JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12060

Fitness and morphological outcomes of many generations of hybridization in the copepod *Tigriopus californicus*

V. L. PRITCHARD[1], V. L. KNUTSON[2], M. LEE, J. ZIEBA & S. EDMANDS

Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA

Keywords:

controlled cross;
hybrid breakdown;
hybrid swarm;
interpopulation cross;
outbreeding;
survivorship;
Tigriopus.

Abstract

Hybridization between genetically divergent populations is an important evolutionary process, with an outcome that is difficult to predict. We used controlled crosses and freely mating hybrid swarms, followed for up to 30 generations, to examine the morphological and fitness consequences of interpopulation hybridization in the copepod *Tigriopus californicus*. Patterns of fitness in two generations of controlled crosses were partly predictive of long-term trajectories in hybrid swarms. For one pair of populations, controlled crosses revealed neutral or beneficial effects of hybridization after the F1 generation, and hybrid swarm fitness almost always equalled or exceeded that of the midparent. For a second pair, controlled crosses showed F2 hybrid breakdown, but increased fitness in backcrosses, and hybrid swarm fitness deviated both above and below that of the parentals. Nevertheless, individual swarm replicates exhibited different fitness trajectories over time that were not related in a simple manner to their hybrid genetic composition, and fixation of fitter hybrid phenotypes was not observed. Hybridization did not increase overall morphological variation, and underlying genetic changes may have been masked by phenotypic plasticity. Nevertheless, one type of hybrid swarm exhibited a repeatable pattern of transgressively large eggsacs, indicating a positive effect of hybridization on individual fecundity. Additionally, both parental and hybrid swarms exhibited common phenotypic trends over time, indicating common selective pressures in the laboratory environment. Our results suggest that, in a system where much work has focused on F2 hybrid breakdown, the long-term fitness consequences of interpopulation hybridization are surprisingly benign.

Introduction

Introgressive hybridization between genetically divergent populations is an important evolutionary process, with an outcome that is difficult to predict. It can increase population fitness and adaptive potential by countering inbreeding depression, increasing genetic diversity and generating novel phenotypes as a result of new allelic combinations (e.g. Schweitzer *et al.*, 2002; Song *et al.*, 2011; Pardo-Diaz *et al.*, 2012). Thus, introgression may act to reduce the extinction risk of a population. Hybrid populations may be more fit than their parents, and introgression has been implicated in the emergence of invasiveness (e.g. Fitzpatrick & Shaffer, 2007; Darling, 2011). Conversely, introgressive hybridization can reduce the fitness of a population, by the introduction of maladaptive traits or disruption of co-adapted gene complexes (e.g. Keller *et al.*, 2000; Lancaster *et al.*, 2007; Muhlfeld *et al.*, 2009). Such outbreeding depression may act to increase barriers to gene flow between hybridizing forms via reinforcement (e.g. Bimová *et al.*, 2011). However, it may also lead to the functional extinction of one or both of the parental populations. The potentially deleterious effects of introgression are of particular concern to those dealing with species of conservation concern, for example when considering

Correspondence: Suzanne Edmands, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.
Tel.: +1 213 740 5548; fax: +1 213 740 8123;
e-mail: sedmands@usc.edu
[1]Present address: Department of Biology (Genetics), University of Turku, FI-20014, Finland.
[2]Present address: Department of Invertebrate Zoology and Geology, California Academy of Sciences, San Francisco, CA 94118, USA.

interpopulation transplants to alleviate inbreeding depression (e.g. Madsen *et al.*, 2004; Miller *et al.*, 2012), or managing populations containing non-native genetic material as a result of anthropogenically induced range expansions (Vilà *et al.*, 2000; Wolf *et al.*, 2001).

Studies of interpopulation hybridization can both improve our understanding of how new species form and guide management of threatened taxa. Many such studies have focused on existing hybrid zones, where contemporary patterns may not be reflective of processes that occurred when the taxa first made contact. Rather few studies have simulated contact between genetically divergent populations *de novo* and followed the outcome over an extended time period. Of those that have, most have focused on controlled crosses over relatively few generations (e.g. Rieseberg *et al.*, 1996; Erickson & Fenster, 2006; but see Hercus & Hoffman, 1999; Ranganath & Aruna, 2003; Pekkala *et al.*, 2012). Secondary contact between fully interfertile populations in the wild is expected to initially generate a hybrid swarm, with matings between different hybrid genotypes across multiple and potentially overlapping generations. It is unclear to what extent the outcome of controlled crosses is predictive of fitness and phenotypic trajectories in such a situation.

The intertidal copepod *Tigriopus californicus*, which occurs as numerous genetically distinct populations along the Pacific coast of North America, has become an important model for studies of interpopulation hybridization. F2 hybrid individuals of crosses between populations typically exhibit a pattern of reduced fitness compared to the parental lines, as measured by characters such as fecundity, development time and survivorship (Burton, 1990; Edmands *et al.*, 2005), fertility (Willett, 2008), response to osmotic stress (Burton, 1986) and cytochrome oxidase activity (Edmands & Burton, 1999). Reduced fitness is generally not observed in the F1 generation (although see Ganz & Burton, 1995), indicating that this hybrid breakdown is due to the disruption of co-adapted gene complexes. Co-adaptation between the nuclear and mitochondrial genomes, mediated through the oxidative phosphorylation pathway and the mitochondrial transcription apparatus, is known to play a major role (e.g. Edmands & Burton, 1999; Ellison & Burton, 2008a). However, there is evidence both that nuclear–mitochondrial co-adaptation is incomplete in some populations and that nuclear–nuclear interactions may also contribute to this hybrid breakdown (Harrison & Edmands, 2006; Willett, 2006, 2011; Edmands *et al.*, 2009).

Tigriopus californicus has a short generation time (minimum 22 days at 20 °C, Burton, 1987), is easily maintained and reproduces freely in the laboratory, making it an ideal subject in which to follow the long-term trajectories of hybrid swarms. Hwang *et al.* (2011, 2012) previously found temporal trends in fitness of two hybrid swarms to be partly reflective of fitness patterns observed in controlled crosses. As in other species (e.g. Martin & Willis, 2010), *T. californicus* exhibits a wide range of reproductive isolation between populations (Ganz & Burton, 1995; Edmands, 1999) that may be mediated by different interactions across different parental genomes. It is unknown therefore to what extent the results of Hwang *et al.* (2011, 2012) can be extrapolated to predict long-term outcomes in other hybrid swarms.

Here, we investigated this further by creating freely mating hybrid swarms between two additional pairs of *T. californicus* populations, more morphologically divergent that those used by Hwang *et al.* (2011, 2012), and following their trajectory over a longer time period than the previous studies. We addressed the following questions: (i) Can the fitness and morphological trajectories of hybrid swarms be predicted from the outcome of controlled crosses between the same populations over a limited number of generations? (ii) Are these trajectories repeatable amongst replicates of the same cross? and (iii) Does hybridization generate increased phenotypic diversity and more potential for phenotypic change over time? We found that, once again, patterns of fitness in two generations of controlled cross were partly predicative of trajectories in hybrid swarms. Nevertheless, individual swarm replicates exhibited different fitness trajectories over time, which were not related in a simple manner to their hybrid genetic composition. Hybridization did not generally increase overall phenotypic variation for any swarm type, and underlying genetic changes may have been masked by phenotypic plasticity.

Materials and methods

We used three reproductively compatible populations of *T. californicus* (Burton, 1990; Ganz & Burton, 1995). San Diego, California, USA (SD, 32°45′N, 117°15′W), and Punta Baja, Baja California, Mexico (PBJ, 29°58′N, 115°48′W), are 336 km apart, 23% divergent at the mitochondrial Cytochrome Oxidase I locus (Edmands, 2001) and 3.5% divergent across 50 nuclear loci (Pritchard *et al.*, 2011). Santa Cruz, California, USA (SC, 36°57′N, 122°03′W), is 640 km from SD, and these two populations are 21% divergent over the mitochondrial genome (Burton *et al.*, 2007), 3.8% divergent across 50 nuclear loci (Pritchard *et al.*, 2011) and 2.7% divergent across the transcriptome (Barreto *et al.*, 2011). Adult F2 hybrids between SD and SC exhibit transmission ratio distortion at markers distributed across the genome, caused by selection against hybrid genotypes between hatching and adulthood (Pritchard *et al.*, 2011).

Tigriopus californicus biology

Tigriopus californicus is sexually dimorphic (Fig. S1). An adult male possesses modified antennules with which he clasps an immature female; the female is carried

until her terminal moult, when she is inseminated and released. Virgin females can therefore be obtained by separating clasped pairs. Studies examining mating discrimination between *T. californicus* populations have found no preference (Brown, 1991; Ganz & Burton, 1995; Palmer & Edmands, 2000). Females mate only once (Burton, 1985) and use stored sperm to fertilize sequential clutches of eggs, each of which is carried until hatching. A single female can produce several hundred progeny (Vittor, 1971). Sex determination may involve both additive genetic and environmental components (Voordouw & Anholt, 2002a,b; Voordouw *et al.*, 2005), and no recombination occurs in females (Ar-rushdi, 1963).

Establishment and maintenance of swarms

Several thousand *T. californicus* were collected from SD, SC and PBJ and maintained for 2 months as large populations in the laboratory. Eight freely mating swarm replicates were then established for each of five swarm types: pure PBJ ('A' replicates); pure SD ('B' replicates); pure SC ('C' replicates); hybrid swarms initially comprising 50% SD and 50% PBJ ('D' replicates); and hybrid swarms initially comprising 50% SD and 50% SC ('E' replicates). Three hundred gravid females were used to initiate each replicate. Swarms were maintained in 1 L beakers containing 800 mL of swarm culture medium (400 mL each of live cultures of the algae *Platymonas* and *Monochrysis*, 0.08 g ground Tetramin fish food, 0.08 g powdered *Spirulina*) and housed in an incubator at 20 °C with a 12 h light/12 h dark cycle. Further culture details are provided in the studies by Hwang *et al.* (2011, 2012) and Pritchard & Edmands (2012).

Controlled crosses

To examine fitness and morphology of known genotypes, three generations of controlled cross were also performed between SD and PBJ and SD and SC. Crosses included reciprocal F1, F2 and F3 intercrosses, all eight F2 backcross types and intrapopulation (parental) controls for each generation (Tables 1a,b). Each cross was initiated by placing an adult male with a nonsibling virgin female in a 60 × 15 mm Petri dish containing 10 mL of dish culture medium (1 L filtered seawater, 0.1 g ground Tetramin, 0.1 g powdered Spirulina). Mated females were monitored until their first eggsac hatched; 10 newly hatched larvae (nauplii) were then transferred to a new Petri dish containing fresh culture medium. Dishes were maintained under the temperature and light regimen described above. Fitness was quantified by counting the number of larvae surviving after 14 days. Not all controlled cross pairs produced offspring; to investigate whether this infertility was associated with cross type, we recorded this as a binary trait (1 = offspring present, 0 = offspring absent). For morphological analysis, we photographed a sample of nonsibling females for each cross type and collected morphological data as described below. Male morphology was not recorded for the controlled crosses.

Swarm morphology and fitness

Prior to swarm establishment ('Month 0'), we recorded morphology of 40 males and 40 females from each of the three pure populations. Subsequently, we recorded morphology of up to 20 males and 20 females from each surviving swarm replicate at 3 month intervals. Swarm replicates were split into two blocks, such that replicates 1–4 (first assay block) were assayed 3 weeks earlier than replicates 5–8 (second assay block). Occasionally, we omitted a replicate or sampled fewer individuals due to low population size. Nonpaired males were selected haphazardly. To minimize the time between photography and egg hatching, we preferentially selected females with late development stage (orange) eggsacs, but used those with earlier stage (brown or green) eggsacs where necessary. Morphology was recorded by placing each individual in a drop of seawater and photographing it from the dorsal aspect using a Leica MZ12 dissecting microscope with attached camera (Leica Microsystems, Wetzlar, Germany; 32× magnification). Five females and five males each from replicates C5, D7 and E5 were photographed three times to assess the repeatability of measurements.

Following photography, males were frozen for future molecular analysis. Females were transferred to individual Petri dishes containing 10 mL of dish culture medium and allowed to hatch eggs. Within 24 h of hatching, we transferred up to 10 nauplii to a new dish and quantified 14 day survivorship as described above. We did not include cases where females died (PBJ: 1.7% of individuals; SD: 2.7%; SC: 1.1%; SD × PBJ: 0.9%; SD × SC: 0.9%) or failed to produce four live nauplii from their eggsac (PBJ: 1.7%; SD: 1.9%; SC: 0.4%; SD × PBJ: 0.3%; SD × SC: 0.7%). For females at Month 21, we additionally counted total number of hatchlings. Females were frozen following nauplii transfer.

Morphological measurements (Fig. S1) were taken from photographs using UTHSCSA ImageTool (University of Texas Health Science Center, San Antonio, TX, USA; swarms) or Optimas 5.2 (Meyer Instruments, Inc., Houston, TX, USA; controlled cross). Cephalothorax length (CTL), cephalothorax width (CTW), male urosome length (UL) and clasper length (CLL) were measured using landmark points. For eggsac area (ESA), we manually traced the edge of each eggsac where it overlaid the body and used a 'threshold' command to define the complete border. Swarms photographs were measured in random order without knowledge of an individual's identity. Occasionally, a measurement was

Table 1 Design and results for (a) SD × PBJ and (b) SD × SC controlled cross experiments. Gen., generation; Mit, mitochondrial background of cross; R, recombinant backcross, heterozygous parent is male; NR, nonrecombinant backcross, heterozygous parent is female. Boldface indicates the values significantly deviating from the midparent mean (intercrosses) or three-quarter parent mean (backcrosses) following correction for multiple testing. Recombinant backcrosses with the same female parent were pooled for statistical analysis. A dash indicates that a statistical test was not performed.

					Survivorship			CTL (mm)			CTW (mm)			ESA (mm^2)		
Gen.	Cross	Mit.	Female parent	Male parent	*n*	Mean ± SD	*P*	*n*	Mean ± SD	*P*	*n*	Mean ± SD	*P*	*n*	Mean ± SD	*P*
(a)																
F1	Parental	SD	SD	SD	55	0.72 ± 0.35	–	35	0.56 ± 0.04	–	35	0.38 ± 0.03	–	34	0.050 ± 0.014	–
	Parental	PBJ	PBJ	PBJ	46	0.55 ± 0.46	–	14	0.56 ± 0.05	–	14	0.37 ± 0.02	–	14	0.049 ± 0.017	–
	Intercross	SD	SD	PBJ	43	0.69 ± 0.350	0.025	26	0.56 ± 0.06	0.802	26	0.38 ± 0.04	0.012	25	0.048 ± 0.016	0.592
	Intercross	PBJ	PBJ	SD	53	**0.54 ± 0.41**	0.001	18	0.57 ± 0.05	0.686	18	0.39 ± 0.02	0.015	18	0.050 ± 0.017	0.866
F2	Parental	SD	SD × SD	SD × SD	36	0.90 ± 0.19	–	25	0.51 ± 0.03	–	25	0.35 ± 0.02	–	25	0.046 ± 0.010	–
	Parental	PBJ	PBJ × PBJ	PBJ × PBJ	13	0.42 ± 0.39	–	7	0.48 ± 0.05	–	7	0.34 ± 0.03	–	7	0.039 ± 0.016	–
	Intercross	SD	SD × PBJ	SD × PBJ	48	0.63 ± 0.32	0.777	36	0.50 ± 0.04	0.548	36	0.35 ± 0.02	0.688	36	0.043 ± 0.014	0.973
	Intercross	PBJ	PBJ × SD	PBJ × SD	42	**0.77 ± 0.23**	0.000	25	0.49 ± 0.04	0.822	25	0.35 ± 0.03	0.790	25	0.047 ± 0.017	0.247
	R Backcross	SD	SD × SD	SD × PBJ	11	**0.90 ± 0.12**	0.000	11	0.53 ± 0.06	0.458	11	0.37 ± 0.03	0.891	11	0.051 ± 0.015	0.571
	R Backcross	SD	SD × SD	PBJ × SD	14	0.89 ± 0.12		10	0.50 ± 0.04		10	0.34 ± 0.02		9	0.043 ± 0.013	
	R Backcross	PBJ	PBJ × PBJ	SD × PBJ	4	**0.88 ± 0.13**	0.001	4	0.50 ± 0.06	0.564	4	0.34 ± 0.03	0.270	4	0.051 ± 0.018	0.262
	R Backcross	PBJ	PBJ × PBJ	PBJ × SD	12	0.60 ± 0.33		5	0.49 ± 0.03		5	0.34 ± 0.02		5	0.041 ± 0.006	
	NR Backcross	SD	SD × PBJ	SD × SD	13	0.67 ± 0.32	0.045	13	0.54 ± 0.04	0.011	13	0.36 ± 0.03	0.237	13	0.044 ± 0.008	0.357
	NR Backcross	PBJ	PBJ × SD	PBJ × PBJ	8	**0.78 ± 0.35**	0.000	7	0.51 ± 0.05	0.181	7	0.35 ± 0.04	0.101	7	0.047 ± 0.016	0.523
	NR Backcross	SD	SD × PBJ	PBJ × PBJ	10	0.66 ± 0.20	0.010	8	0.49 ± 0.04	0.849	8	0.34 ± 0.02	0.590	8	0.042 ± 0.012	0.460
	NR Backcross	PBJ	PBJ × SD	SD × SD	11	0.72 ± 0.29	0.400	8	0.51 ± 0.03	0.516	8	0.36 ± 0.03	0.852	8	0.043 ± 0.011	0.588
F3	Parental	SD	(SD × SD) × (SD × SD)	(SD × SD) × (SD × SD)	47	0.69 ± 0.35	–	40	0.50 ± 0.05	–	40	0.35 ± 0.03	–	40	0.048 ± 0.016	–
	Parental	PBJ	(PBJ × PBJ) × (PBJ × PBJ)	(PBJ × PBJ) × (PBJ × PBJ)	11	0.68 ± 0.25	–	9	0.48 ± 0.04	–	9	0.33 ± 0.03	–	9	0.048 ± 0.021	–
	Intercross	SD	(SD × PBJ) × (SD × PBJ)	(SD × PBJ) × (SD × PBJ)	26	0.69 ± 0.26	0.940	23	0.47 ± 0.03	0.175	23	0.32 ± 0.02	0.080	23	0.046 ± 0.011	0.649
	Intercross	PBJ	(PBJ × SD) × (PBJ × SD)	(PBJ × SD) × (PBJ × SD)	20	0.75 ± 0.23	0.101	18	0.51 ± 0.04	0.172	18	0.36 ± 0.03	0.078	18	0.054 ± 0.018	0.322
(b)																
F1	Parental	SD	SD	SD	55	0.72 ± 0.35	–	35	0.56 ± 0.04	–	35	0.38 ± 0.03	–	34	0.050 ± 0.014	–
	Parental	SC	SC	SC	54	0.44 ± 0.46	–	13	0.65 ± 0.05	–	13	0.43 ± 0.02	–	13	0.069 ± 0.020	–
	Intercross	SD	SD	SC	36	0.53 ± 0.42	0.131	20	0.57 ± 0.04	0.015	20	0.38 ± 0.03	0.992	20	0.050 ± 0.019	0.142
	Intercross	SC	SC	SD	46	0.52 ± 0.45	0.038	20	0.59 ± 0.05	0.288	20	0.38 ± 0.10	0.125	19	0.053 ± 0.017	0.303
F2	Parental	SD	SD × SD	SD × SD	36	0.87 ± 0.19	–	25	0.51 ± 0.03	–	25	0.35 ± 0.02	–	25	0.046 ± 0.010	–
	Parental	SC	SC × SC	SC × SC	52	0.75 ± 0.31	–	35	0.57 ± 0.04	–	35	0.40 ± 0.03	–	35	0.049 ± 0.013	–
	Intercross	SD	SD × SC	SD × SC	25	**0.67 ± 0.28**	0.000	30	0.53 ± 0.03	0.407	30	0.37 ± 0.02	0.211	30	0.050 ± 0.013	0.377
	Intercross	SC	SC × SD	SC × SD	40	**0.73 ± 0.28**	0.003	36	0.53 ± 0.04	0.100	36	0.37 ± 0.02	0.436	36	0.047 ± 0.017	0.932
	R Backcross	SD	SD × SD	SD × SC	8	0.79 ± 0.23	0.254	7	0.51 ± 0.04	0.079	7	0.35 ± 0.03	0.560	7	0.039 ± 0.008	0.869
	R Backcross	SD	SD × SD	SC × SD	11	0.93 ± 0.10		11	0.50 ± 0.03		11	0.36 ± 0.02		11	0.051 ± 0.020	
	R Backcross	SC	SC × SC	SD × SC	8	**0.88 ± 0.22**	0.000	9	0.54 ± 0.05	0.257	9	0.38 ± 0.02	0.786	9	0.056 ± 0.021	0.010
	R Backcross	SC	SC × SC	SC × SD	14	0.92 ± 0.10		13	0.55 ± 0.04		13	0.38 ± 0.03		13	0.056 ± 0.014	
	NR Backcross	SD	SD × SC	SD × SD	6	0.73 ± 0.37	0.069	4	0.50 ± 0.04	0.291	4	0.35 ± 0.03	0.524	4	0.043 ± 0.005	0.957
	NR Backcross	SC	SC × SD	SC × SC	9	**0.90 ± 0.10**	0.000	7	0.56 ± 0.06	0.807	7	0.385 ± 0.04	0.668	7	0.048 ± 0.010	0.960
	NR Backcross	SD	SD × SC	SC × SC	7	0.73 ± 0.35	0.370	5	0.54 ± 0.04	0.421	5	0.366 ± 0.04	0.113	5	0.061 ± 0.013	0.018
	NR Backcross	SC	SC × SD	SD × SD	12	**0.69 ± 0.26**	0.001	7	0.54 ± 0.03	0.533	7	0.360 ± 0.02	0.349	7	0.043 ± 0.013	0.243
F3	Parental	SD	(SD × SD) × (SD × SD)	(SD × SD) × (SD × SD)	47	0.70 ± 0.35	–	40	0.50 ± 0.05	–	40	0.345 ± 0.03	–	40	0.048 ± 0.016	–
	Parental	SC	(SC × SC) × (SC × SC)	(SC × SC) × (SC × SC)	18	0.73 ± 0.32	–	12	0.54 ± 0.04	–	12	0.383 ± 0.03	–	12	0.050 ± 0.017	–
	Intercross	SD	(SD × SC) × (SD × SC)	(SD × SC) × (SD × SC)	12	0.77 ± 0.27	0.180	13	0.53 ± 0.06	0.332	13	0.371 ± 0.04	0.964	13	0.053 ± 0.013	0.305
	Intercross	SC	(SC × SD) × (SC × SD)	(SC × SD) × (SC × SD)	17	**0.82 ± 0.19**	0.002	14	0.55 ± 0.03	0.032	14	0.394 ± 0.03	0.022	14	0.058 ± 0.016	0.330

SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

not collected due to insufficient photograph quality (e.g. incorrect orientation of individual).

Genotyping

Molecular data were obtained for a subset of photographed males and females: all individuals from hybrid swarm replicates D4, D6, D7, D8, E4, E6 and E7 at months 3, 6, 9, 15 and 21. Individuals were genotyped for 54 putatively population diagnostic SNPs (51 nuclear and three mitochondrial) as described in the study by Pritchard *et al.* (2011). We calculated nuclear hybrid index (HI; pure SD = 0, pure SC or PBJ = 1) and heterozygosity (HZ) for each individual from simple genotype counts using Microsoft Excel. Further details of the genomic trajectory of the hybrid swarms are provided in Pritchard & Edmands (2012).

Statistical analysis

Survivorship and presence/absence of offspring in the controlled crosses were treated as binomial responses. Morphological measurements were log-transformed prior to analysis. To correct for overall size dependence of morphological features, we included CTL as a covariate in the analyses for all other measurements; measurements corrected in this way are henceforth termed 'relative'. Swarm replicates with < 5 data points at a time point were removed from the analysis for that time point. Unless otherwise indicated, statistical tests were performed using SPSS Statistics 19 (IBM Corporation, Armonk, NY, USA).

Pure populations

To examine morphological variation amongst pure populations at Month 0, we used AN(C)OVA with sex and population as fixed factors. To examine differences in survivorship amongst populations, we pooled pure individuals from the three generations of controlled cross and fit a generalized linear model using a logistic link function, with population as a fixed factor. Where results were significant, we examined how populations or sexes differed using linear contrasts.

Controlled cross

We compared fertility of cross types within each generation by fitting a generalized linear model to the offspring presence/absence data and using linear contrasts to investigate significant effects. We examined deviation from the midparent mean (intercrosses) or three-quarter parent mean (backcrosses) for each hybrid cross type at each generation using a generalized linear model (survivorship), or AN(C)OVA (morphology) with planned contrasts. Expected genomic composition of backcrosses differs depending upon whether the heterozygote parent is a male (producing recombinant gametes) or a female (producing nonrecombinant gametes). Recombinant backcrosses, such as female SD × SD crossed with male SD × SC, produce offspring with a mixture of homozygous and heterozygous genotypes across chromosome pairs, and one complete haplotype, from the female parent, that matches the mitochondrial background. Nonrecombinant backcrosses (e.g. female SD × SC crossed with male SD × SD) produce offspring either completely homozygous or completely heterozygous across a chromosome pair. Depending upon the female parent, the offspring either have 100% probability of one complete nuclear haplotype matching the mitochondrial background (e.g. female SD × SC crossed with male SD × SD) or $< 0.025\%$ probability (e.g. female SD × SC crossed with male SC × SC). Where there is cytonuclear co-adaptation within a population, the latter backcross type is expected to exhibit lower mean fitness than the former, despite on average having the same nuclear complement. We therefore pooled samples for the two different types of recombinant backcross, but examined the two types of nonrecombinant backcross separately. Three-quarter parent value was calculated as ($^3/_4$ Parent 1 mean + $^1/_4$ Parent 2 mean). We corrected for multiple testing over all contrasts within each trait and parental pair (SD and PBJ or SD and SC) using the Holm–Bonferroni procedure (Holm, 1979).

Swarms

For all analyses, we treated replicate as a random factor nested within swarm type. To examine overall differences in survivorship amongst swarm types, we fit a generalized linear mixed model using the package lme4 implemented in R 2.15 (R Development Core Team, 2011; Bates *et al.*, 2012). Influence of swarm type was tested by comparing the likelihood of the full model to that of the model with replicate only, using a chi-square test. We tested for overall morphological differences amongst swarm types using AN(C)OVA. In all cases, where we observed a significant effect of swarm type, we used linear contrasts to examine how they differed. We also used planned linear contrasts to examine each hybrid swarm replicate independently for survivorship deviating from the midparent mean expectation at each time point. We corrected for multiple testing using the Holm–Bonferroni approach, over all months within each trait and parental pair.

We examined the morphological data for each hybrid replicate at each time point for transgressive phenotypes, that is, values outside the phenotypic range of the parental swarms at that same time point. Amount of transgression for each replicate was calculated, following Stelkens *et al.* (2009), as (total range − parental range)/parental range.

We examined the level of variability of traits at each time point by calculating coefficients of variation for

each replicate and used ANOVAs to test for differences amongst swarm types. To examine whether hybrid swarms exhibited greater morphological or fitness variation over time than did pure parental swarms, we also compared total coefficients of variation over Months 3–21 for the three longest surviving replicates for each of the swarm types (PBJ: A5, A7, A8; SD: B3, B4, B7; SC: C2, C3, C5; SD × PBJ: D4, D7, D8; SD × SC: E4, E6, E7).

Assessment of swarm fitness trends at Month 15 was complicated by unusually poor survival and slow development of nauplii in the second assay block, probably due to an unknown contaminant in the dish culture medium. This block contained all three surviving pure PBJ swarm replicates. We therefore performed additional tests comparing survivorship between swarm types within this block alone (replicates A7, A8, B7, C6, C8, D6, D6, D7, D8, E6, E7).

We investigated the relationship between ESA, hatch number and swarm type at Month 21 using ANOVA with ESA as a covariate and swarm type as a fixed factor.

Genetic influences on fitness and morphology

We examined the relationships between genotype, morphology and fitness within swarms. To reduce the chance of including the original parental individuals in the analysis, we excluded data from the Month 3 time point.

First, we used Pearson's tests to investigate correlations between mean HI or HZ of each genotyped replicate at each time point and mean offspring survivorship. We controlled for overall differences in survivorship between time points by expressing this as proportional deviation from the midparent mean. Second, we used a linear regression to investigate the relationship between individual HI and CTL. We again controlled for the observed decrease in CTL over time and differences in CTL between the sexes by expressing this value as proportional deviation from the midparent mean of the relevant sex. Finally, we used a stepwise linear regression model, with CTL and HI as factors, to investigate the relationship between HI and relative ESA. To control for the possibility of different environmental effects between beakers, we analysed replicates independently.

Previous research has demonstrated co-adaptation within populations between the mitochondrial genome and the nuclear-encoded ribosomal polymerase gene (mtRPOL; Ellison & Burton, 2008a, 2010). We therefore examined whether correspondence between mtDNA and mtRPOL genotypes (identified by population diagnostic SNPs within the mitochondrion and the mtRPOL gene) influenced an individual's CTL or relative ESA. We used ANOVA with mitochondrial and RPOL genotypes as factors, and HI included as a covariate. We restricted our analysis to the SD × SC swarms, as the PBJ mitochondrial genotype was largely lost from the SD × PBJ swarms by Month 6 (Pritchard & Edmands, 2012).

Results

Pure populations

Repeatabilities of morphological measurements were as follows: CTL: 0.91; CTW: 0.92; UL: 0.73; CLL: 0.26; ESA: 0.82. Given the low repeatability of the clasper measurement, we did not analyse this feature past Month 0. We found differences amongst populations at Month 0 for all morphological features except relative UL. We observed a strong influence of population and sex on CTL, with females being larger than males and SC > SD > PBJ (pop: $F_{2,232} = 714.4$, $P < 0.001$; sex: $F_{1,232} = 332.8$, df = 1, $P < 0.001$; sex*pop: $F_{2,232} = 2.01$, $P = 0.14$). Population, but not sex, overall influenced relative CTW (pop: $F_{2,231} = 48.9$, $P < 0.001$; sex: $F_{1,231} = 0.08$, $P = 0.78$; SC > SD and PBJ). There was, however, a sex–population interaction, with SC and PBJ males, but not SD males, having a greater relative width than females (sex * pop: $F_{2,231} = 2.9$, $P = 0.054$). Population also affected relative ESA (pop: $F_{2,112} = 34.6$, $P = 0.007$; SC > SD > PBJ) and relative male CLL, despite the low repeatability of this measurement (pop: $F_{2,114} = 7.4$, $P = 0.001$; SC > PBJ > SD). We found clear differences in nauplii survival amongst pure strains in the controlled crosses ($\chi^2_2 = 101.7$, $P < 0.001$; SD > SC > PBJ).

Controlled crosses

A substantial proportion of controlled cross pairs (0–45%, depending upon cross type, Fig. S2) produced no offspring. Within SD × PBJ, we found no significant fertility differences amongst cross types (F1: $\chi^2_3 = 7.47$, $P = 0.058$; F2: $\chi^2_9 = 9.41$, $P = 0.400$; F3: $\chi^2_3 = 1.58$, $P = 0.664$). We found differences amongst cross types within SD × SC; however, these were not overall in the direction of decreased fertility of interpopulation hybrids (F1: $\chi^2_3 = 15.09$, $P = 0.002$; F2: $\chi^2_9 = 20.28$, $P = 0.016$; F3: $\chi^2_3 = 12.06$, $P = 0.007$; Fig. S2). Fitness, as measured by 14 day survivorship, showed substantial variation amongst cross types for both pairs of parents, with significant deviations both above and below the midparent mean (Table 1a,b, Fig. 1).

We observed few strong deviations from the midparent mean in the morphological data, and none were significant following correction for multiple testing (Tables 1a,b, Fig. S3). F1 females for both directions of the SD × PBJ cross tended to have relatively wider cephalothoraxes than the midparent. Relative ESA was increased in two backcrosses, with differing mitochondrial backgrounds, in the SD × SC cross. We also observed a trend for larger eggsacs in hybrid individuals with a PBJ mitochondrial background for all three generations of the SD × PBJ cross.

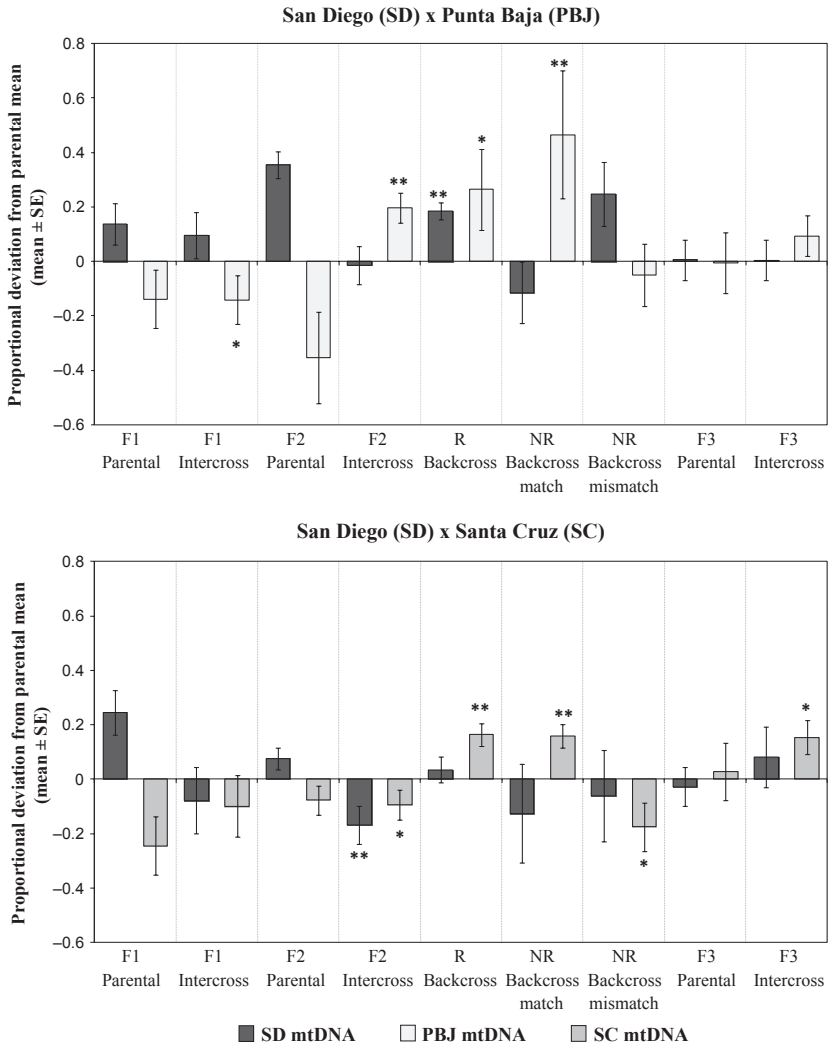

Fig. 1 Nauplii survivorship for the controlled crosses, expressed as proportional deviation from the midparent mean (all except backcrosses) or the three-quarter parent mean (backcrosses). Significance of deviations from the midparent or three-quarter parent is indicated by $*P < 0.05$ following correction for multiple testing; $**P < 0.001$. SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

Hybrid swarms

Swarms differed in their early survival, with three PBJ (A) replicates, three SD (B) replicates and four SD × SC (E) replicates, but no SC (C) or SD × PBJ (D) replicates, dying off before Month 6 (Table S1). Three PBJ, two SD, five SC, three PBJ × SD and three SD × SC replicates were maintained through 21 months. Although we did not perform census counts, we observed large changes in population density between time points in all replicates, similar to those quantified by Hwang *et al.* (2011, 2012). As only one pure PBJ replicate remained at Month 24, data were not analysed for the SD × PBJ swarms at this time point.

Swarm fitness

The majority of survivorship counts were based on 10 nauplii (SD: 96% of counts; PBJ: 92%; SC: 98%; SD × PBJ: 98%; SD × SC: 96%). Swarm type rarely had a significant influence on survivorship when variation amongst replicates was taken into account (Table 2a,b, Fig. 2). In the SD × PBJ experiment, we observed overall lower survivorship in the pure PBJ swarms compared to the pure SD or hybrid swarms at Months 3, 15 and 21. Contrast tests examining individual replicates revealed multiple significant deviations from the midparent mean following FDR correction (Fig. 2). Deviations within SD × PBJ swarms were, with a single exception, in the direction of increased survivorship compared to the midparent; SD × SC swarms showed deviations in survivorship both above and below the midparent value.

Examination of the second assay block alone at Month 15 revealed lower survivorship in SD × SC swarms, but higher survivorship in SD × PBJ swarms, compared to either parent (full model vs. model without swarm: SD × PBJ, $\chi^2_2 = 8.47$, $P = 0.014$, SD × PBJ > SD > PBJ; SD × SC, $\chi^2_2 = 9.02$, $P = 0.011$, SD and SC > SD × SC).

Table 2 Influence of swarm type: (a) pure SD, pure PBJ or hybrid SD × PBJ ('D'), (b) pure SD, pure SC or hybrid SD × SC ('E'), and replicate, on survivorship and morphological traits. Survivorship was examined using a generalized linear mixed model; cephalothorax length (CTL) using ANOVA; and cephalothorax width (CTW), eggsac area (ESA) and urosome length (UL) using ANCOVA with CTL as the covariate. Boldface type indicates a significant result following correction for multiple testing. 'Contrasts' shows swarm types found to differ in planned contrasts. A dash indicates that a contrast test was not performed.

	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24
(a) SD × PBJ experiment								
Survivorship								
Type	$\chi^2_2 = 14.22$ $P = \mathbf{0.001}$	$\chi^2_2 = 2.97$ $P = 0.226$	$\chi^2_2 = 1.02$ $P = 0.599$	$\chi^2_2 = 2.58$ $P = 0.274$	$\chi^2_2 = 11.97$ $P = \mathbf{0.003}$	$\chi^2_2 = 0.64$ $P = 0.727$	$\chi^2_2 = 16.74$ $P = \mathbf{0.000}$	
Contrasts	SD/PBJ, PBJ/D	–	–	–	SD/PBJ, PBJ/D	–	ALL	
Female CTL								
Type	$F_{2,20.2} = 1.73$ $P = 0.203$	$F_{2,12.8} = 4.99$ $P = 0.025$	$F_{2,8.0} = 2.38$ $P = 0.140$	$F_{2,7.0} = 1.28$ $P = 0.336$	$F_{2,7.2} = 2.18$ $P = 0.172$	$F_{2,5.0} = 0.52$ $P = 0.624$	$F_{2,4.0} = 0.46$ $P = 0.654$	
Rep(Type)	$F_{20,381} = 7.02$ $P = \mathbf{0.000}$	$F_{12,262} = 9.27$ $P = \mathbf{0.000}$	$F_{8,185} = 5.29$ $P = \mathbf{0.000}$	$F_{7,185} = 3.85$ $P = \mathbf{0.001}$	$F_{7,176} = 6.29$ $P = \mathbf{0.000}$	$F_{5,142} = 27.80$ $P = \mathbf{0.000}$	$F_{4,117} = 18.99$ $P = \mathbf{0.000}$	
Contrasts	–	–	–	–	–	–	–	
Female CTW								
Type	$F_{2,20.5} = 2.86$ $P = 0.080$	$F_{2,14.1} = 3.63$ $P = 0.054$	$F_{2,8.1} = 1.01$ $P = 0.395$	$F_{2,7.1} = 2.25$ $P = 0.174$	$F_{2,7.3} = 4.34$ $P = 0.048$	$F_{2,5.1} = 6.98$ $P = 0.034$	$F_{2,3.7} = 1.16$ $P = 0.381$	
Rep(Type)	$F_{20,380} = 3.59$ $P = \mathbf{0.000}$	$F_{12,261} = 5.51$ $P = \mathbf{0.000}$	$F_{8,184} = 5.20$ $P = \mathbf{0.000}$	$F_{7,184} = 1.76$ $P = 0.098$	$F_{7,175} = 4.47$ $P = \mathbf{0.000}$	$F_{5,141} = 2.48$ $P = 0.035$	$F_{4,116} = 2.12$ $P = 0.068$	
Contrasts	–	–	–	–	–	–	–	
Female ESA								
Type	$F_{2,20.2} = 0.56$ $P = 0.570$	$F_{2,12.8} = 0.74$ $P = 0.496$	$F_{2,8.0} = 3.33$ $P = 0.081$	$F_{2,7.0} = 8.42$ $P = 0.014$	$F_{2,7.0} = 0.89$ $P = 0.446$	$F_{2,5.0} = 0.68$ $P = 0.550$	$F_{2,3.9} = 0.25$ $P = 0.787$	
Rep(Type)	$F_{20,379} = 9.21$ $P = \mathbf{0.000}$	$F_{12,260} = 15.11$ $P = \mathbf{0.000}$	$F_{8,184} = 27.54$ $P = \mathbf{0.000}$	$F_{7,184} = 11.87$ $P = \mathbf{0.000}$	$F_{7,174} = 29.78$ $P = \mathbf{0.000}$	$F_{5,141} = 23.91$ $P = \mathbf{0.000}$	$F_{4,115} = 6.48$ $P = \mathbf{0.000}$	
Contrasts	–	–	–	–	–	–	–	
Male CTL								
Type	$F_{2,21.6} = 2.92$ $P = 0.076$	$F_{2,15.6} = 27.00$ $P = \mathbf{0.000}$	$F_{2,9.2} = 2.46$ $P = 0.140$	$F_{2,8.3} = 2.00$ $P = 0.196$	$F_{2,8.1} = 4.24$ $P = 0.055$	$F_{2,6.0} = 0.95$ $P = 0.438$	$F_{2,5.1} = 3.59$ $P = 0.106$	
Rep(Type)	$F_{21,380} = 15.51$ $P = \mathbf{0.000}$	$F_{14,276} = 3.18$ $P = \mathbf{0.000}$	$F_{9.197} = 5.49$ $P = \mathbf{0.000}$	$F_{8.184} = 4.17$ $P = \mathbf{0.000}$	$F_{8.198} = 5.50$ $P = \mathbf{0.000}$	$F_{6.156} = 23.08$ $P = \mathbf{0.000}$	$F_{5,125} = 5.59$ $P = \mathbf{0.000}$	
Contrasts	–	ALL	–	–	–	–	–	
Male CTW								
Type	$F_{2,22.8} = 4.41$ $P = 0.024$	$F_{2,17.6} = 3.66$ $P = 0.047$	$F_{2,9.4} = 1.98$ $P = 0.192$	$F_{2,8.4} = 0.93$ $P = 0.431$	$F_{2,9.3} = 4.46$ $P = 0.044$	$F_{2,6.5} = 4.59$ $P = 0.057$	$F_{2,5.8} = 5.99$ $P = 0.039$	
Rep(Type)	$F_{21,379} = 4.20$ $P = \mathbf{0.000}$	$F_{14,275} = 3.66$ $P = \mathbf{0.000}$	$F_{9.196} = 5.22$ $P = \mathbf{0.000}$	$F_{8.183} = 3.55$ $P = \mathbf{0.001}$	$F_{8.197} = 1.56$ $P = 0.138$	$F_{6,155} = 1.99$ $P = 0.070$	$F_{5,124} = 2.43$ $P = 0.039$	
Contrasts	–	–	–	–	–	–	–	
Male UL								
Type	$F_{2,23.3} = 0.44$ $P = 0.649$	$F_{2,21.2} = 1.82$ $P = 0.183$	$F_{2,12.5} = 3.16$ $P = 0.077$	$F_{2,9.7} = 1.11$ $P = 0.370$	$F_{2,9.2} = 1.98$ $P = 0.192$	$F_{2,6.3} = 0.63$ $P = 0.565$	$F_{2,5.8} = 0.11$ $P = 0.894$	
Rep(Type)	$F_{21,378} = 3.37$ $P = \mathbf{0.000}$	$F_{14,275} = 1.39$ $P = 0.155$	$F_{9.196} = 0.56$ $P = 0.827$	$F_{8.183} = 0.92$ $P = 0.499$	$F_{8.197} = 1.64$ $P = 0.115$	$F_{6,154} = 3.78$ $P = \mathbf{0.002}$	$F_{5,124} = 2.29$ $P = 0.050$	
Contrasts	–	–	–	–	–	–	–	
(b) SD × SC experiment								
Survivorship								
Type	$\chi^2_2 = 2.59$ $P = 0.274$	$\chi^2_2 = 1.55$ $P = 0.460$	$\chi^2_2 = 1.83$ $P = 0.400$	$\chi^2_2 = 0.81$ $P = 0.669$	$\chi^2_2 = 6.56$ $P = 0.038$	$\chi^2_2 = 1.72$ $P = 0.424$	$\chi^2_2 = 6.57$ $P = 0.037$	$\chi^2_2 = 1.10$ $P = 0.576$
Contrasts	–	–	–	–	–	–	–	–
Female CTL								
Type	$F_{2,20.2} = 18.70$ $P = \mathbf{0.000}$	$F_{2,11.4} = 11.11$ $P = \mathbf{0.002}$	$F_{2,9.4} = 9.29$ $P = \mathbf{0.005}$	$F_{2,10.0} = 16.88$ $P = \mathbf{0.001}$	$F_{2,9.0} = 5.63$ $P = 0.026$	$F_{2,8.0} = 10.37$ $P = \mathbf{0.006}$	$F_{2,6.1} = 5.95$ $P = 0.038$	$F_{2,6.1} = 18.89$ $P = \mathbf{0.002}$
Rep(Type)	$F_{20,412} = 13.07$ $P = \mathbf{0.000}$	$F_{11,218} = 11.70$ $P = \mathbf{0.000}$	$F_{9,198} = 7.85$ $P = \mathbf{0.000}$	$F_{10,231} = 13.82$ $P = \mathbf{0.000}$	$F_{9,222} = 23.65$ $P = \mathbf{0.000}$	$F_{8,209} = 18.53$ $P = \mathbf{0.000}$	$F_{6,150} = 16.61$ $P = \mathbf{0.000}$	$F_{6,155} = 20.03$ $P = \mathbf{0.000}$
Contrasts	ALL	ALL	ALL	ALL	–	ALL	–	ALL

Table 2 (*Continued*)

	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24
Female CTW								
Type	$F_{2,25.5} = 6.56$ $P = \mathbf{0.005}$	$F_{2,13.0} = 2.28$ $P = 0.141$	$F_{2,11.2} = 0.84$ $P = 0.459$	$F_{2,14.8} = 2.42$ $P = 0.124$	$F_{2,10.5} = 1.59$ $P = 0.249$	$F_{2,10.4} = 7.51$ $P = 0.010$	$F_{2,7.5} = 3.05$ $P = 0.107$	$F_{2,7.3} = 0.83$ $P = 0.474$
Rep(Type)	$F_{20,411} = 2.71$ $P = \mathbf{0.000}$	$F_{11,217} = 6.78$ $P = \mathbf{0.000}$	$F_{9,197} = 5.31$ $P = \mathbf{0.000}$	$F_{10,230} = 1.87$ $P = 0.051$	$F_{9,221} = 3.72$ $P = \mathbf{0.000}$	$F_{8,208} = 2.86$ $P = \mathbf{0.005}$	$F_{6,149} = 2.25$ $P = 0.042$	$F_{6,154} = 6.19$ $P = \mathbf{0.000}$
Contrasts	SD/SC, SD/E	–	–	–	–	–	–	–
Female ESA								
Type	$F_{2,23.8} = 9.88$ $P = \mathbf{0.001}$	$F_{2,10.6} = 6.48$ $P = 0.012$	$F_{2,8.4} = 2.34$ $P = 0.147$	$F_{2,10.9} = 3.40$ $P = 0.071$	$F_{2,9.2} = 0.85$ $P = 0.458$	$F_{2,9.0} = 2.00$ $P = 0.058$	$F_{2,6.4} = 0.74$ $P = 0.512$	$F_{2,7.0} = 0.76$ $P = 0.504$
Rep(Type)	$F_{20,404} = 3.91$ $P = \mathbf{0.000}$	$F_{11,217} = 14.82$ $P = \mathbf{0.000}$	$F_{9,197} = 12.98$ $P = \mathbf{0.000}$	$F_{10,229} = 9.59$ $P = \mathbf{0.000}$	$F_{9,220} = 28.81$ $P = \mathbf{0.000}$	$F_{8,208} = 6.82$ $P = \mathbf{0.000}$	$F_{6,149} = 7.77$ $P = \mathbf{0.000}$	$F_{6,154} = 8.48$ $P = \mathbf{0.000}$
Contrasts	SD/SC, SD/E	–	–	–	–	–	–	–
Male CTL								
Type	$F_{2,21.4} = 18.85$ $P = \mathbf{0.000}$	$F_{2,16.5} = 7.15$ $P = \mathbf{0.006}$	$F_{2,10.1} = 15.00$ $P = \mathbf{0.001}$	$F_{2,10.0} = 19.81$ $P = \mathbf{0.000}$	$F_{2,9.0} = 5.93$ $P = \mathbf{0.023}$	$F_{2,8.0} = 6.54$ $P = \mathbf{0.021}$	$F_{2,8.1} = 43.81$ $P = \mathbf{0.000}$	$F_{2,6.2} = 14.40$ $P = \mathbf{0.005}$
Rep(Type)	$F_{21,376} = 15.73$ $P = \mathbf{0.000}$	$F_{11,201} = 4.84$ $P = \mathbf{0.000}$	$F_{10,210} = 5.82$ $P = \mathbf{0.000}$	$F_{10,243} = 10.42$ $P = \mathbf{0.000}$	$F_{9,227} = 19.85$ $P = \mathbf{0.000}$	$F_{8.204} = 19.16$ $P = \mathbf{0.000}$	$F_{8,176} = 4.58$ $P = \mathbf{0.000}$	$F_{6,120} = 10.19$ $P = \mathbf{0.000}$
Contrasts	ALL	ALL	ALL	ALL	ALL	ALL	ALL	ALL
Male CTW								
Type	$F_{2,24.9} = 7.52$ $P = \mathbf{0.003}$	$F_{2,16.1} = 1.45$ $P = 0.264$	$F_{2,12.1} = 12.77$ $P = \mathbf{0.001}$	$F_{2,12.4} = 6.56$ $P = \mathbf{0.011}$	$F_{2,10.4} = 9.98$ $P = \mathbf{0.004}$	$F_{2,9.2} = 16.35$ $P = \mathbf{0.001}$	$F_{2,11.9} = 17.30$ $P = \mathbf{0.000}$	$F_{2,7.9} = 5.15$ $P = 0.037$
Rep(Type)	$F_{21,375} = 6.38$ $P = \mathbf{0.000}$	$F_{11,200} = 6.02$ $P = \mathbf{0.000}$	$F_{10,209} = 2.38$ $P = \mathbf{0.011}$	$F_{10,242} = 3.76$ $P = \mathbf{0.000}$	$F_{9.226} = 3.78$ $P = \mathbf{0.000}$	$F_{8.203} = 4.07$ $P = \mathbf{0.000}$	$F_{8.175} = 2.74$ $P = \mathbf{0.007}$	$F_{6,119} = 4.34$ $P = \mathbf{0.001}$
Contrasts	ALL	–	SD/SC, SD/E	ALL	SD/SC, SD/E	SD/SC, SD/E	ALL	–
Male UL								
Type	$F_{2,27.7} = 0.22$ $P = 0.802$	$F_{2,19.4} = 0.11$ $P = 0.898$	$F_{2,12.2} = 2.57$ $P = 0.117$	$F_{2,14.1} = 0.83$ $P = 0.455$	$F_{2,12.0} = 0.66$ $P = 0.534$	$F_{2,10.2} = 0.41$ $P = 0.673$	$F_{2,16.2} = 0.38$ $P = 0.693$	$F_{2,8.5} = 2.35$ $P = 0.155$
Rep(Type)	$F_{21,375} = 3.75$ $P = \mathbf{0.000}$	$F_{11,200} = 3.82$ $P = \mathbf{0.000}$	$F_{10,209} = 2.29$ $P = 0.014$	$F_{10,242} = 2.26$ $P = 0.015$	$F_{9.226} = 1.79$ $P = 0.072$	$F_{8.202} = 2.33$ $P = 0.021$	$F_{8.175} = 1.41$ $P = 0.197$	$F_{6,119} = 3.33$ $P = 0.005$
Contrasts	–	–	–	–	–	–	–	–

SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

Swarm morphology

We observed a long-term trend of decreasing CTL and increasing relative CTW in females, but not in males, for all swarm types (Fig. 3a,b Table S1). Statistical analyses (Tables 2a,b) revealed highly significant variation between replicates within a swarm type at almost every time point, but fewer consistent differences between swarm types. In the SD × SC experiment, both males and females in hybrid swarms were intermediate in CTL to pure SD (shorter) and pure SC (longer). Hybrid males tended to exhibit relative CTW s equivalent to pure SC (wider) and larger than pure SD (narrower); however, we did not observe such a pattern in females.

Comparison of phenotypes at each time point revealed multiple hybrid replicates to contain individuals outside of the parental range (Figs 3a,b and 4). Although few demonstrated any systematic pattern, a clear trend emerged in the SD × PBJ experiment: females in hybrid swarm replicates carried larger eggsacs relative to their size than females in parental swarm replicates, particularly at earlier time points (Fig. 4). Maximum relative eggsac size in replicate D7 at Month 9 was larger than that observed within any pure SD or PBJ individual over the entire swarms experiment.

Tests comparing coefficients of variation for survivorship and morphological traits revealed few significant differences between swarm types at any time point, and these were never in the direction of increased variability within hybrid swarms (Table S2).

Hatch number at Month 21 was strongly related to ESA, with no influence of swarm type (ANOVA: area, $F_{1,232} = 210.3$, $P < 0.001$; type, $F_{4,232} = 0.837$, $P = 0.503$). Thus, larger eggsacs indicated a higher number of offspring rather than larger hatchling size.

Genetic influences on swarm fitness and morphology

Swarm types differed in their overall level of hybridity. SD × SC swarms retained both SD and SC alleles, with the proportion varying between replicates; SD × PBJ swarms were dominated by SD alleles, with the frequency of PBJ alleles decreasing over the course of the experiment (Fig. 5; further details in Pritchard & Edmands, 2012). We found no significant relationship between mean nuclear HI or HZ and mean

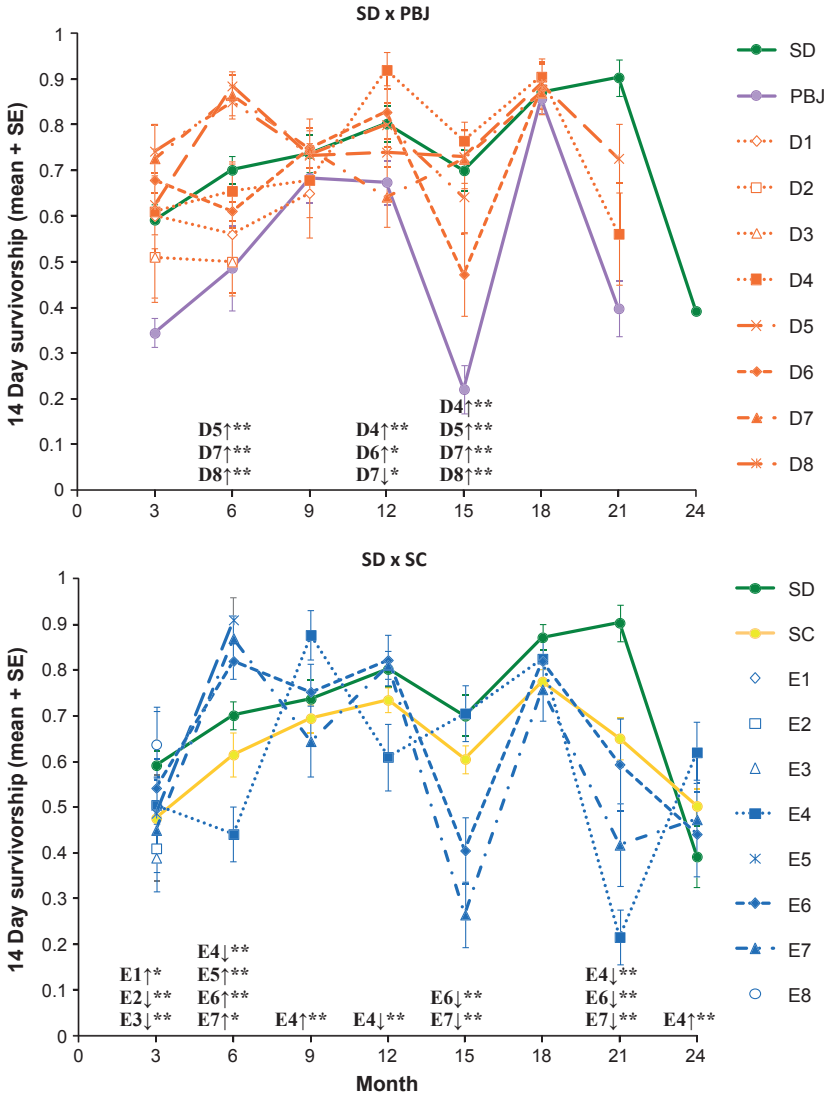

Fig. 2 Nauplii survivorship for each hybrid swarm replicate (D: SD × PBJ; E: SD × SC) compared to nauplii survivorship over all pure parental swarm replicates (PBJ; SD; SC). Standard errors for pure parental lines are calculated from pooled individuals from all replicates. Deviations of hybrid swarm replicates above (↑) or below (↓) midparent survivorship are indicated by $*P < 0.05$ following correction for multiple testing; $**P < 0.001$. SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

survivorship within a replicate, for either hybrid swarm type (Pearson's correlation: SD × PBJ: HI, $P = 0.35$; HZ, $P = 0.43$; SD × SC: HI, $P = 0.55$; HZ, $P = 0.67$). In the SD × PBJ swarms, we found a weakly negative relationship between HI and CTL (expressed as deviation from the midparent mean) in replicate D7 ($r^2_{1,112} = 0.12$, b = −0.029, $P < 0.001$) and no relationship in any other replicate (D4: $P = 0.17$; D6: $P = 0.63$; D8: $P = 0.59$). We found a weakly positive relationship between HI and CTL in all SD × SC swarm replicates (E4: $F_{1,139} = 14.4$, $P < 0.001$, $r^2 = 0.09$, b = 0.019; E6: $F_{1,145} = 6.8$, $P = 0.01$, $r^2 = 0.04$, b = 0.012; E7: $F_{1,149} = 8.5$, $P = 0.004$, $r^2 = 0.05$, b = 0.011). We observed a weakly negative relationship between HI and relative ESA in replicate D7 ($t_{1,49} = -3.01$, $P = 0.004$; b = −0.352) and no significant relationship ($P < 0.05$) in any other replicate. We found no significant influence ($P < 0.05$) of either mitochondrial genotype, mtRPOL genotype or their interaction on either CTL or ESA within the SD × SC swarm experiment.

Discussion

Controlled crosses between different populations exhibit different patterns of fitness

In this study, we performed controlled crosses and monitored long-term hybrid swarms between three genetically divergent populations of *T. californicus*, which differed both in their morphology and in their fitness, as measured by 14 day larval survivorship in the laboratory. Our results demonstrated that F2 hybrid breakdown and cytonuclear co-adaptation, frequently

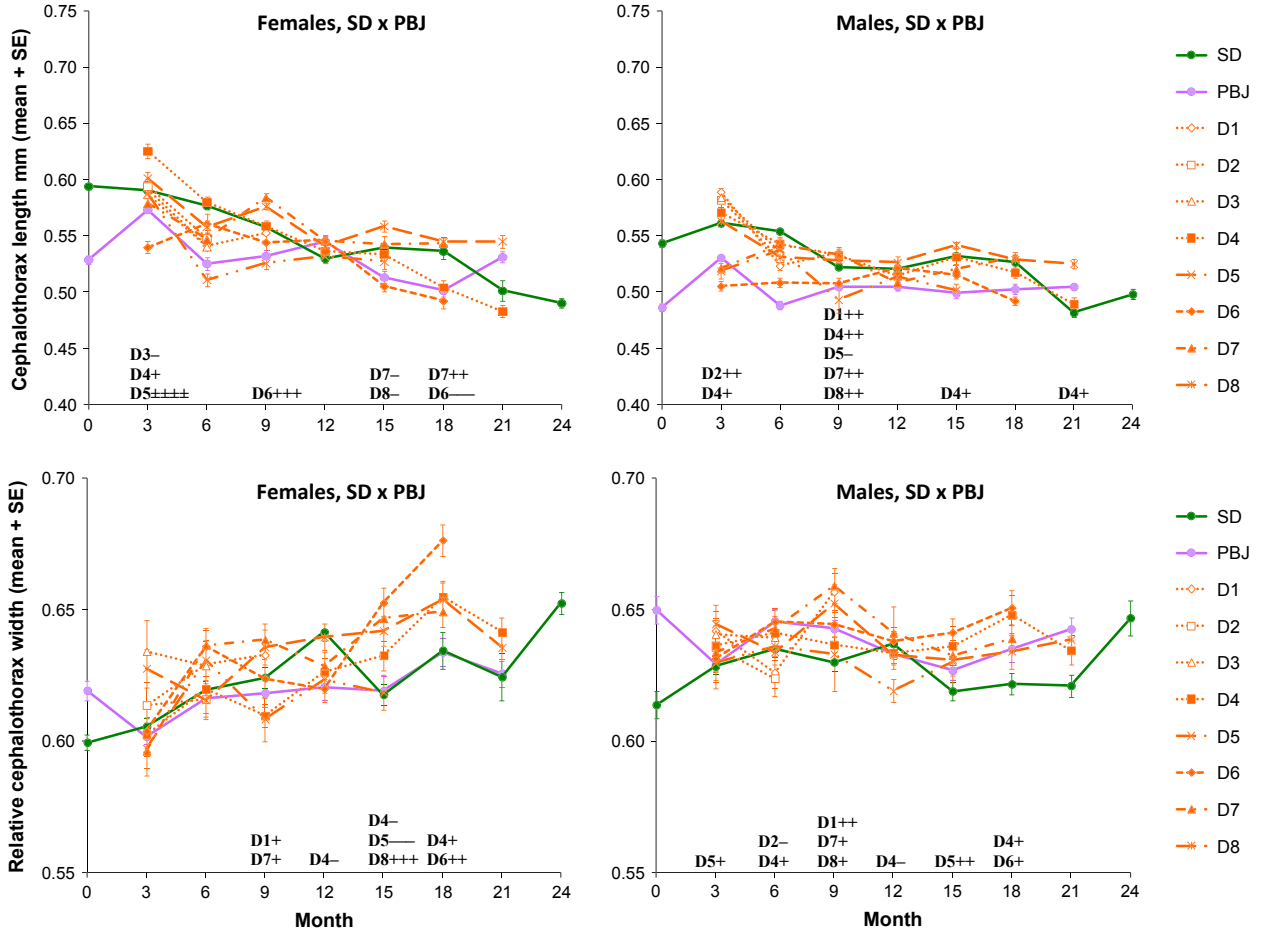

Fig. 3 (a) Cephalothorax length (top) and relative cephalothorax width (CTW) (bottom) within each SD × PBJ ('D') hybrid swarm replicate, compared to values over all pure SD and PBJ replicates. Standard errors for pure parental lines are calculated from pooled individuals from all replicates. Transgressive phenotypes at each time point are indicated by + for values larger than the parental range; – for values smaller than the parental range; ± for values both smaller and larger than the parental range. Number of symbols indicate the amount of transgression: + 1–9.9%; ++ 10–19.9%; +++ 20–20.9%; ++++ 30–30.9%. SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

observed in *T. californicus* interpopulation crosses (e.g. Edmands, 1999; Ellison & Burton, 2008b), is not ubiquitous. While we observed the classic pattern of no fitness reduction in the F1 followed by reduced survivorship in the F2 in the SD × SC cross, this was not seen in the SD × PBJ cross: hybrids with a PBJ mitochondrial background showed slightly decreased fitness in the F1, but increased fitness in the F2 (Fig. 1). Backcross results provided evidence for co-adaptation between the SC and PBJ mitochondrial genomes and their associated nuclear genomes; hybrids without at least one complete nuclear haplotype matching the mitochondrion were less fit than those with one. However, there was no similar evidence for nuclear–cytoplasmic co-adaptation involving the SD mitochondrial genome. The SD × SC cross showed full fitness recovery in the F3, a pattern also observed in a different pair of *T. californicus* populations (Hwang *et al.*, 2011) and suggesting strong selection against the hybrid genetic combinations reducing fitness in the F2. Our SD × SC results contrast with those of Ellison & Burton (2008b), who found evidence for both continued fitness reduction in the F3 generation and nuclear–cytoplasmic co-adaptation within the SD population. This may reflect culture differences between studies: replicated controlled crosses between *T. californicus* populations have been observed to produce variable outcomes (Willett, 2007), suggesting that small environmental changes can have substantial effects.

Our results demonstrate very different patterns of reproductive isolation between SD and PBJ and SD and SC, despite the populations having a similar level of genetic divergence over both the mitochondrial and nuclear genome. Level of genetic divergence between

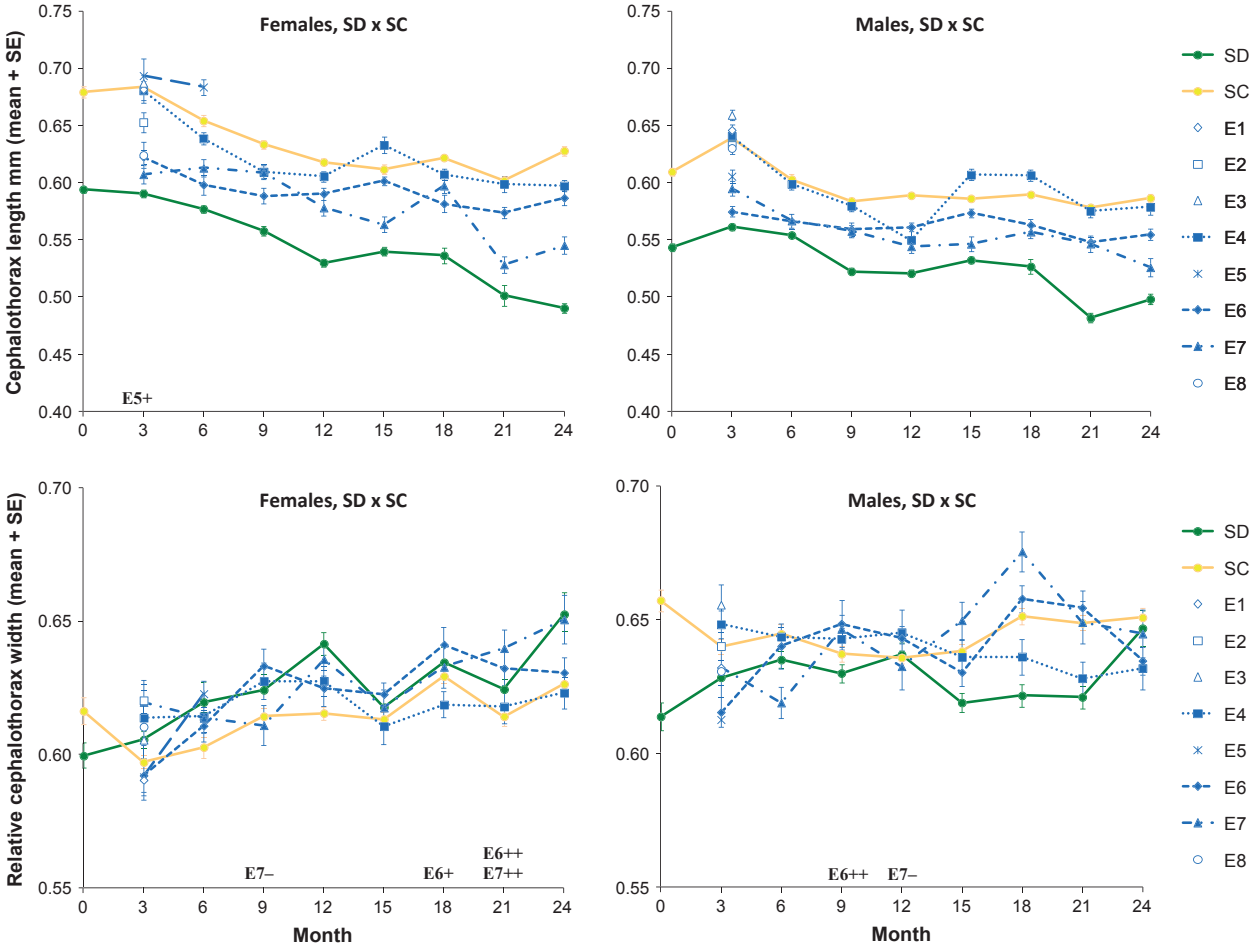

Fig. 3 *Continued*
(b) Cephalothorax length (top) and relative CTW (bottom) within each SD × SC ('E') hybrid swarm replicate, compared to values over all pure SD and SC replicates. Standard errors for pure parental lines are calculated from pooled individuals from all replicates. Transgressive phenotypes are indicated as in (a).

populations has frequently been found to be predictive of intrinsic barriers to gene flow between them, suggesting that these barriers arise due to the accumulation of genomic incompatibilities over time (e.g. Pereira *et al.*, 2011). Our observation adds to other results (Edmands, 2002), demonstrating that this is not always the case.

Fitness trajectories of hybrid swarms partly reflect patterns in controlled crosses

Fitness patterns observed within the controlled crosses were partly predictive of those observed in hybrid swarms. In the SD × PBJ controlled cross, hybridization beyond the F1 had a neutral or beneficial effect on fitness; pure PBJ were the least fit at every generation, whereas certain F2 intercrosses and backcrosses exhibited increased fitness. Correspondingly, mean fitness of SD × PBJ hybrid swarms was rarely lower than pure PBJ, and in many cases exceeded that of the fitter parent, SD. In contrast, the SD × SC controlled cross demonstrated both beneficial and detrimental effects of hybridization, with fitness reduced in the F2 and increased in certain backcrosses. Correspondingly, SD × SC hybrid swarm replicates exhibited significant deviations in fitness both above the fitter parent and below the less fit one. Despite this overall pattern, we observed substantial fitness variation between replicates of the same swarm type at each time point. This variation was observed in both pure and hybrid swarms, and mean fitness of hybrid swarm replicates was not correlated with HZ or HI for either swarm type. Thus, we found no evidence that this variation was related in a simple manner to differences in genetic composition amongst replicates.

Interpretations of fitness patterns in our *T. californicus* populations at Month 15 were complicated by an

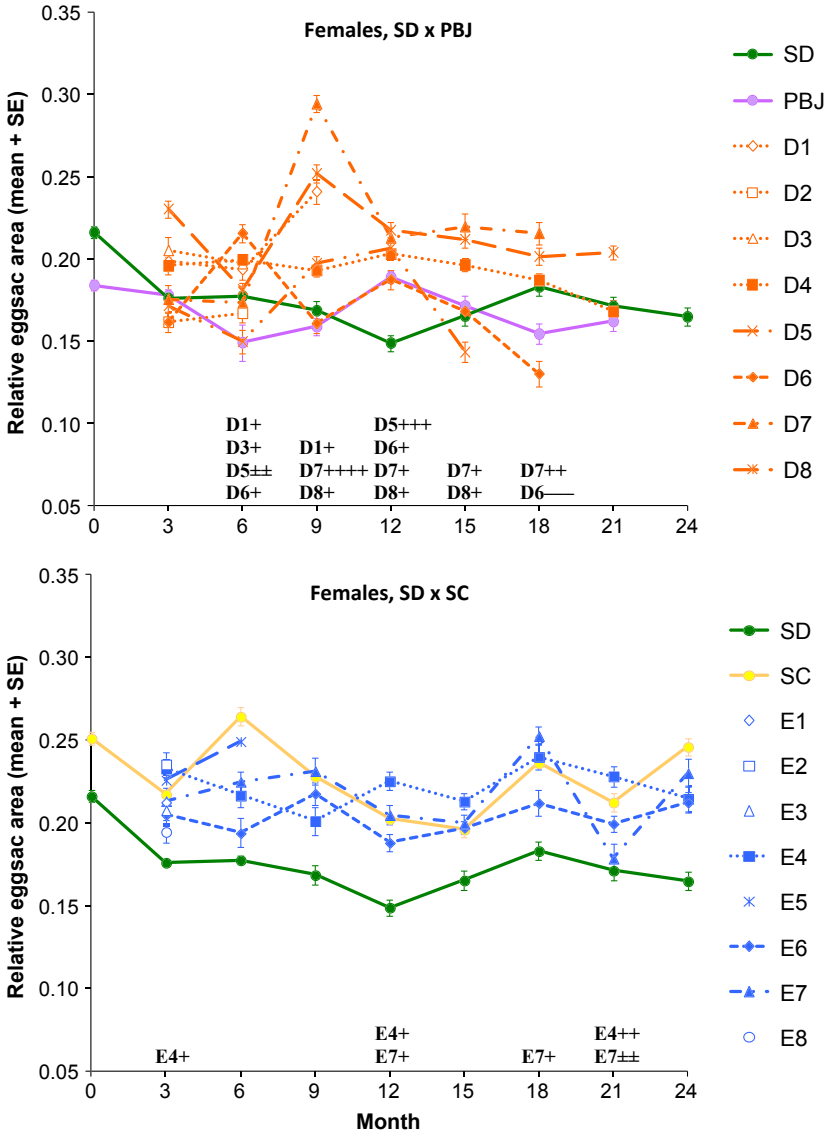

Fig. 4 Relative eggsac area for each hybrid swarm replicate (D: SD × PBJ E: SD × SC) compared to all pure parental swarm replicates (PBJ; SD; SC). Standard errors for pure parental lines are calculated from pooled individuals from all replicates. Transgressive phenotypes are indicated as in Fig. 3a. SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

unknown contaminant affecting one of the two assay blocks. Analysis of this block alone shows that, compared to the parental lines, fitness of SC × SD hybrid swarms was more strongly reduced by this stressor, but fitness of SD × PBJ hybrid swarms less strongly reduced. Studies investigating the effect of interpopulation hybridization on response to environmental stress in *T. californicus* have given varying results. Ellison & Burton (2008a) showed that F2 hybrids could have reduced ability to up-regulate OXPHOS transcription in response to salinity stress. On the other hand, several studies have shown F2 hybrid breakdown to be reduced under conditions of heat stress (Edmands & Deimler, 2004; Willett, 2012). Many other studies have demonstrated hybrid fitness to be contingent on the environment (e.g. Johansen-Morris & Latta, 2008; Carson *et al.*, 2012); our results demonstrate that the outcome of such studies may similarly depend upon the parental populations utilized.

Early fitness trajectories in our hybrid swarms contrast with those documented in the study by Hwang *et al.* (2011, 2012). Using a pair of populations showing hybrid breakdown in the F2 followed by recovery in the F3, as seen in SD × SC, they found an initial decrease in swarm fitness between Month 3 and Month 6, corresponding to when most F2 hybrid individuals might be present in the population. Using another pair of populations exhibiting F1 hybrid breakdown, although much more severely than that observed between SD and PBJ, they found greatly decreased fitness at Month 3. In contrast, we do not observe any such consistent temporal trend of reduced

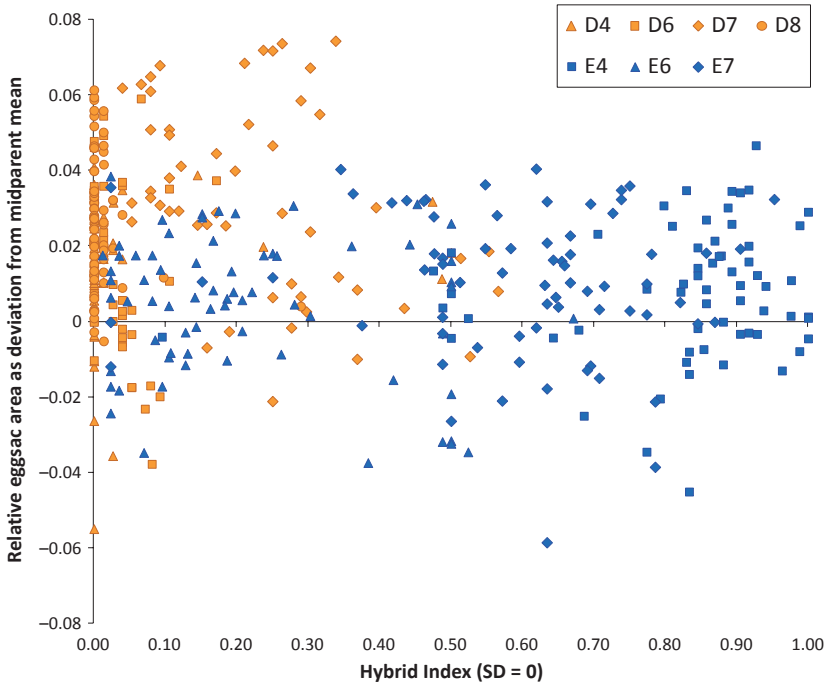

Fig. 5 Relative eggsac area, expressed as deviation from the midparent mean at each time point, plotted against individual hybrid index (0 = pure SD). SD × PBJ replicates: D4, D6, D7, D8; SD × SC replicates: E4, E6, E7. SD, San Diego, California, USA; PBJ, Punta Baja, Baja California, Mexico; SC, Santa Cruz, California, USA.

fitness within either hybrid swarm type at Months 3, 6 or beyond (Fig. 2). Genetic data have previously shown almost all adults in the swarms at Month 3 to be either pure parental or F1 individuals (Pritchard & Edmands, 2012); thus, it is unlikely that such a period of reduced swarm fitness occurred prior to our first assay point. Our results demonstrate that the occurrence of hybrid breakdown in controlled crosses between populations does not necessarily manifest as an overall fitness reduction when those same populations come into contract as a freely mating hybrid swarm. This result is not unexpected: in such a situation, the first few generations following initial contact will comprise of a variety of pure, intercross and backcross individuals. As observed in the controlled crosses between our study populations, these may differ widely in their fitness, and overall fitness of the swarms will be a function of the individual genotypes present.

Hwang *et al.* (2011) observed rapid recovery of hybrid swarms: fitness at the Month 12 and 15 time points surpassed the mean of the parental swarms, suggesting fixation of advantageous hybrid genotypes. Hwang *et al.* (2012), in contrast, monitored swarms for a longer time period and found no long-term increase in fitness. The lack of hybrid breakdown in the F3 generation of our controlled crosses, and the absence of seriously deleterious long-term consequences of hybridization in either hybrid swarm type, suggests efficient selection against hybrid incompatibilities, at least within those swarms that survived past 6 months. Nevertheless, we did not observe a sustained fitness increase above the midparent for either the SD × SC or SD × PBJ swarms over the 21 months (16–33 generations) of the experiment.

There are indications from studies of *de novo* interpopulation hybridization in other species that a pattern of fitness reduction followed by recovery above the midparent is not a general rule, although few have followed trajectories for as long as Hwang *et al.* (2011, 2012). Bijlsma *et al.* (2010) and Pekkala *et al.* (2012) crossed inbred lines of *Drosophila* and found increased fitness of hybrid populations to be maintained over 7–10 generations, although in these studies hybrid fitness is unlikely to be influenced by the disruption of co-adapted gene complexes. Hercus & Hoffman (1999) found the fitness of interspecific hybrid lines of *Drosophila* to equal that of the fitter parent at generations 17 and 30, although they did not examine changes in viability at earlier time points. Johnson *et al.* (2010) showed that approximately 20 generations of introgressive hybridization between two species of the salamander *Ambystoma* in the wild had not resulted in an increase in fitness: individuals from long-term hybrid populations were less fit under laboratory conditions than either parental populations or F1 hybrids. In contrast, Erickson & Fenster (2006), performing controlled crosses between adaptively divergent populations of the legume *Chamaecrista fasciculata*, found an increase in fitness between the F2 and F6 hybrid generations, indicating selection on recombinant genotypes. Long-term increase in fitness in a hybrid population compared to its parents requires the fixation of fitter genotypic combinations; the likelihood that this will occur

is a function of many factors, including the type of genetic variation underlying fitness traits, and the demography of the population. Such fixation may require hundreds of generations (Buerkle & Rieseberg, 2008). In particular, fluctuations in effective population size, which were previously found for all genotyped replicates over the course of this experiment (Pritchard & Edmands, 2012), are expected to impede the efficacy of selection on hybrid genotypes.

Amount of morphological variation is similar amongst swarm types, but hybrid swarms exhibit some repeatable morphological differences from their parents

We observed much variation between replicates and time points for morphological traits, in both pure and hybrid swarms. All swarm types showed a temporal trend of decreasing CTL in both sexes, and increasing relative CTW in females, suggesting similar longer-term selective pressures in all replicates. Decreases in CTL over time were also observed by Hwang *et al.* (2011, 2012). Hybrid swarms neither exhibited more morphological variation than pure swarms nor showed greater phenotypic change over time. Thus, we find little evidence that the creation of novel genetic combinations in hybrids is increasing the phenotypic variation available to selection (e.g. Lucek *et al.*, 2010) or producing transgressive phenotypes (Rieseberg *et al.*, 1999, but see discussion of eggsac size below). Our results suggest either a relatively high phenotypic plasticity or a considerable amount of standing genetic variation for the morphological traits measured within the parental swarms. Supporting the former possibility, Voordouw *et al.* (2005) found that food availability, manipulated by changing larval density, significantly affected adult size in sibling pairs of *T. californicus*. Similarly, Vittor (1971) found clutch size, and thus eggsac size, to vary with population density. Population density in our swarm beakers was observed to fluctuate widely over time, and this could account for much of the morphological variance observed amongst replicates. However, the presence of common morphological trends over time suggests some directional selection. Despite the low level of intrapopulation genetic diversity and high population subdivision generally observed at neutral markers, there does appear to be some quantitative trait variation available for selection within *T. californicus* populations. Edmands & Harrison (2003) found larger differentiation between populations in neutral marker loci than in quantitative traits, suggesting that stabilizing or fluctuating selection is causing phenotypically expressed genetic variation to be retained.

The SD × SC experiment involved two populations differing in overall size, as indicated by CTL. The populations also differed in sexual dimorphism, with SC males, but not SD males, having relatively wider cephalothoraces than females. Individuals in the hybrid swarms tended to be intermediate in length to the parentals, with CTL weakly but positively related to HI, indicating an additive genetic component to this trait. We did not document male morphology in the controlled crosses; however within SD × SC hybrid swarms, relative CTW of males either equalled or exceeded that of the wider parent, SC, suggesting a nonadditive genetic contribution. Such a nonadditive influence on relative CTW was also suggested in the SD × PBJ controlled cross: F1 hybrid females in both directions of cross were relatively wider than the midparent mean. Within all swarm types, females, but not males, became relatively wider over successive generations; thus, sexual dimorphism changed over time. Taken together, these results suggest that CTW is a trait under selection, both under the experimental conditions and in the wild, and that this selection varies between populations and between the sexes. In the *T. californicus* system, where males guard immature females prior to copulation, there may be sexual conflict at the initiation of mate guarding (Jormalainen, 1998), which may drive the evolution of both female cephalothorax morphology and male clasper morphology. Unfortunately, the low repeatability of the clasper measurement in this study, due to the poor resolution of our imaging method at smaller scales, precluded examination of such potential co-evolutionary interactions within the hybrid swarms in more detail.

We observed a striking pattern in the SD × PBJ replicates of increased eggsac size in the hybrid swarm individuals compared to the controls. Eggsac size is strongly correlated with hatchling number: hence, this implies increased fecundity of hybrid swarm individuals. This pattern persisted through all months and within most replicates; however, it was most pronounced at Month 9, and within replicate D7, which retained more genetic material from PBJ over time than the other swarm replicates (Fig. 5; Pritchard & Edmands, 2012). Declines in relative eggsac size below the midparent within SD × PBJ replicate swarms were generally associated with subsequent extirpation of the line and may have reflected adverse environmental conditions rather than genetic influences. Intriguingly, we observed relative eggsac size greatly exceeding the midparent mean in a substantial number of SD × PBJ swarm individuals that, on the basis of our 51 population diagnostic SNPs, contained only genetic material from SD (Fig. 5). This suggests either the presence of a rapidly introgressing portion of the PBJ genome unlinked to our SNP markers, or the influence of an epigenetic or environmental effects linked to the presence of the PBJ genes in the swarm beaker or in the ancestral lineage of individual copepods. Epigenetic factors, which may persist over multiple generations, have been demonstrated to have an important role in

generating phenotypic variation in hybrids in both plants (e.g. Shivaprasad *et al.*, 2011) and mammals (e.g. Han *et al.*, 2008) and have previously been suggested to alter gene transcription in interpopulation hybrids of *T. californicus* (Flowers & Burton, 2006).

Long-term fitness consequences of interpopulation hybridization in *T. californicus* may be surprisingly benign

An understanding of the long-term consequences of genetic admixture between genetically divergent populations is important to the management of populations of conservation concern. This is both because such populations may be threatened by introgressive hybridization from invasive taxa (e.g. Fitzpatrick & Shaffer, 2007; Muhlfeld *et al.*, 2009) and because introduction of genetic material from divergent lineages has been proposed as a way of rescuing a population from inbreeding (Edmands, 2007; Frankham *et al.*, 2011). Recent studies have shown that the effect of such interpopulation hybridization varies according to the parental populations involved. For example, both Pekkala *et al.* (2012) and Heber *et al.* (2012) crossing artificially inbred populations of *Drosophila* found the fitness benefits of hybridization to depend on the level of divergence between the parents. In these studies, fitness changes are primarily expected to be due to the masking of recessive deleterious alleles that have become fixed between alternative lines. While this process may similarly influence fitness of interpopulation hybrids in *T. californicus* (Edmands *et al.*, 2009), the disruption of co-adapted gene complexes that have become established within the populations over evolutionary time is also known to play a large role. Hence, our study may more closely reflect the outcome of hybridization between genetically divergent populations and taxa in the wild. Previously, Hwang *et al.* (2011, 2012) found that, despite the occurrence of hybrid breakdown in controlled crosses, interpopulation hybrid swarms of *T. californicus* could recover from an initial reduction in population fitness to equal or exceed parental values. Here, using a second pair of interpopulation crosses similarly exhibiting reduced fitness in early controlled cross generations, we again find no long-term detrimental effects of interpopulation hybridization. Thus, in a system where much work has focused on outbreeding depression, the longer-term fitness consequences of interpopulation hybridization appear surprisingly benign. Nevertheless, molecular studies (Hwang *et al.*, 2012; Pritchard & Edmands, 2012) have found that some hybrid swarms become 'genetically swamped' by a single parental population. Such an outcome may be highly deleterious to a taxon of conservation concern, even in the absence of overall reduced population fitness.

References

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

Barreto, F.S., Moy, G.W. & Burton, R.S. 2011. Interpopulation patterns of divergence and selection across the transcriptome of the copepod *Tigriopus californicus*. *Mol. Ecol.* **20**: 560–572.

Bates, D., Maechler, M. & Bolker, B. 2012. lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-0. Available at: http://lme4.r-forge.r-project.org/

Bijlsma, R., Westerhof, M.D.D., Roekx, L.P. & Pen, I. 2010. Dynamics of genetic rescue in inbred *Drosophila melanogaster* populations. *Conserv. Genet.* **11**: 449–462.

Bimová, B.V., Macholán, M., Baird, S.J.E., Munclinger, P., Dufková, P., Laukaitis, C.M. *et al.* 2011. Reinforcement selection acting on the European house mouse hybrid zone. *Mol. Ecol.* **20**: 2403–2424.

Brown, A.F. 1991. Outbreeding depression as a cost of dispersal in the harpacticoid copepod, *Tigriopus californicus*. *Biol. Bull.* **181**: 123–126.

Buerkle, C.A. & Rieseberg, L.H. 2008. The rate of genome stabilization in homoploid hybrid species. *Evolution* **62**: 266–275.

Burton, R.S. 1985. Mating system of the intertidal copepod *Tigriopus californicus*. *Mar. Biol.* **86**: 247–252.

Burton, R.S. 1986. Evolutionary consequences of restricted gene flow amongst natural populations of the copepod, *Tigriopus californicus*. *Bull. Mar. Sci.* **39**: 526–535.

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

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

Burton, R.S., Byrne, R.J. & Rawson, P.D. 2007. Three divergent mitochondrial genomes from California populations of the copepod *Tigriopus californicus*. *Gene* **403**: 53–59.

Carson, E.W., Tobler, M., Minckley, W.L., Ainsworth, R.J. & Dowling, T.E. 2012. Relationships between spatio-temporal environmental and genetic variation reveal an important influence of exogenous selection in a pupfish hybrid zone. *Mol. Ecol.* **21**: 1209–1222.

Darling, J.A. 2011. Interspecific hybridization and mitochondrial introgression in invasive *Carcinus* shore crabs. *PLoS ONE* **6**: 17828.

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

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

Edmands, S. 2002. Does parental divergence predict reproductive compatibility? *Trends Ecol. Evol.* **17**: 520–527.

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

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

Edmands, S. & Deimler, J.K. 2004. Local adaptation, intrinsic coadaptation and the effects of environmental stress on

interpopulation hybrids in the copepod *Tigriopus californicus*. *J. Exp. Mar. Biol. Ecol.* **303**: 183–196.

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

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

Edmands, S., Northrup, S.L. & Hwang, A.S. 2009. Maladapted gene complexes within populations of the intertidal copepod *Tigriopus californicus*? *Evolution* **63**: 2184–2192.

Ellison, C.K. & Burton, R.S. 2008a. Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. *Proc. Natl Acad. Sci. USA* **105**: 15831–15836.

Ellison, C.K. & Burton, R.S. 2008b. Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution* **62**: 631–638.

Ellison, C.K. & Burton, R.S. 2010. Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *J. Evol. Biol.* **23**: 528–538.

Erickson, D.L. & Fenster, C.B. 2006. Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution* **60**: 225–233.

Fitzpatrick, B.M. & Shaffer, H.B. 2007. Hybrid vigor between native and introduced salamanders raises new challenges for conservation. *Proc. Natl Acad. Sci. USA* **104**: 15793–15798.

Flowers, J.M. & Burton, R.S. 2006. Ribosomal RNA gene silencing in interpopulation hybrids of *Tigriopus californicus*: nucleolar dominance in the absence of intergenic spacer subrepeats. *Genetics* **173**: 1479–1486.

Frankham, R., Ballou, J.D., Eldridge, M.D., Lacy, R.C., Ralls, K., Dudash, M.R. *et al.* 2011. Predicting the probability of outbreeding depression. *Conserv. Biol.* **25**: 465–475.

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

Han, Z., Mtango, N.R., Patel, B.G., Sapienza, C. & Latham, K.E. 2008. Hybrid vigor and transgenerational epigenetic effects on early mouse embryo phenotype. *Biol. Reprod.* **79**: 638–648.

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

Heber, S., Briskie, J.V. & Apiolaza, L.A. 2012. A test of the 'genetic rescue' technique using bottlenecked donor populations of *Drosophila melanogaster*. *PLoS ONE* **7**: 43133.

Hercus, M.J. & Hoffman, A.A. 1999. Does interspecific hybridization influence evolutionary rates? An experimental study of laboratory adaptation in hybrids between *Drosophila serrata* and *Drosophila birchii*. *Proc. R. Soc. Lond. B* **266**: 2195–2220.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* **6**: 65–70.

Hwang, A.S., Northrup, S.L., Alexander, J.K., Vo, K.T. & Edmands, S. 2011. Long-term experimental hybrid swarms between moderately incompatible *Tigriopus californicus* populations: hybrid inferiority in early generations yields to hybrid superiority in later generations. *Conserv. Genet.* **12**: 895–909.

Hwang, A.S., Northrup, S.L., Peterson, D.L., Kim, Y. & Edmands, S. 2012. Long-term experimental hybrid swarms between nearly incompatible *Tigriopus californicus* populations: persistent fitness problems and assimilation by the superior population. *Conserv. Genet.* **13**: 567–579.

Johansen-Morris, A.D. & Latta, R.G. 2008. Genotype by environment interactions for fitness in hybrid genotypes of *Avena barbata*. *Evolution* **62**: 573–585.

Johnson, J.R., Fitzpatrick, B.M. & Shaffer, H.B. 2010. Retention of low-fitness genotypes over six decades of admixture between native and introduced tiger salamanders. *BMC Evol. Biol.* **10**: 147.

Jormalainen, V. 1998. Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. *Q. Rev. Biol.* **73**: 275–304.

Keller, M., Kollmann, J. & Edwards, P.J. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. *J. Appl. Ecol.* **37**: 647–659.

Lancaster, M., Bradshaw, C.J.A., Goldsworthy, S.D. & Sunnucks, P. 2007. Lower reproductive success in hybrid fur seal males indicates fitness costs to hybridization. *Mol. Ecol.* **16**: 3187–3197.

Lucek, K., Roy, D., Bezault, E., Sivasundar, A. & Seehausen, O. 2010. Hybridization between distant lineages increases adaptive variation during a biological invasion: stickleback in Switzerland. *Mol. Ecol.* **19**: 3995–4011.

Madsen, T., Ujvari, B. & Olsson, M. 2004. Novel genes continue to enhance population growth in adders (*Vipera berus*). *Biol. Conserv.* **120**: 145–147.

Martin, N.H. & Willis, J.H. 2010. Geographic variation in postzygotic isolation and its genetic basis within and between two *Mimulus* species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **365**: 2469–2478.

Miller, J.M., Poissant, J., Hogg, J.T. & Coltman, D.W. 2012. Genomic consequences of genetic rescue in an insular population of bighorn sheep (*Ovis canadensis*). *Mol. Ecol.* **21**: 1583–1596.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F. *et al.* 2009. Hybridization rapidly reduces fitness of a native trout in the wild. *Biol. Lett.* **5**: 328–331.

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

Pardo-Diaz, C., Salazar, C., Baxter, S.W., Merot, C., Figueiredo-Ready, W., Joron, M. *et al.* 2012. Adaptive introgression across species boundaries in *Heliconius* butterflies. *PLoS Genet.* **8**: 1002752.

Pekkala, N., Knott, K.E., Kotiaho, J.S., Nissinen, K. & Puurtinen, M. 2012. The benefits of interpopulation hybridization diminish with increasing divergence of small populations. *J. Evol. Biol.* **25**: 2181–2193.

Pereira, R.J., Monahan, W.B. & Wake, D.B. 2011. Predictors for reproductive isolation in a ring species complex following genetic and ecological divergence. *BMC Evol. Biol.* **11**: 194.

Pritchard, V.L. & Edmands, S. 2012. The genomic trajectory of hybrid swarms: outcomes of repeated crosses between populations of *Tigriopus californicus*. *Evolution* doi: 10.111/j.1558-5646.2012.01814.x.

Pritchard, V.L., Dimond, L., Harrison, J.S., Velázquez, C.C., Zieba, J.T., Burton, R.S. *et al.* 2011. Interpopulation hybridization results in widespread viability selection across the genome in *Tigriopus californicus*. *BMC Genet.* **12**: 54.

R Development Core Team. 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical

Computing, Vienna Austria. Available at www.R-project.org/. Accessed September 1 2012.
Ranganath, H.A. & Aruna, S. 2003. Hybridization, transgressive segregation and evolution of new genetic systems in *Drosophila*. *J. Genet.* **82**: 163–177.
Rieseberg, L.H., Sinervo, B., Linder, C.R., Ungerer, M.C. & Arias, D.M. 1996. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. *Science* **272**: 741–745.
Rieseberg, L.H., Arche, M.A. & Wayne, R.K. 1999. Transgressive segregation, adaptation and speciation. *Heredity* **83**: 363–372.
Schweitzer, J.A., Martinsen, G.D. & Whitham, T.G. 2002. Cottonwood hybrids gain fitness traits of both parents: a mechanism for their long-term persistence? *Am. J. Bot.* **89**: 981–990.
Shivaprasad, P.V., Dunn, R.M., Santos, B.A.C.M., Bassett, A. & Baulcombe, D.C. 2011. Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs. *EMBO J.* **31**: 257–266.
Song, Y., Endepols, S., Klemann, N., Richter, D., Matuschka, F.-R., Shih, C.H. *et al.* 2011. Adaptive introgression of anticoagulant rodent poison resistance by hybridization between old world mice. *Curr. Biol.* **21**: 1296–1301.
Stelkens, R.B., Schmid, C., Selz, O. & Seehausen, O. 2009. Phenotypic novelty in experimental hybrids is predicted by the genetic distance between species of cichlid fish. *BMC Evol. Biol.* **9**: 283.
Vilà, M., Weber, E. & D'Antonio, C.M. 2000. Conservation implications of invasion by plant hybridization. *Biol. Invasions* **2**: 207–217.
Vittor, B.A. 1971. Effects of the environment on fitness-related life history characters in *Tigriopus californicus*. Ph. D. dissertation, University of Oregon. Available at scholarsbank.uoregon.edu. Accessed September 1 2012.
Voordouw, M.J. & Anholt, B.R. 2002a. Environmental sex determination in a splash pool copepod. *Biol. J. Linn. Soc.* **76**: 511–520.
Voordouw, M.J. & Anholt, B.R. 2002b. Heritability of sex tendency in a harpacticoid copepod, *Tigriopus californicus*. *Evolution* **56**: 1754–1763.
Voordouw, M.J., Robinson, H.E. & Anholt, B.R. 2005. Paternal inheritance of the primary sex ratio in a copepod. *J. Evol. Biol.* **18**: 1304–1314.
Willett, C.S. 2006. Deleterious epistatic interactions between electron transport system protein-coding loci in the copepod *Tigriopus californicus*. *Genetics* **173**: 1465–1477.
Willett, C.S. 2007. Significant variation for fitness impacts of ETS loci in hybrids between populations of *Tigriopus californicus*. *J. Hered.* **99**: 56–65.
Willett, C.S. 2008. No evidence for faster male hybrid sterility in population crosses of an intertidal copepod (*Tigriopus californicus*). *Genetica* **133**: 129–136.
Willett, C.S. 2011. Complex deleterious interactions associated with malic enzyme may contribute to reproductive isolation in the copepod *Tigriopus californicus*. *PLoS ONE* **6**: 12.
Willett, C.S. 2012. Hybrid breakdown weakens under thermal stress in population crosses of the copepod *Tigriopus californicus*. *J. Hered.* **103**: 103–114.
Wolf, D.E., Takebayashi, N. & Rieseberg, L.H. 2001. Predicting the risk of extinction through hybridization. *Conserv. Biol.* **15**: 1039–1053.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Number of samples, mean and standard deviation for morphological measurements and 14-day larval survivorship for each swarm replicate at each time point.

Table S2 Results of ANOVAs testing for an influence of swarm type on coefficient of variation within swarm replicates. Footnotes show results of *post-hoc* LSD tests.

Figure S1 Female (left) and male (right) *T. californicus*, showing morphometric measurements taken.

Figure S2 Proportion of total controlled cross pairs producing no live offspring.

Figure S3 Morphological variation within the controlled crosses.

Data deposited at Dryad: doi: 10.5061/dryad.2bj90

Received 4 August 2012; revised 23 October 2012; accepted 24 October 2012