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THE GENOMIC TRAJECTORY OF HYBRID SWARMS: OUTCOMES OF REPEATED CROSSES BETWEEN POPULATIONS OF *TIGRIOPUS CALIFORNICUS*

Victoria L. Pritchard[1] and Suzanne Edmands[1,2]

[1]*Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-0371*

[2]*E-mail: sedmands@usc.edu*

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Introgressive hybridization between genetically divergent populations is an important evolutionary process. The degree to which repeated hybridization events between the same parental taxa lead to similar genomic outcomes is unknown. This study addressed this question by following genomic trajectories of replicate hybrid swarms of the copepod *Tigriopus californicus* over many generations of free mating. Swarm composition was determined both by differential reproductive success of founder individuals and subsequent selection on hybrid genotypes. For one cross, between two populations showing differential fitness in the laboratory and no hybrid breakdown, the genetic trajectory was highly repeatable: replicates rapidly became dominated by alleles from the fitter parent. In a second cross, between two populations showing similar fitness and significant F2 hybrid breakdown, alleles from alternative populations dominated different replicates. Swarms exhibited a general temporal trend of decreasing cytonuclear mismatch. Some patterns of differential introgression across the genome were strikingly congruent amongst swarm replicates, both within and between cross types, and reflected patterns of segregation distortion previously observed within controlled crosses between the same parental populations. Differences in heterozygosity between the sexes, and evidence for a previously suspected sex-distortion locus, suggest that complex interactions between sex and genotype influence hybrid swarm outcome.

KEY WORDS: **Cytonuclear coadaptation, copepod, genomic clines, hybrid zone, introgression, segregation distortion.**

Introgressive hybridization between genetically divergent populations is an important evolutionary process that occurs commonly in nature. The consequences of such hybridization are difficult to predict, and depend on the genetic composition of the interacting populations and the nature of intrinsic barriers to gene flow between them. Introgression can reduce population fitness by modifying locally adaptive traits or bringing together Bateson—Dobzhansky–Muller (BDM) incompatibilities (e.g., Martin and Willis 2010; Verhoeven et al. 2011). Conversely it can counter inbreeding depression and make novel genetic combinations available to selection, thus increasing overall population fitness (e.g., Stelkens et al. 2009; Lucek et al. 2010). Hybrid populations may outperform their parents, and introgressive hybridization has been implicated in the emergence of invasiveness (e.g., Keller and Taylor 2010; Turgeon et al. 2011). New, evolutionarily independent lineages can arise from hybrid populations (e.g., Hermansen et al. 2011; Stemshorn and Reed, 2011). From a practical perspective, these various effects of introgressive hybridization are relevant to the management of threatened taxa, for example, when considering transplantation between populations to alleviate inbreeding depression (Tallmon et al. 2004), or managing populations subject to non-native introgression as a result of

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anthropogenically induced range expansions (Allendorf et al. 2001).

Studies of genomic interactions in hybridizing populations can both improve our understanding of the evolutionary process and inform conservation management decisions. Rapid advances in genetic tools and analytical methods are making such studies increasingly feasible (Payseur 2010). Introgression between parental populations is not expected to proceed at equal rates across the genome: rather, regions influencing hybrid fitness will have patterns of introgression different from selectively neutral regions. In extreme cases, loci may spread rapidly beyond the initial hybrid zone (e.g., Fitzpatrick et al. 2010). Thus, investigation of differential introgression across the genome can provide insights into the location and nature of loci contributing to reproductive isolation and, conversely, to hybrid fitness. Such loci include those subject to extrinsic selection, for example, conferring adaptive advantages in the local environment, and those subject to intrinsic selection within the hybrid genome. Studies have found overlap between regions of the genome exhibiting elevated interpopulation divergence, implicating local selection, and regions showing non-neutral patterns of introgression when those groups hybridize (Gompert et al. 2012a; Lamaze et al. 2012), although this is by no means complete (Luttikhuizen et al., 2012). Analyses of natural hybrid zones in *Populus* (Lexer et al. 2010) and *Mus* (Teeter et al. 2010; Janoušek et al. 2012) have identified numerous loci, distributed across the genome, as candidates for BDM incompatibilities based on introgression patterns indicative of epistatic interactions.

Of particular interest is whether replicated hybridizations over many generations have a repeatable genomic outcome, and whether that outcome can be predicted from observations of controlled crosses (e.g., Karrenberg et al. 2007). Laboratory studies have demonstrated that independent hybridizations can indeed lead to repeatable outcomes, which may mirror patterns in hybrid species naturally generated from the same parents (Rieseberg et al. 1996). However, comparisons of hybrid populations independently originating from the same parental taxa in the wild have revealed substantial incongruence in patterns of introgression across loci (Nolte et al. 2009; Teeter et al. 2010; Janoušek et al. 2012). In *Mus,* while some loci identified from controlled crosses as being involved in reproductive isolation showed non-neutral patterns of introgression in natural hybrid zones, others did not (Janoušek et al. 2012). Such incongruence may reflect uncontrolled factors such as genetic substructure within the parental taxa or divergent environmental conditions; alternatively it may reflect a more general pattern of stochasticity in the outcome of hybridization events. Studies of replicate hybrid populations with the same parent populations, maintained under common garden conditions, can shed more light on these questions.

The copepod *Tigriopus californicus* is an ideal laboratory model for such studies. The species occurs in supralittoral tidepools on rocky outcrops along the Pacific coast of North America. Its mating system is well studied (Burton 1985): a male guards a female until she becomes sexually mature; females mate only once, and produce up to several hundred eggs; recombination occurs only in males. In common with other copepods, sex appears to be determined by genetic and environmental factors (Voordouw and Anholt 2002a,b; Gusmao and McKinnon 2009), although specific genes have not yet been identified. Gene flow between populations on different outcrops is highly restricted, and interpopulation genetic divergence frequently exceeds that observed between species in other taxa. Despite this, most populations are completely interfertile. The second generation (F2) hybrid offspring of interpopulation crosses typically exhibit reduced fitness, as measured by such traits as development time, fecundity and survivorship (Burton 1990, Edmands et al. 2005), cytochrome oxidase activity (Edmands and Burton 1999), and mitochondrial ATP production (Ellison and Burton 2008a). Degree of fitness reduction is positively related to genetic and geographic distance between the parental populations (Edmands 1999). Individuals in the first hybrid generation (F1) generally do not suffer reduced fitness (although see Ganz and Burton 1995), indicating that F2 hybrid breakdown results from the disruption of gene complexes via segregation and recombination. Adaptation between the nuclear and mitochondrial genomes, mediated through the oxidative phosphorylation pathway and the mitochondrial transcription apparatus, is known to play a role (e.g., Edmands and Burton 1999; Ellison and Burton 2008b). However, unexpected patterns of fitness within certain crosses (e.g., Edmands et al. 2009) and studies of segregation distortion in F2 hybrid adults (Harrison and Edmands 2006; Edmands et al. 2009; Pritchard et al. 2011; Willett 2011) have demonstrated that nuclear-mitochondrial coadaptation is incomplete in some populations, and that multiple interactions across the nuclear genome also contribute to hybrid breakdown.

Here, we generate replicate hybrid populations, using two different types of *T. californicus* interpopulation cross, and follow their genetic trajectory over many generations. We simulate the situation expected to occur following secondary contact in nature by allowing free mating between individuals. We address two primary questions: whether replicate hybrid swarms progress to a repeatable outcome, and whether patterns of segregation distortion observed in controlled crosses are predictive of patterns of introgression across the genome.

Methods

We established hybrid swarms using two reproductively compatible pairs of *T. californicus* populations, similar in their degree of

genetic divergence, that have been used in previous studies. San Diego, California (SD, 32°45′N, 117°15′W) and Punta Baja, Baja California (PBJ, 29°58′N, 115°48′W), 336 km apart, are 23% divergent at the mitochondrial COI locus (Edmands 2001) and 3.49% divergent across 50 nuclear loci (Pritchard et al. 2011). PBJ, the more southern population, exhibits lower survival between hatching and adulthood under our culture conditions than does SD (Pritchard and Edmands, unpubl. data). Unusually, controlled crosses between SD and PBJ do not show significant hybrid breakdown in the F1, F2, or F3 generations (Pritchard and Edmands, unpubl. data). Santa Cruz, California (SC, 36°57′N, 122°03′W) is 640 km north of SD; these two populations are 21% divergent at the mitochondrial genome (Burton et al. 2007) and 3.76% divergent across 50 nuclear loci (Pritchard et al. 2011). Comparison of transcriptomes from SD and SC has demonstrated a median divergence of 2.71% and identified numerous regions potentially under selection (Barreto et al. 2011). Pure SC and SD exhibit no consistent differences in survivorship or competitive fitness under the rearing conditions applied, however, SC individuals are larger and females produce more offspring per clutch of eggs (Willett 2010; Pritchard and Edmands, unpubl. data). SD × SC exhibits the usual pattern of no fitness reduction in the F1 followed by F2 hybrid breakdown (Ellison and Burton 2008a). F2 adults exhibit strong segregation distortion at genome-wide markers, caused by selection between hatching and adulthood (Willett and Berkowitz 2007; Pritchard et al. 2011). Direction of distortion varies between linkage groups, and also with sex and mitochondrial background (Foley and Edmands, unpubl. data). Although survivorship in the SD × SC backcross shows clear evidence for cytonuclear coadaptation involving the SC mitochondrion, evidence for a similar pattern involving the SD mitochondrion is more equivocal (Ellison and Burton 2008b; Pritchard and Edmands, unpubl. data). Some studies have shown fitness reductions to continue into the F3 generation (Ellison and Burton, 2008a).

ESTABLISHMENT AND MAINTENANCE OF SWARMS

Several thousand *T. californicus* individuals were collected from SD, SC, and PBJ and subsequently maintained as large laboratory populations. Within 2 months of collection, two types of hybrid swarm were initiated: SD × PBJ ("D" swarms) and SD × SC ("E" swarms). Eight replicates were established for each swarm type: 150 gravid females from each contributing population were used to initiate each replicate. Swarms were maintained in 1L glass beakers containing 800 mL of 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*). Beakers were housed in an incubator at 20°C with a 12 h light to 12 h dark cycle. Every 14 days, beakers were systematically rotated within and between incubator shelves and partial water changes were performed: beakers were rehydrated to 800 mL using deionized water, 400 mL of contents were removed through a sieve, retained copepods were returned, and beakers were topped up to 800 mL with fresh culture medium. Replicates with no living copepods were discarded. Where replicates had small numbers (< 20 adults) alive, all surviving individuals were transferred to a beaker containing fresh culture medium in an attempt to prevent loss of the replicate.

GENOTYPING

Every three months, a sample of up to 20 unpaired males and 20 females (with egg sacs removed) was harvested from each swarm beaker and frozen for subsequent analysis. Genetic data were obtained for males and females from the seven longest-surviving hybrid swarm replicates (SD × PBJ: D4, D6, D7, D8; SD × SC: E4, E6, E7), for Months 3, 6, 9, 15, and 21. For comparative purposes, males from the final time point of six short-lived hybrid swarm replicates (SD × PBJ: D1, D2, D3; SD × SC: E1, E2, E3) were also genotyped. Individuals were scored for 54 putatively population-diagnostic SNPs (51 nuclear and three mitochondrial) described in Pritchard et al. (2011). Genotyping was performed at Roswell Park Cancer Institute, using the iPLEX Gold Assay on MassARRAY Compact (Sequenom). We provided unpurified, dried down lysis extracts (see Pritchard et al. 2011). Samples with a SNP call rate < 90% were removed from subsequent analyses. Accuracy of calls in heterozygotes was tested with a blind set of F1 SD × PBJ and SD × SC individuals. As stored sperm could potentially increase observed heterozygosity in mated females, we additionally genotyped 36 pure SD and 23 pure SC females, mated with a male from the alternative population, to investigate whether the alternative alleles were observed. We also genotyped 65–95 individuals from each parental population to check the diagnostic utility of SNPs. Finally, we genotyped a small archived sample of SD × PBJ F2 individuals ($n = 26$), enabling us to assign SNPs TC058 and TC097, diagnostic between SD and PBJ but not mapped in Pritchard et al. (2011), to linkage group.

STATISTICAL ANALYSIS

We performed linkage group analysis for TC058 and TC097 using Map Manager QTX (Manly et al. 2001). We calculated hybrid indices for swarm individuals using simple allele counts. Cytonuclear mismatch within an individual was defined as the proportion of nuclear alleles derived from a population other than that providing the mitotype. We examined each locus in each replicate at each time point for deviations from Hardy–Weinberg equilibrium using exact tests implemented in Genepop (Raymond and Rousset 1995). We examined each replicate for evidence of between-chromosome epistatic interactions by pooling genotypic data for Months 9, 15, and 21 and examining each pair of physically unlinked loci for linkage disequilibrium using Genepop. Tests were

performed for sexes pooled and separately. We corrected for multiple testing within each type of test within each cross-type using the false discovery rate (FDR) method of Benjamini and Hochberg (1995), with $\alpha = 0.05$.

We applied *t*-tests to examine each replicate for overall differences in heterozygosity between the sexes. We used ANOVA followed by post-hoc least significant difference (LSD) tests to compare hybrid index and cytonuclear mismatch in samples of males from surviving and nonsurviving swarm replicates at Months 3, 6, or 9, depending on the final time point of the nonsurviving replicates.

We used the moments-based approach (Waples 1989), implemented in NeEstimator (Ovenden et al. 2007) to estimate effective population size (N_e) from the variation in allele frequencies between successive samples from each replicate. We assumed one generation per month. Replicates at Month 0 were assumed to have equal allele frequencies of 0.5 over all loci.

Finally, we used Introgress (Gompert and Buerkle 2010), implemented in R 2.9 (R Development Core Team 2009), to identify loci with introgression patterns deviating from the overall hybrid background within each swarm replicate. We assessed significance of deviations using the permutation approach. As the number of individuals genotyped at each time point was small, we treated time points as multiple samples from within the same multigenerational hybrid zone and combined them for the analysis. We excluded Month 3 from all replicates as this sample primarily contained parental and F1 individuals. We ran analyses both for sexes combined, and separately. The type of selection acting on each locus was implied by visually comparing the genotypic clines for this locus with the 95% confidence intervals for clines generated on all loci combined, following Nolte et al. (2009). We used the FDR approach to correct for multiple testing, within each of the two sets of tests ($n = 274$ for sexes combined; $n = 548$ for sexes separately).

SIMULATIONS

We examined whether observed changes in hybrid index and cytonuclear mismatch over time could occur without selection by modeling hybridizing populations using simuPOP 1.0.7 (Peng and Kimmel 2005). For each simulation we generated an initial population reflecting genotypes within actual swarms and maintained this population by random mating through generation 21. We specified 36 or 43 nuclear markers, corresponding to SNPs diagnostic between parental populations, plus a single population-diagnostic mitochondrial marker. Map distances were taken from Pritchard et al. (2011), with crossover frequency specified as 0.01 per cM per generation. We implemented an effective population size <80, based on the mean N_e estimated for real swarm replicates using data from Month 3 and the final time point. To do this we specified a constant population size of $n = 80$, with number of offspring produced by each breeding pair following a Poisson distribution with $k = 2$. Females mated only once but males could mate multiple times; to minimize runtime errors caused by insufficient number of female parents we applied a within-population sex ratio of two females to one male. We generated 1000 replicates for each scenario: at generations 3, 6, 12, and 18 (representing Months 6, 9, 15, and 21), we randomly selected 40 individuals from each replicate and calculated nuclear hybrid index, mitochondrial hybrid index, and cytonuclear mismatch. Two different scenarios were implemented. First, we generated initial populations with equal numbers of nuclear and mitochondrial haplotypes derived from each founder population, simulating the distribution of parental and F1 genotypes expected at Month 3 with equal numbers of randomly mating pure parents at Month 0. Second, we specified initial genotype frequencies corresponding to those observed at Month 3 in each replicate, thus assuming differential reproductive success of pure founder individuals. Observed mean hybrid indices and cytonuclear mismatch for each actual replicate at each time point were compared to the distribution of means estimated from the corresponding simulated replicates. Finally, we estimated N_e actually simulated in our model by applying the Waples (1989) approach, using genetic data from generations 3 and 18 of the first 20 simulated populations initialized with equal haplotypic frequencies.

Results

Following Foley et al. (2011), chromosomes A, B, C, and D of Pritchard et al. (2011) are renamed Chromosomes 3, 4, 12, and 6, respectively. Examination of pure populations and known heterozygotes confirmed $n = 36$ nuclear loci reliably diagnostic between SD and PBJ, $n = 43$ nuclear loci reliably diagnostic between SD and SC, and $n = 3$ diagnostic mitochondrial loci. Two additional loci described as diagnostic between SD and SC in Pritchard et al. (2011) exhibited rare variation in pure SD samples. Linkage group analysis using SD × PBJ F2 individuals indicated that TC058 was most closely linked to TC012 (Chromosome 6, LOD = 7.2), and TC097 most closely linked to TC102 (Chromosome 3, LOD = 8.4). For modeling purposes, we specified an arbitrary distance of 10 cM between these two locus pairs. In mated females, we observed unexpected heterozygotes in <0.4% at total sites assayed, distributed over multiple markers, and comprising a single heterozygote call in each of 11 individuals. This was slightly higher than the apparent overall genotyping error rate, estimated from unexpected heterozygote calls in known pure males as <0.1%. Examination of loci with three different alleles fixed in SD, PBJ, and SC confirmed no accidental cross-contamination between hybrid swarm types.

SWARM TRAJECTORIES

Due to fluctuations in swarm density and other factors, some time points had <40 genotyped individuals per replicate. All SD × PBJ (D) swarm replicates persisted through Month 6, with two lost before Month 9 and a third before Month 12. Swarm D7, suffered a fungal infection prior to Month 3, which appeared to greatly reduce census population size. Three SD × SC (E) swarm replicates had died out by Month 6, with another lost before Month 9. ANOVA demonstrated significant variation in cytonuclear mismatch and hybrid index amongst SD × PBJ replicates at Months 6 and 9, and SD × SC replicates at Month 3, however, we found no overall difference between surviving replicates and those that died out following these time points.

Hybrid swarms at Month 3 consisted almost entirely of pure individuals and apparent F1 hybrids (Fig. 1). The two different cross-types followed different temporal trajectories (Figs. 1, S1). In the SD × PBJ swarms, the PBJ mitotype was entirely lost by Month 9. SD allele frequencies generally increased over all time points, with PBJ alleles almost completely lost from D4 and D8 by Month 21. In contrast, both SD and SC mitotypes remained in the SD × SC hybrid swarms through Month 15. The composition of different SD × SC replicates tended to different outcomes. By Month 21, E4 contained 100% SC mtDNA and 91% SC nuclear alleles; in contrast, E6 contained 100% SD mtDNA and 87% SD alleles, whereas E8 retained both mitotypes and 68% SC alleles. For both cross-types, hybrid trajectory apparently reflected relative proportions of pure and F1 individuals at Month 3.

HETEROZYGOSITY

We observed significantly greater heterozygosity in females than males at multiple time points in the SD × SC experiment (Table 1), which was not explained by the few extra heterozygous SNP calls in fertilized individuals. Further examination showed this to be driven by a larger proportion of males with pure SD or pure SC genotypes. In contrast, the few significant differences in the SD × PBJ experiment involved greater heterozygosity in males. Hardy–Weinberg tests revealed an overall significant heterozygote deficiency in D4 at Month 3, due to lack of hybrids, and in E7 at Month 6, associated with a large number of pure SD males. This was followed by significant heterozygote excess in E7 females at Month 9. Otherwise, no locus or sample exhibited a systematic pattern of significant deviation from Hardy–Weinberg equilibrium either for sexes together or separately. Regarding both statistically significant and nonsignificant deviations, we observed a trend in the SD × SC swarms, but not in the SD × PBJ swarms, for females to have more loci with heterozygote excess (E4: 91% of tests in females vs. 65% of tests in males; E6: 79% vs. 43%; E7: 59% vs. 47%).

LINKAGE EQUILIBRIUM

Following FDR correction, we found 18 significant deviations from linkage equilibrium amongst the 2445 total tests within the SD × PBJ swarms, all within D7 (Table S1). In males, these occurred between multiple loci on two chromosome pairs: 1 and 8, and 7 and 10. In females they involved loci on Chromosomes 8 and 10. In the SD × SC swarms, we found 466 significant deviations from linkage equilibrium amongst 5238 tests. Approximately 70% of these occurred in E6 males, involving 37 of 66 possible chromosome pairs and nine chromosomal–mitochondrial interactions. A further 10% were in E6 females: two-thirds involved chromosome pairs with no significant interactions in males. Most other significant deviations were in E7, again with little congruence between the sexes, and including no chromosomal–mitochondrial interactions. Few significant deviations occurred in E4, and there was overall little congruence between replicates in linkage disequilibrium patterns.

EFFECTIVE POPULATION SIZE

Estimates of variance effective population size fluctuated greatly within all replicates, depending upon the time points examined, and confidence intervals were wide (Table 2). Estimated N_e based on assumed equal allele frequencies at Month 0 and observed sample allele frequencies at Month 3 was generally very small. N_e calculated from differences in allele frequency between Month 3 and the final genotyped time point varied from 36 to 150.

GENOMIC CLINES ANALYSIS

Introgress revealed multiple significant deviations from the null expectation of homogeneous admixture across the genome, with closely linked loci exhibiting congruent patterns (Tables 3, S2 and Figs. S2 and S3). Proportion of loci associated with non-neutral introgression ranged from 0% (D4) to 67% (D7). Within a cross-type, linkage groups frequently exhibited different deviations within different swarm replicates. Nevertheless, we observed some common patterns.

SD × PBJ replicates exhibited genotypic clines suggestive of selection against the SD genotype, combined with heterozygote advantage (overdominance), at Chromosome 1; in D6 and D8, but not in D7, this pattern was restricted to males. Two of the replicates, D6 and D7, exhibited clines consistent with positive selection for SD at Chromosome 5 and negative selection for SD at Chromosome 7, with some differences between genders.

Within SD × SC, all replicates exhibited deviations from neutral expectations at one end of Chromosome 8, suggestive of selection against heterozygotes (underdominance) and/or against SD alleles. Examining the sexes separately, we also observed deviations for all replicates at Chromosome 11, however, clines

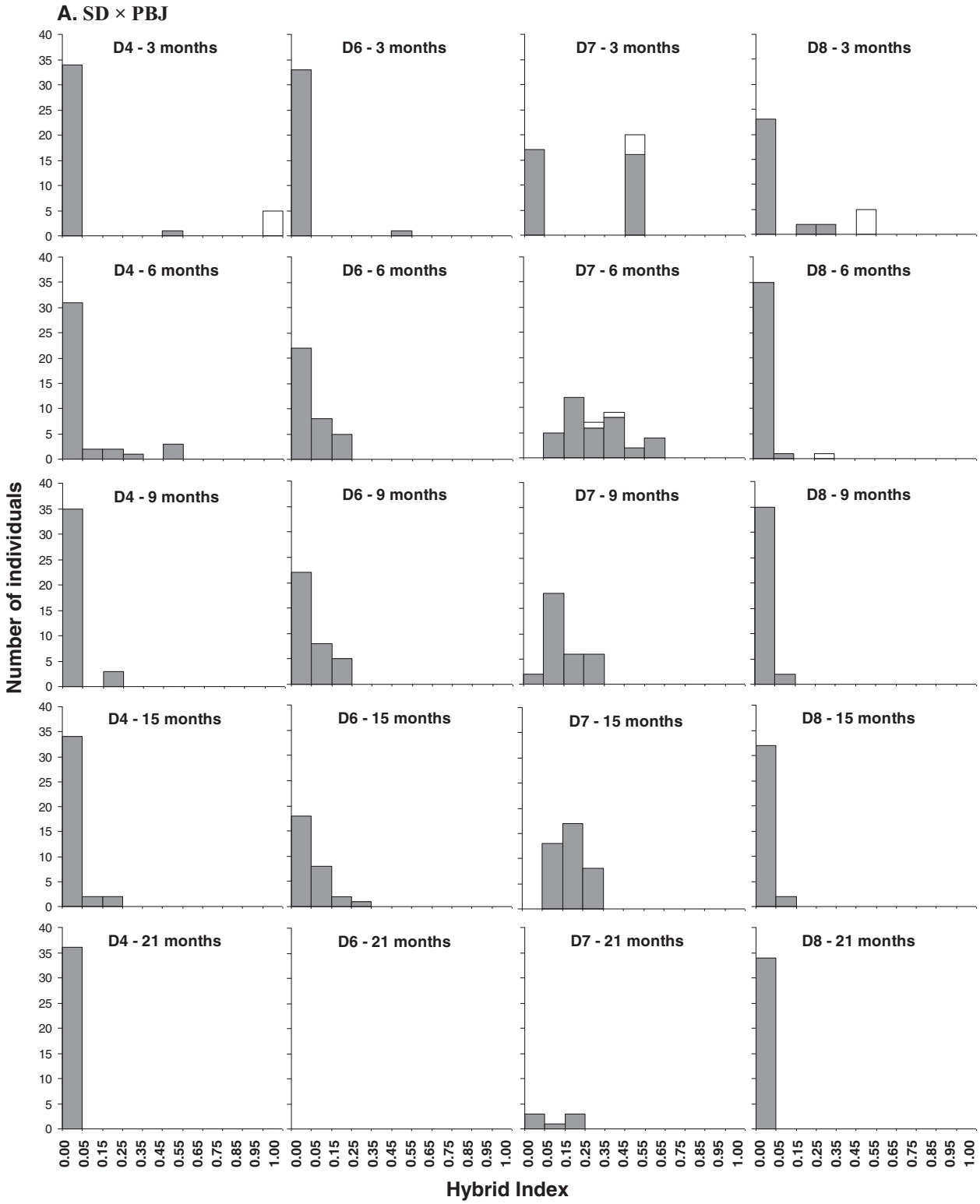

Figure 1. Distribution of nuclear hybrid indices for (A) SD × PBJ swarm replicates and (B) SD × SC swarm replicates at each genotyped time point. A hybrid index of 0 indicates 100% SD alleles, whereas a hybrid index of 1 indicates 100% PBJ/SC alleles. Mitochondrial genotypes are indicated by: □ PBJ mtDNA; ■ SD mtDNA; ▫ SC mtDNA [Correction added on October 25, 2012, after first online publication: In the heading of Figure 1, "A. SB × PBJ" was changed to "A. SD × PBJ".].

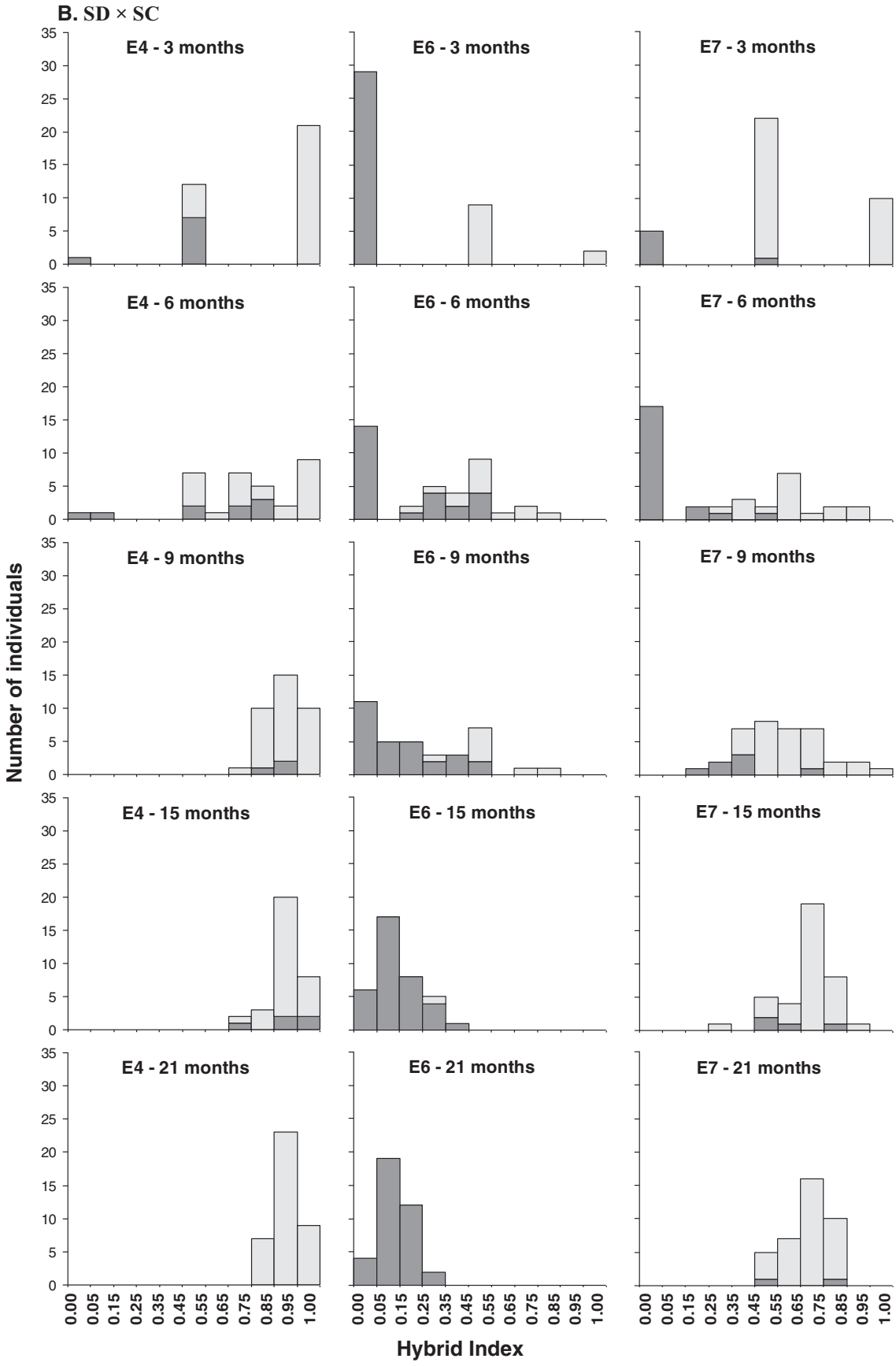

Figure 1. *Continued*.

Table 1. Mean observed male and female heterozygosity within each swarm replicate at each genotyped time point. "p" (values in italics) shows the result of *t*-test comparisons, with bold indicating the gender with significantly higher heterozygosity.

		Month 3			Month 6			Month 9			Month 15			Month 21		
Cross	Rep	Fem	Male	*p*	Fem	Male	*p*	Fem	Male	*p*	Fem	Male	*p*	Fem	Male	*p*
SD × PBJ	D4	0.00	0.06	*0.31*	0.20	0.06	*0.15*	0.01	0.06	*0.07*	0.01	0.07	*0.11*	0.01	0.01	*0.99*
	D6	0.07	0.00	*0.24*	0.04	**0.14**	*0.02*	0.07	**0.13**	*0.05*	0.08	0.16	*0.06*	–	–	–
	D7	0.30	**0.76**	*0.00*	0.43	0.46	*0.57*	0.29	0.20	*0.10*	0.28	0.28	*0.92*	–	–	–
	D8	0.18	0.25	*0.59*	0.04	0.01	*0.38*	0.00	0.02	*0.21*	0.03	0.02	*0.56*	0.02	0.01	*0.20*
SD × SC	E4	0.47	0.22	*0.09*	**0.57**	0.29	*0.02*	**0.25**	0.13	*0.01*	0.20	0.15	*0.20*	**0.20**	0.13	*0.01*
	E6	**0.42**	0.00	*0.00*	**0.61**	0.31	*0.01*	0.28	0.40	*0.31*	0.19	0.23	*0.58*	0.22	0.19	*0.23*
	E7	0.66	0.49	*0.21*	**0.42**	0.17	*0.01*	**0.69**	0.51	*0.00*	0.46	0.44	*0.27*	0.44	0.39	*0.07*

Table 2. Estimates of N_e, and 95% confidence limits (CI), for each swarm replicate.

	Month 0–3		Month 3–6		Month 6–9		Month 9–15		Month 15–21		Month 3 to end	
Rep	N_e	95% CI	N_e	95% CI	N_e	95% CI	N_e	95% CI	N_e	95% CI	N_e	95% CI
D4	**2.8**	1.6, 4.2	**34.7**	16.9, 73.4	**26.5**	13.5, 51.7	∞	339.3, ∞	**520.6**	339.3, ∞	**36.2**	20.9, 57.3
D6	**1.3**	0.8, 2.0	**65.2**	25.8, 270.1	∞	180.7, ∞	**136.3**	51.3, 821.1	–	–	**149.9**	68.0, 379.1
D7	**7.8**	4.4, 12.5	**82.1**	31.1, 463.8	**6.4**	3.7, 10.1	**52.3**	26.0, 105.5	–	–	**47.6**	26.4, 79.8
D8	**2.3**	1.3, 3.5	**10.0**	5.6, 16.6	**837.8**	58.6, ∞	**1441.2**	75.0, ∞	∞	104.0, ∞	**50.7**	28.7, 82.9
E4	**4.3**	2.6, 6.6	∞	∞, ∞	**9.7**	5.6, 15.6	**215.5**	75.0, 14,904.0	**89.0**	42.0, 213.0	**59.9**	34.9, 96.1
E6	**3.1**	1.9, 4.6	**25.6**	13.7, 47.2	**93.7**	35.5, 622.3	**44.7**	24.1, 81.0	**150.4**	60.9, 636.7	**133.7**	72.5, 239.7
E7	∞	278.3, ∞	**5.8**	3.5, 8.9	**5.6**	3.4, 8.7	**56.5**	29.5, 108.5	∞	∞, ∞	**141.7**	76.1, 258.7

in E4 and E6 were consistent with selection against SD while those in E7 suggested selection in the opposing direction. We also observed opposing trends on Chromosome 12, with negative selection on SD inferred in E4 but positive selection for SD inferred within E6. As in SD × PBJ, results suggested selection against SD on Chromosome 7.

All three SD × SC replicates exhibited large deviations from neutral expectations across Chromosome 10. Further examination revealed a strong difference between the sexes: fitted clines indicated positive selection on SD alleles in females and no (E6) or negative selection (E4, E7) on these alleles in males. We also observed evidence for positive selection on Chromosome 10 SD alleles in females but not in males in SD × PBJ replicate D7. We investigated this further for Months 9–21 of the SD × SC swarms (avoiding the confounding influence of large numbers of pure SD males in earlier months) by plotting, for each of the three genotypes at Chromosome 10 locus TC045, the proportion of individuals that were female against the mean hybrid index of the swarm replicate (Fig. 2). Results suggested an interaction of Chromosome 10 genotype with sex and overall hybrid background. Where SC allele frequency was low and hence SC/SC genotypes were rare, SC/SD individuals were more likely to be male than female. With increasing frequency of SC alleles in a population, SC/SD individuals had an increased likelihood of being female, with SC/SC homozygotes tending to be male. This effect appeared independent of an individual's mitotype.

SIMULATIONS

Mean effective population size within a subset of 20 simulated populations, calculated from allele frequencies at Generations 3 and 18, was 56.0, comparable to N_e estimated for actual hybrid swarms. Results of simuPOP simulations showed that, in general, observed nuclear hybrid indices and levels of cytonuclear mismatch were highly unlikely to be obtained with no selection and equal founding contributions from each parental population to the first two swarm generations (Table 4, Fig. 3). In contrast, altering starting genotypic frequencies to reflect those observed in different swarm replicates at Month 3, reflecting unequal genetic contributions from the parental populations, resulted in substantial overlap between observed and simulated values (Table 5, Fig. S4). Nevertheless, swarm replicates exhibited mean cytonuclear mismatch lower than that of 99% of simulated swarms in 64% of tests for SD × PBJ and 17% of tests for SD × SC. From Month 9 onwards, cytonuclear mismatch within all but one hybrid swarm was within the lowest 25% of values observed within the simulated swarms, and generally declined over time. The notable exception was D6, which had very few PBJ alleles at Month 3 and exhibited a trend for increasing hybrid index and cytonuclear mismatch.

Table 3. Results of Introgress analysis on genotyped swarm replicates. Values indicate the probability of a locus corresponding to the null expectation of homogeneous admixture across the genome, estimated from 1000 permutations; significant deviations are shown in bold [Correction added on October 25, 2012, after first online publication: In table 3, the following deviations are significant and are now shown in bold: Column 4, Rows 16 and 18-21; Column 12, Rows 37 and 40-41; and Column 14, Rows 40-41.].

		SD × PBJ							SD × SC						
Ch	Locus	D4	D6		D7		D8		E4		E6		E7		
1	TC016	0.895	**<0.001**	OD,NS[1]	**0.001**	NS	0.065	[1]	**0.001**	PS[1]	0.063		0.039		
	TC033	0.156	**<0.001**	OD,NS[1]	**0.003**	NS	**0.001**	NS	**<0.001**	PS[1]	0.047		0.244		
	TC124	0.880	**<0.001**	OD,NS[1]	**<0.001**	OD,NS	0.065	[1]	**0.006**	PS[1]	0.075		0.051		
	TC084	0.411	**<0.001**	OD,NS[1]	**<0.001**	OD,NS	0.020	[1]	**0.000**	PS[1]	0.075		0.155		
	TC085	0.125	0.753		**0.003**	NS	0.016		0.067		**<0.001**	NS	0.352		
2	TC118	0.809	0.054		**<0.001**	PS	0.316		0.030		0.016		0.085		
	TC060	–	–		–		–		0.155		0.017		0.548		
	TC106	–	–		–		–		0.289		0.045		**0.012**	NS[1]	
	TC107	0.539	0.078		**0.003**	UD,NS	0.513		0.187		0.387		**0.001**	NS[1]	
3	TC099	0.828	**0.001**	E	0.341		0.316		0.302		0.026		0.416		
	TC102	0.493	**0.004**	E	0.170		0.015		0.278		**0.011**	E	0.174		
	TC125	0.853	**0.009**	NS	0.450		**<0.001**	NS	0.231		0.122		0.330		
	TC097	0.256	0.204		0.637		0.708		–		–		–		
4	TC040	0.615	0.242		**<0.001**	NS[1]	0.792		0.666		**0.009**	E	0.165		
	TC169	0.686	0.119		**<0.001**	NS[1]	0.316		0.096		**0.009**	E	0.046		
5	TC074	0.383	**0.004**	PS[1]	**0.003**	PS,E	0.973		0.041		**0.004**	OD,E[1]	0.735		
	TC008	0.815	0.031		**<0.001**	PS,E	0.842		0.084		0.507		0.633		
	TC006	0.263	**<0.001**	PS[1]	**<0.001**	PS,E	0.402		0.020		0.022		0.385		
	TC111	0.685	**0.004**	PS[1]	0.704		0.774		0.294		0.039		0.502		
	TC171	0.079	**<0.001**	PS[1]	**<0.001**	PS,E	0.081		0.044		0.566		0.883		
	TC157	0.168	**0.001**	PS[1]	**<0.001**	PS	0.903		0.219		0.998		0.955		
6	TC012	0.854	0.190		**0.006**	PS	0.153		0.070		**0.015**	NS	0.106		
	TC058	0.854	0.201		0.085		0.923		–		–		–		
7	TC184	0.048	**<0.001**	NS[1]	**0.002**	NS,E[1]	0.153		0.316		**<0.001**	NS	0.819		
	TC103	0.048	**<0.001**	NS[1]	**0.003**	NS,E[1]	0.153		**0.001**	OD	0.068		0.912		
	RPOL	–	–		–		–		0.050		0.292		0.185		
	TC162	0.048	0.682		0.225		0.081		0.231		0.153		0.240		
	TC051	–	–		–		–		0.231		0.096		0.143		
8	TC152	0.785	0.238		0.102		0.316		0.369		0.037		0.379		
	TC180	0.785	0.176		0.102		0.316		0.382		0.022		0.264		
	TC156	0.604	0.754		0.086		0.316		**0.001**	UD,PS	0.037		**<0.001**	UD,PS	
	TC078	0.442	0.343		**<0.001**	NS	0.245		**0.013**	NS	**<0.001**	UD,NS	**<0.001**	UD	
	TC128	–	–		–		–		0.033		**0.001**	UD,NS	**<0.001**	UD, NS	
9	TC073	0.269	0.759		**<0.001**	IA	0.360		0.849		0.348		**0.012**	PS [1]	
	TC011	–	–		–		–		0.492		0.820		**<0.001**	UD,PS	
10	TC188	–	–		–		–		**<0.001**	UD,PS[1]	**0.005**	PS [1]	**<0.001**	UD,PS[1]	
	TC104	–	–		–		–		0.294	[1]	**<0.001**	OD,E[1]	0.037	[1]	
	TC045	0.801	0.827		**0.001**	PS [1]	0.208		0.173	[1]	**0.016**	E[1]	**0.004**	OD,E[1]	
	TC130	0.801	0.886		**0.001**	PS [1]	0.354		0.173	[1]	**0.003**	OD,E[1]	**0.018**	E[1]	
	TC077	0.858	0.423		0.185		0.316		0.222	[1]	**0.001**	OD,E[1]	**0.001**	OD,E[1]	
	TC189	0.858	0.356		0.230		0.316		0.294	[1]	**0.004**	E [1]	**0.006**	E[1]	
11	TC043	0.188	**0.004**	UD	**0.002**	IA	0.153		0.266		**<0.001**	NS	0.723		
	TC046	0.026	0.860		**<0.001**	IA	0.153		0.197		0.019	[1]	**<0.001**	PS	
12	TC112	–	–		–				**0.001**	NS[1]	**<0.001**	PS	0.449		
	TC167	0.957	0.379		**<0.001**	PS,UD	0.316		**<0.001**	NS[1]	**<0.001**	PS	0.288		

Implied patterns of selection are indicated as follows: UD = underdominance; OD = overdominance; NS = negative selection on SD alleles; PS = positive selection for SD alleles; E = epistatic interaction between locus and genomic background; IA = increased admixture: probability of the SD homozygote is decreased at low hybrid indices and increased at high hybrid indices.

[1]A difference between the sexes, that is, the locus exhibits a significant departure from null expectations in at least one sex, and the implied pattern of selection on the locus is different between the sexes. Ch = Chromosome.

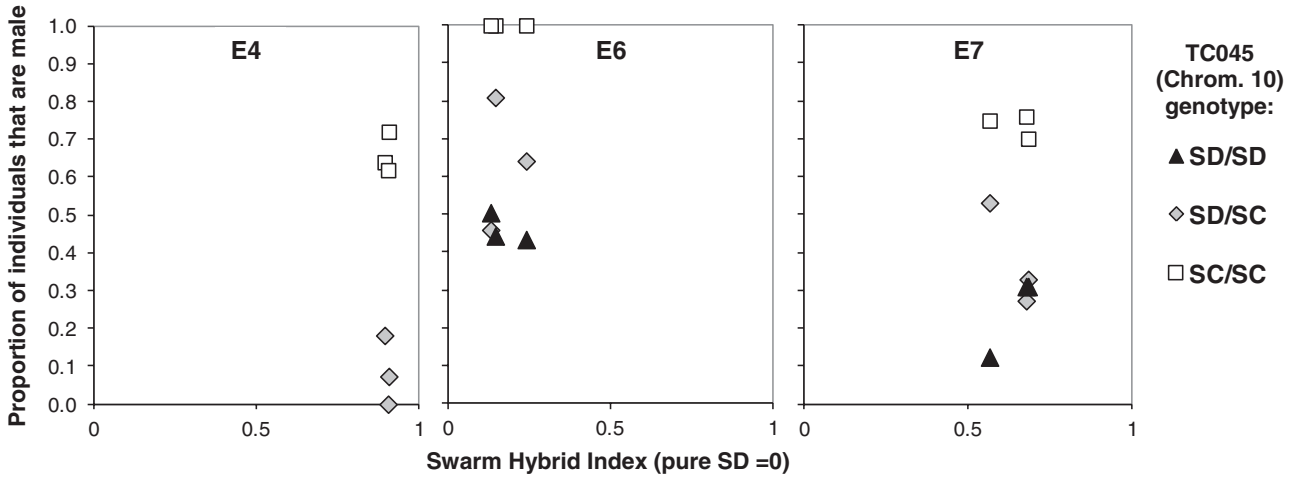

Figure 2. The proportion of individuals carrying a particular genotype at locus TC045 that are male, plotted against overall hybrid index of the swarm replicate, for SD × SC hybrid swarms. Proportions were standardized assuming equal numbers of males and females within each sample. Values for Months 9, 15, and 21 are included.

Table 4. Observed mean hybrid index and cytonuclear mismatch within swarm replicates, and proportion (*P*, values in italics) of simulated values lower than observed values. Simulations (n = 1000) assumed equal starting contributions of parental populations followed by neutral evolution. Observed values outside the 95% confidence interval of simulated values are indicated in bold.

		Month 3		Month 6		Month 9		Month 15		Month 21	
Measure	Rep	Observed	*P*	Observed	*P*	Observed	*P*	Observed	*P*	Observed	*P*
Mitochondrial	**D4**	0.122	***<0.001***	0.000	***<0.001***	0.000	***0.003***	0.000	***0.015***	0.000	*0.048*
Hybrid index	**D6**	0.000	***<0.001***	0.000	***<0.001***	0.000	***0.003***	0.000	***0.015***	–	–
	D7	0.108	***<0.001***	0.051	***<0.001***	0.000	***0.003***	0.000	***0.015***	–	–
	D8	0.156	***<0.001***	0.027	***<0.001***	0.000	***0.003***	0.000	***0.015***	0.000	*0.048*
	E4	0.743	***0.996***	0.727	*0.935*	0.917	***0.989***	0.848	*0.919*	1.000	*0.959*
	E6	0.275	***0.002***	0.342	*0.134*	0.222	*0.064*	0.027	*0.030*	0.000	*0.051*
	E7	0.816	***>0.999***	0.447	*0.319*	0.811	*0.948*	0.895	*0.956*	0.947	*0.933*
Nuclear	**D4**	0.136	***<0.001***	0.065	***<0.001***	0.017	***<0.001***	0.022	***<0.001***	0.003	***<0.001***
Hybrid index	**D6**	0.016	***<0.001***	0.048	***<0.001***	0.050	***<0.001***	0.067	***<0.001***	–	–
	D7	0.272	***<0.001***	0.297	***<0.001***	0.143	***<0.001***	0.185	***<0.001***	–	–
	D8	0.109	***<0.001***	0.011	***<0.001***	0.005	***<0.001***	0.012	***<0.001***	0.006	***<0.001***
	E4	0.784	***>0.999***	0.728	***>0.999***	0.894	***>0.999***	0.906	***>0.999***	0.909	***>0.999***
	E6	0.163	***<0.001***	0.284	***0.001***	0.233	***<0.001***	0.139	***<0.001***	0.125	***<0.001***
	E7	0.563	*0.881*	0.305	***0.003***	0.566	*0.771*	0.680	***0.983***	0.686	***0.985***
Cytonuclear	**D4**	0.014	***<0.001***	0.065	***<0.001***	0.017	***<0.001***	0.022	***<0.001***	0.003	***<0.001***
Mismatch	**D6**	0.016	***<0.001***	0.048	***<0.001***	0.050	***<0.001***	0.067	***<0.001***	–	–
	D7	0.273	***<0.001***	0.315	***<0.001***	0.143	***<0.001***	0.185	***<0.001***	–	–
	D8	0.108	***<0.001***	0.023	***<0.001***	0.005	***<0.001***	0.012	***<0.001***	0.006	***<0.001***
	E4	0.187	*0.043*	0.300	***<0.001***	0.169	***<0.001***	0.216	***<0.001***	0.092	***<0.001***
	E6	0.112	***0.002***	0.279	***<0.001***	0.221	***<0.001***	0.156	***<0.001***	0.131	***<0.001***
	E7	0.306	*0.929*	0.209	***<0.001***	0.391	***0.007***	0.347	***0.006***	0.330	***0.003***

Discussion

Here we investigated whether repeated hybridization events between the same populations lead to convergent genomic outcomes, by following the trajectory of replicated hybrid swarms over many generations of free mating. By using hybrid swarms rather than controlled crosses, we more closely simulated the processes likely to occur when divergent populations meet in the wild. Such processes are expected to include selection for genotypes that are most fit in the local environment and selection against hybrid genotypes that suffer reduced fitness as a result of allelic

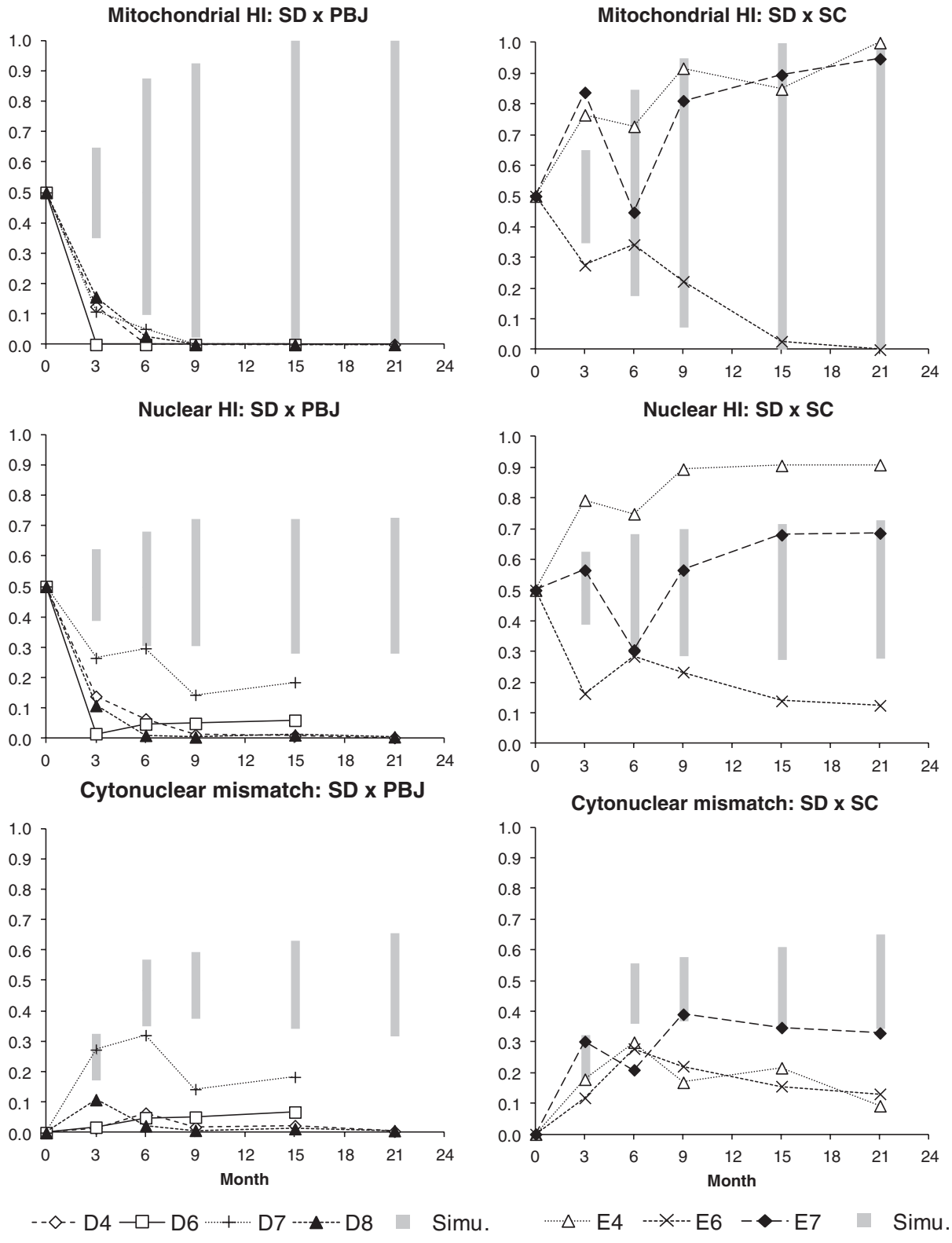

Figure 3. Observed mean hybrid index and cytonuclear mismatch in each swarm replicate, compared to the distribution of mean values from 1000 swarms simulated with equal genetic founding contributions from each parental population. Gray bars indicate 99% confidence intervals of simulated values (Simu.). "Observed" values at Month 0 represent the equal numbers of founding pure individuals from each parental population.

Table 5. Observed mean hybrid index and cytonuclear mismatch within swarm replicates, and proportion (*P*, values in italics) of simulated values lower than observed values. Simulations ($n = 1000$) assumed initial genotype frequencies observed at Month 3, followed by neutral evolution. Observed values outside the 95% confidence interval of simulated values are indicated in bold. Mitochondrial hybrid index was fixed at 0 in the D6 simulation and therefore *P* is not shown.

	Month 3		Month 6		Month 9		Month 15		Month 21	
Measure	Replicate	Observed	Observed	*P*	Observed	*P*	Observed	*P*	Observed	*P*
Mitochondrial	**D4**	0.122	0.000	*0.073*	0.000	*0.175*	0.000	*0.364*	0.000	*0.477*
Hybrid index	**D6**	0.000	0.000	–	0.000	–	0.000	–	–	–
	D7	0.108	0.051	*0.256*	0.000	*0.217*	0.000	*0.399*	–	–
	D8	0.156	0.027	*0.110*	0.000	*0.173*	0.000	*0.333*	0.000	*0.456*
	E4	0.743	0.727	*0.431*	0.917	*0.628*	0.848	*0.580*	1.000	*0.730*
	E6	0.275	0.342	*0.696*	0.222	*0.404*	0.027	*0.175*	0.000	*0.247*
	E7	0.816	0.447	***0.001***	0.811	*0.399*	0.895	*0.519*	0.947	*0.625*
Nuclear	**D4**	0.136	0.065	*0.088*	0.017	***0.003***	0.022	***0.018***	0.003	***0.001***
Hybrid index	**D6**	0.016	0.048	*0.890*	0.050	*0.892*	0.067	*0.938*	–	–
	D7	0.272	0.297	*0.773*	0.143	***0.010***	0.185	*0.078*	–	–
	D8	0.109	0.011	***<0.001***	0.005	***<0.001***	0.012	***0.001***	0.006	***<0.001***
	E4	0.784	0.728	*0.114*	0.894	*0.982*	0.906	***0.989***	0.909	***0.998***
	E6	0.163	0.284	***0.015***	0.233	*0.081*	0.139	*0.395*	0.125	*0.304*
	E7	0.563	0.305	***<0.001***	0.566	*0.418*	0.680	*0.943*	0.686	*0.945*
Cytonuclear	**D4**	0.014	0.065	***0.001***	0.017	***0.001***	0.022	***0.011***	0.003	***<0.001***
Mismatch	**D6**	0.016	0.048	*0.890*	0.050	*0.892*	0.067	*0.938*	–	–
	D7	0.273	0.315	*0.527*	0.143	***0.002***	0.185	*0.078*	–	–
	D8	0.108	0.023	***<0.001***	0.005	***<0.001***	0.012	***0.001***	0.006	***<0.001***
	E4	0.187	0.300	*0.340*	0.169	*0.026*	0.216	*0.241*	0.092	***0.004***
	E6	0.112	0.279	*0.380*	0.221	*0.184*	0.156	*0.132*	0.131	*0.108*
	E7	0.306	0.209	***<0.001***	0.391	*0.153*	0.347	*0.060*	0.330	*0.047*

incompatibilities. Results showed that the outcome depended on both stochastic and deterministic factors, with a number of repeatable evolutionary changes seen in replicate swarms.

HYBRID SWARMS WERE CHARACTERIZED BY LARGE FLUCTUATIONS IN EFFECTIVE POPULATION SIZE AND FREQUENT EXTINCTIONS

Multiple swarm replicates became extinct over the first year of the experiment, with SD × SC swarms dying out at earlier time points than SD × PBJ swarms. We observed no significant genetic differences between replicates that were lost quickly and those that persisted, although we examined only males. We note, however, that SD × SC swarms were compared at Month 3, where most individuals were parentals or first generation hybrids (see below). We therefore cannot rule out a role of F2 hybrid breakdown in subsequent extinctions of some replicates.

Estimates of effective population size, using temporal variation in allele frequency, are likely to have been biased by factors including the presence of overlapping generations, selection and reduced allelic diversity, particularly in SD × PBJ swarms, at later time points. Nevertheless, results clearly show large variations in N_e over time within all replicates. This reflects previous observations by Hwang et al. (2011) of large temporal changes in census size of *T. californicus* hybrid swarms in the laboratory. Such demographic conditions are expected to increase the magnitude of drift and hence impede the efficacy of selection

Large population fluctuations are also common in *T. californicus* in the wild (Vittor 1971; Dybdahl 1994). The species can occur in natural tidepools equivalent or smaller in volume to our culture beakers, and wild populations may not be more food limited or under increased predation pressure (Vittor 1971). However, such wild populations form part of a larger metapopulation, and experience much greater fluctuation in temperature and salinity than under our culture regimen (Vittor, 1971; Dybdahl 1994). We have observed a common long-term decrease in body size in both pure and hybrid swarms maintained for multiple generations, indicative of laboratory-specific selective pressures (Hwang et al. 2011; Pritchard and Edmands, unpubl. data). Thus our results cannot easy be extrapolated to predict outcomes of interpopulation hybridization in the wild. Nevertheless, different *T. californicus* populations are known to be adapted to different osmotic and temperature regimens (Burton 1986; Willett 2010; Kelly et al. 2012) and genes involved in this adaptation may be subject to selection in the experimental swarms.

GENETIC TRAJECTORIES OF HYBRID SWARMS VARIED WITH PARENTAL POPULATIONS, AND WERE STRONGLY INFLUENCED BY DIFFERENTIAL CONTRIBUTIONS OF PURE FOUNDER INDIVIDUALS

Samples taken from hybrid swarm replicates at Month 3 consisted almost entirely of genotypes corresponding to pure individuals and F1 hybrids. Elevated fitness has been observed in backcrosses for both cross-types (Pritchard and Edmands, unpubl. data); thus, this distribution is unlikely to reflect strong selection against later-generation hybrid genotypes. Instead, it suggests a generation time longer than the minimum 3 weeks documented by Burton (1987), such that Month 3 swarms largely comprise the second generation offspring of founder individuals. Mean hybrid indices at this time already deviated greatly from 0.5, suggesting wide variation in survival and reproductive success of pure individuals in the initial swarm generation. This accounts for the very low N_e estimated from allele frequency variation between Months 0 and 3. Notably, by Month 3, all SD × PBJ swarms were dominated by SD mitotypes and nuclear alleles; PBJ exhibits lower survivorship than SD in the laboratory, and may have lower competitive fitness under our temperature regimen (Pritchard and Edmands, unpubl. data; Willett 2010). Swarm D7, which suffered a fungal infection prior to Month 3, subsequently retained more PBJ alleles than other replicates. In contrast genetic composition of the SD × SC swarms at Month 3 varied widely between replicates. This reflects the relative competitive equality of those two populations (Willett 2010), and suggests that difference in hybrid indices at Month 3 resulted from stochastic processes.

Nuclear hybrid indices and associated levels of cytonuclear mismatch in replicates over time were partly determined by their composition at Month 3: observed values often overlapped those seen in simulated populations evolving by drift alone from the same starting conditions. Nevertheless, observed levels of cytonuclear mismatch were significantly below simulated levels for a majority of tests in SD × PBJ and a minority of tests in SD × SC, and overall we observed a trend for relatively low and temporally declining cytonuclear mismatch that could be explained by positive selection for nuclear genotypes coadapted with mitochondrial haplotypes within a swarm. Although cytonuclear coadaptation has been well documented within *T. californicus* populations, it does not appear to be complete (see below), and evidence from phylogenetic studies of other taxa, in which introduced mitotypes have completely replaced native ones (Toews and Brelsford, 2012), shows that selection to reduce cytonuclear mismatch is not a ubiquitous outcome of hybridization events. Fang et al. (2012) have recently suggested that an alternative mechanism, widespread BDM incompatibilities reducing the competitive fitness of hybrid genotypes, can result in progressive loss of genetic material from one parent where the initial contribution of that parent to the hybrid swarm is low. Whichever mechanisms caused our observed trends, it is clear that differential parental contributions at the initial stage of swarm formation can strongly influence subsequent hybrid swarm trajectory.

FEMALES WERE MORE HETEROZYGOUS THAN MALES IN ONE SWARM TYPE, BUT NOT IN THE OTHER

We observed a striking pattern in SD × SC swarms: mean heterozygosity amongst males was lower than that amongst females, particularly in the earlier months. This contrasted with the SD × PBJ swarms, where the few significant differences involved elevated male heterozygosity. Many more SD × SC swarm males than females exhibited entirely nonhybrid genotypes at the earlier time points; particularly notable was a large number of pure SD males in Month 6 in E6 (50% of males pure SD vs. 17% of females) and E7 (65% of males pure SD vs. 17% of females). The presence of so many SD males is intriguing given both that the SDSD genotype at Chromosome 10 in a hybrid background is associated with being female (see below) and that E7 overall exhibits a temporally increasing proportion of SC alleles. Elevated female heterozygosity has also been documented in hybrid swarms and controlled crosses between SD and another population, RP (Harrison and Edmands 2006; Hwang et al. 2011). This pattern may reflect elevated sensitivity of males to deleterious hybrid genetic combinations, or, conversely, higher sensitivity of females to deleterious recessive alleles fixed within pure parental populations (Hwang et al. 2011). We cannot exclude the possibility that of some of these males are individuals from early generations that have greater longevity than hybrid males: Vittor (1971) reported maximum life spans exceeding 130 days. Alternatively, if hybrid males have increased mating success than those with pure genotypes, we may be biasing our results by genotyping males that are not in precopula pairs. *Tigriopus* of both sexes can exercise mate choice (Lazzaretto et al. 1994; Kelly 1998; Ting and Snell 2003), although studies examining mating discrimination between populations have found no preference (Brown 1991; Ganz and Burton 1995; Palmer and Edmands 2000). Further examination of the genetic characteristics of paired and unpaired males within hybrid swarms would be warranted.

CONGRUENCE IN INTROGRESSION PATTERNS ACROSS REPLICATES DEMONSTRATED DETERMINISTIC INFLUENCES ON SWARM EVOLUTION

Genomic clines analysis revealed many markers significantly deviating from the overall genome-wide pattern of introgression. Proportion of non-neutral markers varied amongst swarm replicates: 0–67% of loci within SD × PBJ and 23–43% within SD × SC. Our opportunity to detect differential introgression within SD × PBJ replicates may have been limited by the rapid domination

of SD alleles and associated small range of hybrid genotypes. Although studies of different systems using different molecular markers are not directly comparable, the same statistical approach has implied varying proportions of non-neutral loci in hybrid zones between both genetically divergent populations (57% of loci in *Ovis canadensis*, Miller et al. 2012; 17% of loci in *Salvelinus fontinalis,* Lamaze et al. 2012; 2.5% and 9.5% of loci in *Anguilla marmorata,* Gagnaire et al. 2011), and taxa (37% of loci in *Macoma* bivalves, Luttikhuizen et al. 2012; approximately 50% of loci in *Cottus* sculpins, Nolte et al. 2009; over 85% of loci in *Mus*, Teeter et al. 2010). Particularly where an admixture event is recent and rate of recombination is low, an elevated number of markers may exhibit non-neutral introgression due to physical linkage with loci under selection. Correspondingly, in our hybrid swarms we observe blocks of linked markers with similar introgression patterns. Although this reduces our opportunity to pinpoint specific targets of selection, it also provides confidence that observed patterns are not due to genotyping artifacts.

Swarm replicate D7 exhibited a particularly large number of deviations from null expectations, with widely varying patterns of introgression amongst chromosomes, some difficult to interpret. This replicate is also notable in that females produced transgressively large egg sacs over multiple time points (Pritchard and Edmands, unpubl. data), suggesting increased female fecundity. As noted, D7 contained more F1 hybrids at Month 3 than other SD × PBJ replicates. This may have generated more recombinant genotypes at later swarm generations, potentially bringing together advantageous genomic combinations, which would otherwise not arise due to the rapid loss of PBJ alleles from the swarms.

Although our study controlled for genetic differences between parental populations and most sources of environmental variation, replicate hybrid swarms within the same cross-differed in regard to their dominant parental contribution, demographic conditions, and long-term algal colonization. Recent work on *T. californicus* (Foley and Edmands, unpubl. data) has shown segregation distortion in F2 interpopulation hybrids to vary with sex and mitochondrial background; moreover, patterns of segregation distortion in the same cross may be altered by even small environmental changes (Willett 2007). Furthermore, low and fluctuating N_e increases the likelihood of interlocus differences being generated by drift rather than selection. Despite such sources of variation, we observe some striking congruence in introgression patterns, both across replicates and across different swarm types. Some of these patterns reflect segregation distortion previously observed within F2 individuals. Different types of selection can generate similar genotypic clines (Gompert et al. 2012b), such that the mode of selection inferred from visual inspection of the data is unlikely to give a complete picture of the forces acting on a locus. Nevertheless, as has been seen in other studies, there is clearly a range of different selective pressures acting across the genome, including both positive and negative interactions between alleles from different populations. Our results demonstrate that, despite a substantial influence of stochasticity and relative pure parental fitness on initial hybrid swarm composition, subsequent evolution is also shaped by deterministic factors acting on hybrid genotypes.

Within all three SD × SC swarm replicates, differential introgression implied selection against heterozygotes and positive selection for SC homozygotes, at one end of Chromosome 8. One of the markers contributing to this pattern, TC078, is one of the few to exhibit segregation distortion, a deficiency of heterozygotes, in newly hatched SD × SC F2 larvae (Pritchard et al. 2011). Recent work (Foley and Edmands, unpubl. data) has found a QTL in this region associated with body size and development time, with a substantial nonadditive component. Thus, incompatibilities between SD and SC alleles on Chromosome 8 may be affecting both pre- and postembryonic development. Fitzpatrick et al. (2009) have documented a similar case in *Ambystoma* salamanders, where interspecific heterozygosity at a marker was associated with elevated embryonic mortality, resulting in heterozygote deficiency at this locus in natural hybrid swarms.

Loci on Chromosome 1 also exhibited strongly non-neutral patterns of introgression, across multiple replicates within both hybrid swarm types. Within SD × PBJ, patterns implied heterozygote advantage and selection against SD; within SD × SC, patterns implied selection against SD where the SD mitotype was dominant, but selection against SC where the SC mitotype was dominant. These results oppose what would be expected with cytonuclear coadaptation, both across different hybrid backgrounds and involving different mitotypes. Segregation patterns indicative of overdominance at Chromosome 1 loci were also observed in the F2 by Pritchard et al. (2011) and by Foley and Edmands (unpubl. data).

Patterns also implied negative selection against SD along Chromosome 7 in multiple SD × PBJ swarm replicates, with overdominance inferred in SD × SC replicate E4. Again, Pritchard et al. (2011) observed heterozygote excess at loci along this chromosome in adult SD × SC F2 males with an SD mitochondrial background, and a similar pattern has recently been noted for males with an SC mitochondrial background (Foley and Edmands, unpubl. data). Chromosome 7 contains a locus involved in mitochondrial transcription, mtRPOL, which has been shown to be coadapted with the mitochondrial genome in some populations; however, this coadaptation appears incomplete in SD. Corresponding with our observations, Ellison and Burton (2008b) inferred selection against the SD mtRPOL in F4 hybrids, even where the matching SD mtDNA was present. Although we did not have a SNP within mtRPOL reliably diagnostic between SD and PBJ, the SD-SC diagnostic SNP in mtRPOL deviated less

from neutral expectations than adjacent markers, suggesting that a linked locus and not mtRPOL itself may be the target of selection.

Although we observed a number of loci with patterns of introgression suggestive of their involvement in epistatic interactions with other parts of the genome, evidence for epistasis in the form of statistical linkage disequilibrium between physically unlinked loci within swarms was inconclusive. As has been documented in other taxa (Rieseberg et al. 1996), multiple complex nuclear–nuclear and nuclear–mitochondrial interactions, including BDM incompatibilities, potentially influence fitness in *T. californicus* interpopulation hybrids, however, we lack the power to investigate these further here.

CHROMOSOME 10 WAS ASSOCIATED WITH SEX IN BOTH CONTROLLED CROSSES AND HYBRID SWARMS

Pritchard et al. (2011) found strong segregation distortion in F2 SD × SC males at Chromosome 10, and more recent work (Foley and Edmands, unpubl. data) has shown this to be sex specific, with SD homozygotes tending to be female and SC homozygotes tending to be male, independent of cytoplasmic background. Our results show this sex-specific effect of Chromosome 10 to be maintained in freely mating hybrid swarms: further, its effect appears to depend on the genetic composition of the swarm as a whole, with SDSC heterozygotes being male where the frequency of SC alleles is low, but female where the frequency is high. Thus, the population of origin of Chromosome 10 loci appears to have a role in sex determination of hybrids. Sex ratios in *T. californicus* are frequently male biased and fluctuate widely between families and over time (Voordouw et al. 2005); we did not document them in our hybrid swarms, however, there is no consistent evidence for an unusually large proportion of males in the SC population which would support the hypothesis of an unbalanced masculinization locus on SC Chromosome 10 (Pritchard, Foley and Edmands, unpubl. data). The variation in sex of the SDSC genotype according to overall hybrid background more strongly supports a large-effect sex distortion locus on Chromosome 10 balanced by one or more opposing loci within the nuclear genome of the same population. Interestingly, we also observe an association between Chromosome 10 and sex in replicate D7, the only SD × PBJ replicate to have substantial allelic variation over this chromosome. Moreover, Harrison and Edmands (2006) observed individuals homozygous for SD at Chromosome 10 to have an increased likelihood of being female within a third cross, SD × RP. Thus, the sex distortion effect of Chromosome 10 is not limited to a single hybrid background.

SUMMARY

This study investigated the genomic consequences of secondary contact between genetically divergent, but fully interfertile, populations by creating replicate hybrid swarms and following their trajectory over many generations of free mating. Results demonstrated that long-term outcomes of such contact depended on both stochastic and deterministic factors. Large changes in allele frequencies between swarm initiation and the first genotyped time point indicated considerable variation in survivorship and reproductive success of pure founder individuals. For one cross-type (SD × PBJ), these changes were always in the same direction and reflected higher fitness of the SD population within the laboratory environment. For the other cross-type (SD × SC), changes occurred in different directions between replicates, indicative of random variation in reproductive success. Subsequent genetic trajectories of hybrid swarms were strongly influenced by genetic composition at this 3 month time point. Nevertheless, despite effective population sizes that were low and variable over time, we also observed evidence for common selection pressures across the genome in the hybrid swarms. Most swarm replicates exhibited a temporal trend of decreasing cytonuclear mismatch. A number of patterns of introgression observed across the nuclear genome were strikingly congruent between replicates, both within and between cross-types. These reflected many, but not all, patterns of segregation distortion previously documented within the F2 hybrid offspring of two of the same parental populations. The type of selection implicated varied amongst loci, and included both over- and underdominant interactions between alleles from different populations, and directional selection both corresponding to and opposing that which would be expected with cytonuclear coadaptation within the parental populations. Repeatability of patterns over different cross types demonstrated that the effect of some loci persisted over more than one hybrid genetic background. Results additionally indicated sex-specific effects of recombinant genotypes: within one hybrid swarm type, but not the other, males tended to be less heterozygous than females. We also observed further evidence for a previously documented sex distortion locus of large effect, the influence of which depended on the overall hybrid genetic background. Such sex-specific effects have potential to greatly influence the outcome of introgressive hybridization between populations. To our knowledge, this is the first study to compare the long-term genomic outcome of replicate hybrid swarms initiated with the same parental populations in a common garden environment.

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Supporting Information

Additional Supporting information may be found in the online version of this article at the publisher's website:

Figure S1. Mean hybrid index, separated by sex and chromosome, for all swarm replicates over all genotyped time points.
Figure S2. Fitted genomic clines for each locus, compared to null expectations, for the SD × PBJ swarms.
Figure S3. Fitted genomic clines for each locus, compared to null expectations, for the SD × SC swarms
Figure S4. Observed mean mitochondrial and nuclear hybrid index (HI; pure SD = 0) and cytonuclear mismatch for each swarm replicate, compared to distribution of mean values from 1000 simulated swarms.
Table S1. Pairs of markers in linkage disequilibrium at the FDR corrected $P < 0.05$.
Table S2. Probability of observed genotypic distributions, given a model of homogeneous admixture across the genome, for males and females separately.