	0.693 (0.033)	0.459 (0.013)	0.299 (0.004)	0.323 (0.000)	0.144 (0.001)	0.040 (0.001)	0.667 (0.015)	0.055 (0.000)	0.070 (0.001)
Gen1 midparent	0.853	0.428	0.292	0.278	0.139	0.033	0.653	0.046	0.062
Gen2 midparent	0.486	0.452	0.324	0.314	0.154	0.036	0.679	0.053	0.069
Gen3 midparent	0.775	0.474	0.312	0.325	0.151	0.038	0.675	0.056	0.072

Values are averages among replicates with standard errors in parentheses and all means are compared to midparent values in the same generation. Means greater than midparent values are in normal type. Means less than midparent values are in italics. Means significantly different ($\alpha = 0.05$) according to planned linear contrasts are indicated in boldface

50:50 swarms, and two 80RP:20SD swarms. At that time point, two 50:50 replicates and one 80RP:20SD replicate had higher census counts than the midparent and differed by at least 12-fold.

Survivorship

The 3-month time point showed evidence of heterosis presumably due to the presence of early generation hybrids (Table 2 and Fig. 3). Two 50:50 replicates and one 80RP:20SD replicate had survivorship values significantly greater than the midparent (contrast test, $P = 0.003, 0.038$ and 0.025 , respectively). At the 6-month time point one 50:50 replicate had significantly elevated survivorship while 3 replicates (1 50:50, 2 80:20) had significantly depressed survivorship, with the most severe case (50/50_4) being approximately 55% below the midparent ($P = 0.038$). At month 9, two 50:50 replicates were significantly less than the midparent value by about 26 and 30% ($P = 0.003, 0.038$, respectively). There were no other significant negative deviations from midparent values throughout the course of the experiment. By month 12 three 50:50 replicates and one 80RP:20SD replicate showed significant positive deviations from the midparent. Finally, by month 15, two 50:50 replicates were significantly greater than the midparent by approximately 63 and 66% ($P = 0.007, <0.001$, respectively). One 80RP:20SD replicate exceeded the midparent by about 25%, but this difference was not significant.

Morphological assays

Nested ANOVA followed by planned linear contrasts were performed across all time points to assess morphological variation (SAS, proc glm) (Tables 3, 4). A multivariate test indicated a significant effect of both treatment and replicate for females and males. Both month and treatment had a significant effect on all four morphological characters measured in females as well as the two egg sac measurements. Of the eight male morphological characters, six showed a significant month effect and six differed significantly among treatments. To test for replicate effects one-way MANOVAs were run for each treatment, by month, for all measurements followed by a multivariate Wilks' test. Thirteen of sixteen possible female treatment groups (4 treatments for each time point sampled, minus any treatment groups where only one replicate existed) returned a significant P -value (Wilks' test, $P < 0.05$). Ten of those were highly significant ($P < 0.0001$). For male replicates 10 of 16 different treatment groups had a significant replicate effect, suggesting that the mean of all replicates for a population type is not necessarily indicative of any particular replicate.

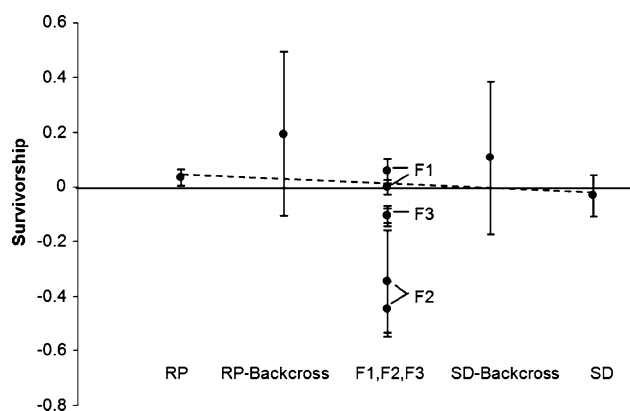


Fig. 1 Survivorship graphed as proportional deviation from the midparent in three-generation controlled cross. Values are averages among replicates, relative to midparent assayed in the same generation, ± 1 standard error. *Dashed line* indicates the additive expectation for each cohort

At 3 months into the experiment, mixed-population females (both 50:50 and 80:20) had two morphological measures significantly larger than midparent values (Table 3). By month 15, both of those measures were significantly smaller than the midparent. Egg sac area decreased over time for all populations and, by month 15, mixed populations had egg sacs with area measurements significantly less than the midparent. For males at month 3, all significant differences observed between mixed populations and midparent values were greater than the midparent but, by month 15, all significant differences were less (Table 4). There was an overall trend across treatments for morphological measurements to decrease over time with the exception of male caudal setae length (CSL). For females, significant decreases were observed between months 3 and 15 for CTL ($P < 0.0001$, $P = 0.0015$, $P < 0.0001$), ESA ($P < 0.0001$, $P = 0.0018$, $P < 0.0001$) and ESL ($P < 0.0001$, $P = 0.0242$, $P < 0.0001$) in the RP,

SD and 50:50 treatments, respectively, and for CTW ($P < 0.0001$, $P < 0.0001$) and UW ($P < 0.0001$, $P < 0.0001$) in the 50:50 and 80RP:20SD treatments. Males from the RP and SD populations showed significant increases in CSL ($P < 0.0238$, $P = 0.0001$, respectively) between months 3 and 6 while 50:50 and 80RP:20SD populations showed nonsignificant increases in CSL ($P = 0.24$, $P = 0.48$, respectively). At the end of the experiment, most differences observed between midparents and hybrid swarms in both males and females indicated that hybrid individuals were smaller.

Microsatellite assays

After 12 months of hybridization, 31 individuals from one 50:50 swarm replicate were scored for nine microsatellite loci. For all loci, alleles deviated from the expected 50:50 starting ratio toward increased frequencies of RP alleles, and seven out of nine of those deviations were significant (Chi-squared test, $P < 0.05$) (Table 5a). None of the loci scored were fixed for either population's alleles. F_{IS} estimates (Weir and Cockerham 1984) indicated trends toward heterozygote excess at 8 of 9 loci, although none of these deviations were statistically significant according to an exact HW test. Heterozygote excess was observed for 6 of 9 loci in females and 4 of 9 loci in males, but none of these deviations were statistically significant. Of a total of 36 locus pairs, 4 showed significant linkage disequilibrium over all samples (Table 6). One pair showed significant linkage disequilibrium in females only and 3 pairs showed significance in males only. Following both Bonferroni correction and sequential Bonferroni correction, only one pair of loci, which are physically linked, showed significant linkage disequilibrium when analyzed for males only as well as across the total population.

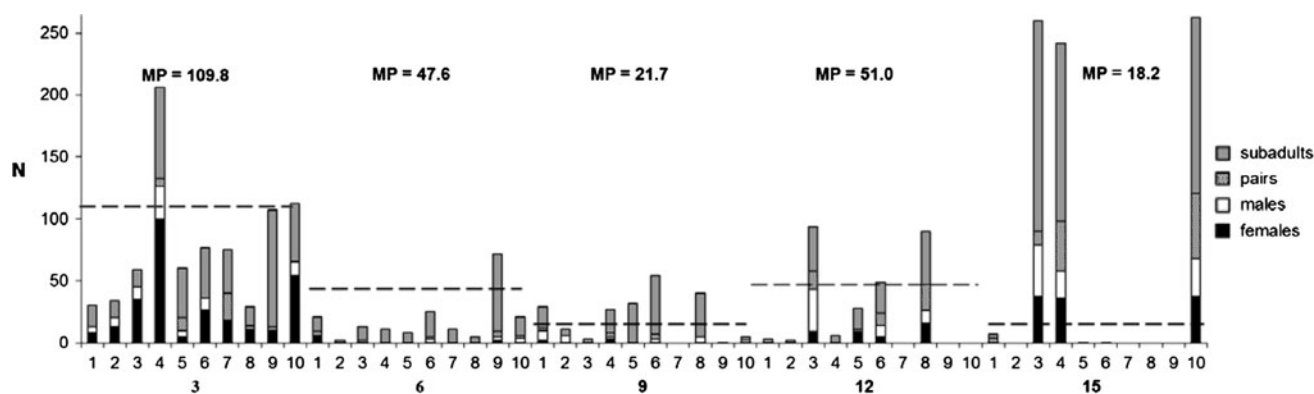


Fig. 2 Census counts for hybrid swarm replicates over 15 months of free mating. *N* number of individuals found in a 200 ml sample; *MP* midparent value. Replicates 1 through 5 are beakers with the initial ratio of 50% RP: 50% SD and 6 thru 10 were initiated with a

ratio of 80% RP: 20% SD. Large bold numbers along *x*-axis indicate month of sampling. *Dashed lines* indicate midparent values (average of the parental means) determined using means of replicate beakers for specific months

Table 2 Mean survivorship values for each hybrid swarm replicate

Month	Replicate					Midparent		
	1	2	3	4	5			
(a)	3	0.67 (0.0624)	0.965 (0.0483) +	0.8211 (0.0595)	0.895 (0.0478) +	0.6632 (0.073)	0.8028 (0.0601)	0.7357895
	6	0.7842 (0.0542)	0.8316 (0.0654) +	0.7264 (0.0656)	0.3458 (0.097) -	0.7842 (0.0659)	0.6944 (0.0887)	0.7606431
	9	0.5381 (0.0889)	0.7608 (0.0676) -	0.7944 (0.0669)	0.7842 (0.0473) +	0.5632 (0.0892)	0.6881 (0.0565) -	0.6979192
	12	1 (0.0408) +	0.75 (0.25)	0.6907 (0.0678)	0.83 (0.0578) +	0.875 (0.0351) +	0.8291 (0.0533) +	0.6145511
	15	0.8037 (0.1564)	nd	0.8785 (0.0416) +	0.8926 (0.046) +	0.7	0.8187 (0.0441) +	0.5742312
(b)	3	0.6421(0.0702)	0.91 (0.0369) +	0.7944 (0.0602)	0.7383 (0.0601)	0.7778 (0.0712)	0.7725 (0.0434)	0.7357895
	6	0.7474 (0.077)	0.6986 (0.0827) -	0.66 (0.1006)	0.6454 (0.061)	0.5927 (0.0585)	0.6688 (0.026) -	0.7606431
	9	0.7519 (0.0703)	nd	0.7994 (0.0468)	nd	0.5714	0.7076 (0.085)	0.6979192
	12	0.7533 (0.0682)	nd	0.7346 (0.0798)	nd	nd	0.744 (0.0093)	0.6145511
	15	nd	nd	nd	nd	0.675 (0.0507)	0.675	0.5742312

(a) 50:50 treatment, (b) 80:20 treatment. Treatment means significantly different from the midparent according to independent linear contrasts are indicated in bold ($\alpha = 0.05$). Values significantly greater than the midparent are marked with a + sign while those significantly less than the midparent are marked by a - sign. Standard errors are in parentheses, except in the few exceptional cases where only one individual from the replicate was measured. Replicates that died out are indicated by “nd” (no data)

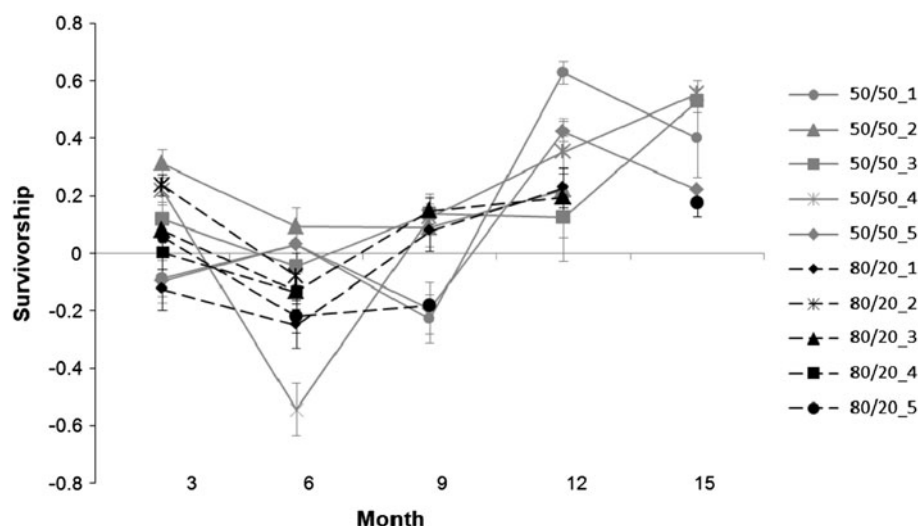
The expected hybrid index for this swarm population in Hardy-Weinberg equilibrium was 0.5, given 50% RP and 50% SD starting allele frequencies. Mean female hybrid index (0.297, $n = 13$) and mean male hybrid index (0.331, $n = 18$) were equivalent to each other (independent t test). Overall the average hybrid index was 0.317 ($n = 31$) and significantly lower than the expected value ($P < 0.0001$, t -test against value of 0.5) (Fig. 4a). No significant correlation ($r = 0.18$) was found between hybrid index and survivorship for this swarm replicate.

After 30 months of hybridization 40 individuals from one swarm replicate (initial starting frequencies of 80% RP: 20% SD copepods) were scored for 11 microsatellite loci. Overall, allele frequencies remained close to the ratio of 80% RP and 20% SD, with six of the diagnostic loci showing deviations toward SD alleles and five loci tending towards RP in character (Table 5b). Two of these 11 loci, TC480 and TC1202, showed particularly extreme deviation from the expected value (Chi-squared test, $P = 0.001$ and $P < 0.0001$, respectively) with TC480 deviating toward more RP alleles than expected while TC1202 shows the opposite pattern. Also showing a significant deviations were loci TC558 and TC62J8 which both had an excess of RP alleles.

Heterozygote deficiency and excess were measured by calculating F_{IS} and deviations from Hardy-Weinberg equilibrium indicated trends toward heterozygote excess at most loci. Significant heterozygote excess, using the HW exact test, was observed in both females and the total population at locus TC1202 ($p_{females} = 0.025$, $p_{tot} = 0.021$) and in females only at locus TC56J2 ($P = 0.0006$). After Bonferroni corrections were performed, only the heterozygote excess in females at locus TC56J2 significantly deviated from Hardy-Weinberg equilibrium. A multi-locus test of heterozygote excess (U -test, Raymond and Rousset 1995) indicated that global heterozygote excess was significant for females ($P = 0.003$) but not for males ($P = 0.788$) or for the total population ($P = 0.100$). Of 55 locus pairs, 3 showed significant linkage disequilibrium over all samples, 3 showed significance in females only and 2 showed significance in males only (Table 6). Following both Bonferroni correction and sequential Bonferroni correction no locus pairs showed significant linkage disequilibrium.

The expected hybrid index for this swarm population in Hardy-Weinberg equilibrium was 0.2, given 80% RP and 20% SD starting allele frequencies. Mean female hybrid index (0.235, $n = 20$) and mean male hybrid index (0.235, $n = 20$) were equivalent to each other. Overall the average hybrid index was 0.235 ($n = 40$) and slightly higher than the expected value ($P = 0.044$, t -test against value of 0.2) (Fig. 4b). No significant correlation ($r = 0.01$) was found between hybrid index and survivorship for this particular replicate.

Fig. 3 Mean proportional deviation from the midparent for survivorship of each hybrid swarm replicate over 15 months of free mating. Midparent values are calculated using control lines at each time point. *Error bars* indicate one standard error



Discussion

Predictions from controlled crosses

For all controlled crosses, only F2s showed significant deviations in survivorship compared to midparent values (Fig. 1). The large and significant F2 declines in survivorship portend fitness problems in the earlier hybrid swarm generations. The lack of depressed survivorship in backcross hybrids implicates homozygote-homozygote interactions as the primary source of fitness problems. Significant recovery between the F2 and F3 cohorts suggests that these deleterious epistatic interactions can be efficiently purged. Yet the precise time course of recovery in freely mating hybrid swarms is difficult to predict because of overlapping generations and the persistence of fertilized females with lifespans up to 95 days (Vittor 1971). Male morphometric characters (Table 1) for F2 and F3 cohorts tended to be smaller than the midparent but this was only significant for one case. For F1 and backcross cohorts most significant differences observed showed increases in size. For morphology, it is more difficult to predict what may happen in the long term because controlled cross hybrids show both patterns of increase and decrease in size and many deviations from the midparent are non-significant.

Duration of outbreeding depression

Other studies have focused on the magnitude of outbreeding depression in controlled crosses (e.g. Burton 1987; Edmands and Burton 1999; Edmands 1999; Fenster and Galloway 2000), partly because knowing the genomic composition of any parent generation allows for comparisons of observed versus expected genotypic frequencies. This is the first multi-generation hybridization study in this

species in which individuals were allowed to freely choose their mates throughout the course of the experiment. In doing so, this mimics a natural hybridization event that may occur if divergent populations have the opportunity to mix.

Survivorship data suggest that after only 3 months of free mating (up to 4 generations in the lab) none of the replicate populations show effects of outbreeding depression (Fig. 3). There are several ways that this may occur: (1) Individuals mate assortatively or hybrids are selected against so that only parental genotypes remain in the population (2) genetic swamping occurs in which one population's genes are selected for while genes from the second population decline in frequency (3) the effects of outbreeding depression may be decreased or delayed because fitness measures include persisting parentals as well as both early generation heterosis and hybrid breakdown and (4) within the first few generations of hybridization selection may have chosen highly fit recombinant genotypes such that outbreeding depression is rapidly purged. Evidence suggests that *Tigriopus* does not avoid outbreeding (Ganz and Burton 1995; Palmer and Edmands 2000). This, combined with the fact that hybrid genotypes were observed at later time points in the experiment (Fig. 4), indicates that scenario number one is not taking place. Genetic swamping could be easily identified, as most individuals would be homozygous for one population's alleles, but we do not see this signature in any of the experimental swarms. Even with the limited amount of molecular data we have for this study, long-term hybrid cultures utilizing these particular parental populations have never shown evidence of genetic swamping (Edmands et al. 2005; Hwang et al. unpublished data).

In the absence of assortative mating, overlapping generations or selection, it would only take about 3 months (three generations) to achieve the largest proportion of F2

Table 3 Mean phenotypic values for female morphometric characters for all control and hybrid swarm treatments

	CTL	CTW	UW	AW	ESA	ESL	
3 months	RP	0.5152 (0.0067)	0.3409 (0.0038)	0.1619 (0.0026)	0.0404 (0.0011)	0.1181 (0.0090)	0.5114 (0.0279)
	SD	0.5486 (0.0052)	0.3638 (0.0039)	0.1830 (0.0023)	0.0432 (0.0008)	0.1346 (0.0031)	0.5399 (0.0078)
	50:50	0.5268 (0.0108)	0.3633 (0.0039)+	0.1870 (0.0027)+	0.0426 (0.0013)	0.1183 (0.0082)-	0.4947 (0.0207)-
	80:20	0.5340 (0.0097)	0.3598 (0.0024)+	0.1867 (0.0033)+	0.0413 (0.0015)	0.1291 (0.0104)	0.5244 (0.0185)
	midparent	0.5319	0.3524	0.1725	0.0418	0.1264	0.5257
6 months	RP	0.4707 (0.0121)	0.3221 (0.0082)	0.1618 (0.0076)	0.0399 (0.0004)	0.0803 (0.0044)	0.4203 (0.0126)
	SD	0.5185 (0.0103)	0.3513 (0.0047)	0.1742 (0.0029)	0.0385 (0.0036)	0.1099 (0.0073)	0.4844 (0.0192)
	50:50	0.5274 (0.0166)+	0.3540 (0.0091)+	0.1729 (0.0032)+	0.0411 (0.0010)	0.1303 (0.0108)+	0.5303 (0.0243)+
	80:20	0.5202 (0.0108)+	0.3525 (0.0064)+	0.1747 (0.0031)+	0.0406 (0.0011)	0.1306 (0.0115)+	0.5347 (0.0265)+
	midparent	0.4946	0.3367	0.168	0.0392	0.0951	0.4524
9 months	RP	0.4650 (0.0107)	0.3141 (0.0141)	0.1497 (0.0069)	0.0371 (0.0023)	0.0750 (0.0087)	0.4030 (0.0261)
	SD	0.4866 (0.0103)	0.3228 (0.0039)	0.1635 (0.0016)	0.0419 (0.0007)	0.0913 (0.0069)	0.4441 (0.0194)
	50:50	0.5044 (0.0154)+	0.3340 (0.0075)+	0.1636 (0.0020)+	0.0399 (0.0009)	0.1152 (0.0057)+	0.4958 (0.0162)+
	80:20	0.4410 (0.0138)-	0.3050 (0.0028)-	0.1509 (0.0042)	0.0376 (0.0009)	0.0691 (0.0068)	0.3768 (0.0156)-
	midparent	0.4758	0.3185	0.1566	0.0395	0.0832	0.4236
12 months	RP	0.4937	0.3271	0.1612	0.0379	0.0916	0.4471
	SD	0.4791	0.3477	0.1663	0.0433	0.0982	0.4814
	50:50	0.4947 (0.0133)+	0.3346 (0.0084)	0.1677 (0.0039)	0.0396 (0.0005)	0.1144 (0.0059)+	0.5053 (0.0176)
	80:20	0.4917 (0.0086)	0.3221 (0.0212)	0.1723 (0.0060)	0.0359 (0.0024)	0.0971 (0.0142)+	0.4601 (0.0264)
	midparent	0.4864	0.3374	0.1637	0.0406	0.0949	0.4642
15 months	RP	0.4769	0.3314	0.1675	0.0376	0.0737	0.3989
	SD	0.5222	0.3602	0.1759	0.0436	0.1193	0.5094
	50:50	0.4634 (0.0140)-	0.3259 (0.0023)-	0.1555 (0.0025)-	0.0419 (0.0031)	0.0916 (0.0156)-	0.4565 (0.0482)+
	80:20	0.4470 (0.0788)-	0.3095 (0.0236)-	0.1313 (0.0256)-	0.0344 (0.0097)	0.0833 (0.0565)-	0.3978 (0.1669)-
	midparent	0.4995	0.3458	0.1717	0.0406	0.0965	0.4542

Units are in millimeters. Treatment means significantly different from the midparent according to independent linear contrasts are indicated in bold ($\alpha = 0.05$). Values significantly greater than the midparent are marked with a + sign while those significantly less than the midparent are marked by a - sign. Standard errors are in parentheses with the exception of months 12 and 15 where only one replicate remained for parental treatments

Table 4 Mean phenotypic values for male morphometric characters in hybrid swarm replicates

Month	Treatment	CTL	UL	CTW	UW	TW	CSL	AW	CLW
3	RP	0.4716 (0.0091)	0.3380 (0.0008)	0.3169 (0.0055)	0.1522 (0.0025)	0.0331 (0.0008)	0.6495 (0.0122)	0.0490 (0.0011)	0.0655 (0.0008)
	SD	0.5145 (0.0045)	0.3612 (0.0041)	0.3413 (0.0028)	0.1619 (0.0015)	0.0356 (0.0014)	0.6456 (0.0055)	0.0494 (0.0007)	0.0658 (0.0013)
	50:50	0.5053 (0.0045) +	0.3583 (0.0021)	0.3526 (0.0044) +	0.1557 (0.0009)	0.0327 (0.0006)	0.6966 (0.0114) +	0.0488 (0.0005)	0.0665 (0.0007)
	80:20	0.4967 (0.0050)	0.3534 (0.0037)	0.3495 (0.0037) +	0.1626 (0.0016) +	0.0341 (0.0011)	0.7330 (0.0092) +	0.0493 (0.0011)	0.0679 (0.0009) +
	midparent	0.4931	0.3496	0.3291	0.1571	0.0343	0.6475	0.0492	0.0657
	RP	0.4610 (0.0050)	0.3250 (0.0020)	0.3157 (0.0045)	0.1629 (0.0005)	0.0335 (0.0016)	0.6673 (0.0264)	0.0526 (0.0013)	0.0651 (0.0010)
6	SD	0.4954 (0.0082)	0.3538 (0.0113)	0.3420 (0.0042)	0.1653 (0.0038)	0.0353 (0.0007)	0.7168 (0.0174)	0.0516 (0.0008)	0.0672 (0.0008)
	50:50	0.4747 (0.0069)	0.3400 (0.0066)	0.3379 (0.0065) +	0.1628 (0.0032)	0.0340 (0.0011)	0.7051 (0.0136)	0.0495 (0.0011) -	0.0668 (0.0014)
	80:20	0.4794 (0.0054)	0.3480 (0.0047)	0.3408 (0.0051) +	0.1588 (0.0018) -	0.0330 (0.0005)	0.7093 (0.0153)	0.0504 (0.0011)	0.0690 (0.0009)
	midparent	0.4782	0.3394	0.3289	0.1641	0.0344	0.692	0.0521	0.0661
	RP	0.4496 (0.0156)	0.3292 (0.0179)	0.3329 (0.0074)	0.1483 (0.007)	0.0326 (0.0006)	0.7159 (0.0205)	0.0478 (0.0016)	0.0652 (0.0006)
	SD	0.4857 (0.008)	0.3485 (0.0076)	0.3393 (0.0033)	0.1607 (0.0021)	0.0333 (0.0007)	0.7217 (0.0349)	0.0502 (0.0013)	0.0671 (0.0009)
9	50:50	0.4667 (0.008)	0.3485 (0.0039)	0.3347 (0.005)	0.1557 (0.0013)	0.0337 (0.0006)	0.7415 (0.0052)	0.0508 (0.0004)	0.068 (0.0006)
	80:20	0.4469 (0.0084) -	0.3229 (0.0033) -	0.3299 (0.0042) -	0.1525 (0.0023) -	0.035 (0.0017)	0.707 (0.0212) -	0.048 (0.0017)	0.0644 (0.0006)
	midparent	0.4676	0.3389	0.3361	0.1545	0.0329	0.7188	0.049	0.0662
	RP	0.4631	0.36530	0.3470	0.14820	0.03530	0.73320	0.0570	0.07250
	SD	0.4785	0.35810	0.34080	0.15610	0.03560	0.74970	0.0490	0.06670
	50:50	0.4637 (0.0128)	0.3360 (0.0074) -	0.3314 (0.0095)	0.149 (0.0042)	0.0338 (0.0005)	0.728 (0.0055)	0.0496 (0.001)	0.0649 (0.0008)
12	80:20	0.4567 (0.0019)	0.3256 (0.0087) -	0.3408 (0.0058)	0.1531 (0.0009)	0.0345 (0.0012)	0.7437 (0.0038)	0.0472 (0.0004) -	0.0714 (0.0022)
	midparent	0.4708	0.3617	0.3439	0.1521	0.0355	0.7415	0.053	0.0696
	RP	0.467	0.34090	0.34020	0.15410	0.03540	0.71320	0.05720	0.07120
	SD	0.4829	0.34920	0.35350	0.16230	0.03710	0.7830	0.04790	0.06690
	50:50	0.4626 (0.0025) -	0.3465 (0.0064)	0.3352 (0.0039) -	0.1502 (0.0008) -	0.0325 (0.0022)	0.7217 (0.0116) -	0.0515 (0.0008)	0.0699 (0.0011)
	80:20	0.4581 (0.0131)	0.3278 (0.0154)	0.3346 (0.0102)	0.1472 (0.0013) -	0.032 (0.0024)	0.7346 (0.0113)	0.0464 (0.0031)	0.0653 (0.0023)
midparent	0.4749	0.3451	0.3469	0.1582	0.0363	0.7481	0.0526	0.069	

Units are millimeters. Treatment means significantly different from the midparent according to independent linear contrasts are indicated in bold ($\alpha = 0.05$). Values significantly greater than the midparent are marked with a + sign while those significantly less than the midparent are marked by a - sign. Standard errors are in parentheses with the exception of months 12 and 15 for which only one control replicate of each population remained

Table 5 Estimated population allele frequencies in (a) one 50:50 replicate after 12 months of free mating and (b) one 80:20 replicate after 30 months

Locus	Allele frequencies	
	RP	SD
(a)		
228*	0.89	0.12
1814*	0.67	0.33
56J2	0.55	0.45
1203*	0.69	0.31
558*	0.68	0.32
197*	0.71	0.29
1555	0.62	0.38
62J8*	0.63	0.36
1202	0.61	0.39
(b)		
228	0.81	0.19
1814	0.83	0.18
56J2	0.79	0.21
1203	0.81	0.19
558*	0.70	0.30
197	0.74	0.26
1555	0.79	0.21
62J8*	0.65	0.35
480*	0.97	0.03
1202*	0.49	0.51
30	0.88	0.13

Bold numbers indicate alleles in excess of expected frequencies. Asterisks indicate a statistically significant deviation (Chi-squared test, $P < 0.05$)

individuals: a maximum of 25% in a 50:50 replicate and 10% in an 80RP:20SD replicate. Yet depressed hybrid survivorship persists through months six and nine (Fig. 3). This finding would be consistent with scenario 3, in which the manifestations of outbreeding depression are delayed due to the presence of a significant proportion of parentals and F1s in early generations. The delay of F2 individuals might also be expected because 23 days is a minimum generation time estimate and development is slower in generation 2 hybrids. It is possible that it could take up to 9 months (12 generations) for the replicate to reach its maximum capacity of F2 individuals. It is not certain, however, whether the greatest effects of outbreeding depression have taken place before month three, or if the decline in fitness observed at months six and nine represents the deepest adaptive valley that the mixed populations may encounter.

What stands out as most remarkable is that, not only do mixed populations recover to expected midparent values but, by month fifteen, two out of four surviving replicates significantly exceed midparent fitness (Fig. 3, Table 2). As seen from the controlled crosses (Fig. 1), the backcrosses produced by this particular pair of populations showed high fitness and this may have largely contributed to the recovery from outbreeding depression. Recent evidence shows that *Tigriopus* populations may harbor an epistatic load of maladapted gene combinations so that hybridization may create gene combinations that are both better and worse than those of the parental populations (Edmands et al. 2009). This is consistent with scenario number four in which selection promotes recombinant genotypes with

Table 6 Pairwise tests of linkage disequilibrium for 50:50 and 80:20 replicates

Locus 1	Locus 2	50:50 replicate, 12 months			80:20 replicate, 30 months		
		Females	Males	Total	Females	Males	Total
228	1814	ns	ns	ns	ns	ns	*
62J8	1814	ns	ns	ns	ns	ns	*
1203	1814	ns	*	ns	ns	ns	ns
558	197	ns	**	ns	ns	ns	ns
228	197	ns	ns	ns	*	ns	**
558	1202	ns	ns	**	*	ns	ns
197	1202	ns	ns	ns	**	ns	ns
1555	1202	*	ns	ns	ns	ns	ns
558	228	ns	ns	*	ns	ns	ns
*62J8	1203	ns	ns	**	ns	*	ns
*197	1814	ns	***	***	ns	ns	ns
*558	30	ns	ns	ns	ns	*	ns

Significant deviations are shown in bold. Asterisks next to locus numbers indicate pairs of loci that are physically linked. After Bonferroni correction, only 197/1814 showed significant LD at 12 months and no locus pairs showed significant LD at 30 months. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

superior fitness. Additionally, scenarios 3 and 4 are not mutually exclusive and may be taking place simultaneously.

Morphological and molecular patterns

Morphological data indicate that, over 15 months or a maximum of ~20 generations, hybrids experience a trade-off between survivorship and size. For females the means of all morphometric characters for all treatments decreased or remained equivalent over 15 months. In addition to individuals becoming smaller over time, both body size and egg sac area of hybrid swarms are significantly smaller than the midparent at month 15, though survivorship is higher. Male morphometric characters show the same patterns of decreased measures over time with the one exception being caudal setae length (CSL) which either increased in size or remained unchanged for all treatments by month 15 (Table 3). Although CSL increased in size over time, by month 15, the 50:50 treatment is still significantly smaller than the midparent for this metric. The 80RP:20SD treatment is also smaller than the midparent but this difference is not significant. Further investigation needs to be conducted to determine whether smaller individuals are truly more fit in the lab environment, or if the most fit hybrid genotypes are constrained to those phenotypes that trade body size for enhanced survivorship.

Overall, microsatellite analysis shows extensive introgression for both swarm treatments that were genotyped (Fig. 4), consistent with Edmands et al. (2005) who used the same two populations and showed that, after 1 year, hybridity increased in all mixed population replicates. Heterozygote advantage may have contributed to the results of the present study, as microsatellite data for both populations that were genotyped showed a trend toward heterozygote excess. For both replicates genotyped, the number of loci showing heterozygote excess was greater in females than in males and one locus, for females of the 80RP:20SD population showed heterozygote excess that was statistically significant. Greater heterozygote excess for females was also reported by Harrison and Edmands (2006). This may be explained by reduced viability of homozygous females due to gender-specific effects of inbreeding. If the isolated pools of *T. californicus* harbor a genetic load, females may benefit more from the masking of deleterious alleles. Alternatively, sex determining factors might also have negative epistatic interactions with homozygous loci, resulting in differential viability between males and females if females are the heterogametic sex. While it is not known which sex in *Tigriopus* is heterogametic, females are the stronger candidate in that they lack recombination, a pattern that has long been noted to correlate with heterogamety (Haldane 1922; Huxley 1928).

Another possibility for overall heterozygote excess is that there could be a fitness advantage associated with specific combinations of the two parental genomes.

Based upon fitness results from controlled crosses and the fact that RP alleles increased in frequency at most loci examined, there may be enhanced fitness associated with alleles from the RP population. The comparison between the two treatments (Table 5) where RP alleles increase at all loci in the 50:50 replicate but only at half of the loci in the 80:20 replicate suggests selection for high but intermediate frequencies of RP alleles. Similar outcomes of increased RP alleles were observed in Edmands et al. (2005). The increase in RP alleles in this previous study cannot be attributed to cytonuclear interactions, since the same pattern was found on both cytoplasmic backgrounds. In the current study, three of the 50:50 replicates survived the duration of the experiment compared to one remaining

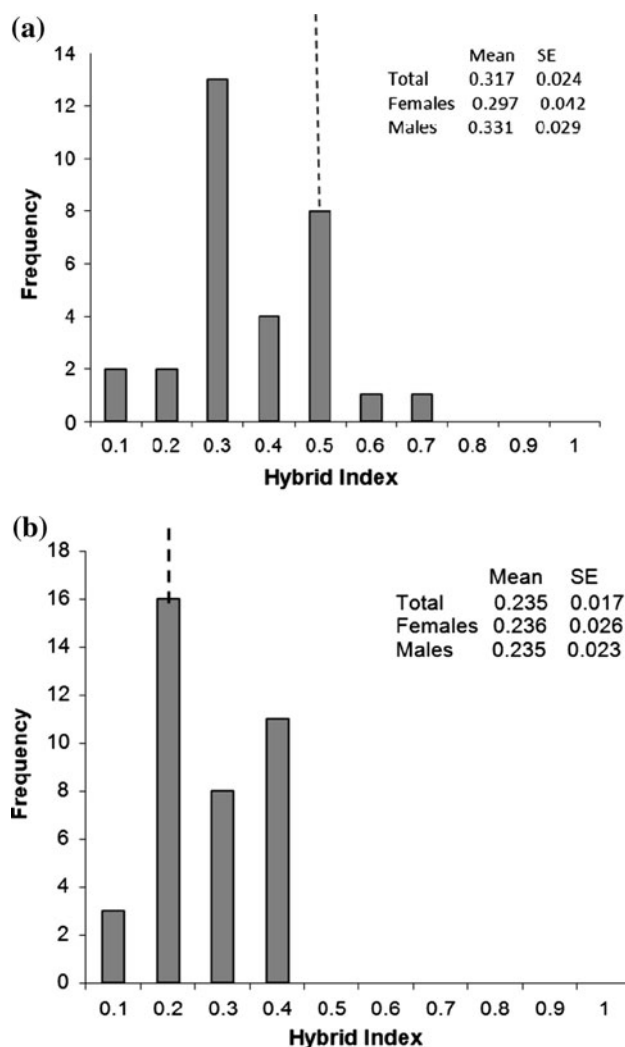


Fig. 4 Frequencies of hybrid indices for genotyped replicates. **a** 50:50, 12 months. **b** 80:20 30 months. Vertical dashed lines indicate expected mean hybrid index: 0.5 for (a) and 0.2 for (b)

80RP:20SD replicate, and the 50:50 replicates had the highest fitness (Fig. 3). However, it was the 80RP:20SD replicate that survived long past the duration of the experiment to month 30 while all other swarm and parental replicates went extinct. Even if there is a fitness advantage associated with increased RP alleles, it seems that the initial starting contributions of each source population do not aid in predicting the final outcome of hybridization.

Is the outcome of hybridization repeatable?

We were particularly interested in assessing the nature of repeatability in this hybridized system. In other words, given the same set of starting circumstances, does evolution tend to arrive upon the same outcome? This knowledge would be useful for managers attempting to restore multiple populations of the same species, especially since there are several examples showing that repeated hybridization events have led to similar results (Brochmann et al. 2000; Schwarzbach and Rieseberg 2002). One of the strongest examples comes from a study by Rieseberg et al. (1996) in which three different hybrid sunflower species were experimentally created using the same two parental species. Conservation of large linkage blocks and evidence for strong epistatic interactions were observed among three synthetic hybrid species as well as a fourth ancient hybrid. Chromosomal rearrangements that resist recombination in the parental species may have contributed to the observed repeatability, but the overall implication is that strong deterministic forces are involved in driving the consequences of hybridization. Conversely, a study using *Tigriopus* found only partial concordance among four hybrid replicates with each replicate taking a different pathway of molecular evolution to arrive at a similar conclusion of increased RP allele frequencies (Edmands et al. 2005).

A thorough observation of the individual morphometric characters of each experimental replicate in this study indicates that the path of morphological evolution is not necessarily repeatable, as replicates have distinct trajectories. MANOVAs for morphometric characters followed by multivariate Wilk's tests showed that there was a significant effect of replicate within treatment for at least one of the two types of hybrid swarms at all months.

While replicate populations showed differences for several morphometric characters, surviving 50:50 replicates all exceeded the midparent survivorship value and, in our study, like that of Edmands et al. (2005), arrived at RP allele frequencies higher than expected. Patterns of linkage disequilibrium were not concordant between the two swarm replicates genotyped nor with the 2005 study. The individual replicates that were able to overcome the threat of extinction were those with the potential to exceed fitness values of parental controls. Further studies that integrate

detailed accounts of both molecular and phenotypic data would help to resolve the questions surrounding hybrid repeatability. It is possible that there are deterministic forces for morphological and molecular evolution, but that they are hampered by drift that may take place as population sizes fluctuate, which clearly occurred in this study (Fig. 2). *T. californicus*' native habitat is composed of the highest tidepools that experience extreme fluctuations in variables such as temperature and salinity so it is entirely possible that the evolution of this species is strongly influenced by drift compared to selection. This would be consistent with the little evidence of local adaptation found (Edmands and Deimler 2004; Hwang et al. unpublished data; but see also Willett 2010) as well as levels of molecular subdivision that exceed measures of quantitative subdivision (Edmands and Harrison 2003).

Conclusion

In conclusion, this study offers extensive experimental evidence that, given an environment to thrive and large enough initial population sizes, hybrid swarms can recover from the effects of severe early-generation outbreeding depression. It lends support to the notion that outbreeding depression may be a temporary phenomenon (Templeton 1986; Rieseberg et al. 1996; Carney et al. 2000; Christiansen 2008; but see also Johnson et al. 2010) and offers a ray of hope for managers faced with situations where the introduction of genetically divergent individuals may be the only remaining option to bolster a dwindling population. Managers should focus efforts on assessing whether the mixing of populations is likely to result in hybrid persistence or genetic swamping, as results from an additional study show that the amount of divergence between the populations at hand, and the fitness of the individual populations in a particular environment, may have a large influence on the outcome of hybridization (Hwang et al. in prep). It is also worth noting that, for the pair of populations studied here, both reciprocal backcrosses had uncharacteristically high fitness. This may have contributed to the rapid recovery observed. Finally, while we have clearly demonstrated the ability to purge the deleterious effects of outbreeding, the long-term outcome of any particular hybrid swarm appears to be largely unpredictable with respect to the initial ratios of individuals from source populations. Additional experimental studies for a range of taxa are needed before we might attempt to apply generalizations about the timing and certainty of recovery.

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