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ORIGINAL ARTICLE

Reproductive and phylogenetic divergence of tidepool copepod populations across a narrow geographical boundary in Baja California

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ABSTRACT

Aim Previous work on the tidepool copepod *Tigriopus californicus* revealed a curious case of incipient speciation at the southern end of the species' range in Baja California, Mexico. The present study expands on the geography of this pattern and tests for congruence between reproductive and phylogenetic patterns.

Location The Pacific coast of North America, from central Baja California to south-eastern Alaska (27–57° N), including the full range of *T. californicus*.

Methods Primary techniques included mating experiments (> 4000 crosses), phylogeny reconstruction (mitochondrial cytochrome *c* oxidase subunit I) and screening of single nucleotide polymorphisms (SNPs, 42 loci). Analyses used > 8000 copepods for the mating experiments, 86 copepods for the phylogeny and 41 copepods for the SNP assays. Phylogenies were constructed using Bayesian, maximum likelihood and maximum parsimony methods.

Results Populations were found to fall into three reproductive groups: northern and southern groups that were reproductively isolated from each other, and an intermediate group that could serve as a conduit for gene flow. The northern and intermediate populations fell into one clade while all southern populations fell into a second clade. These two clades are now separated by less than 12 km at latitude 29.35° N. Nuclear SNP data for a subset of locations confirmed striking divergence between populations on either side of this boundary. The second (southern) clade was further subdivided into two clades separated by the lagoon region of Guerrero Negro (latitude 28° N).

Main conclusions Reproductive assays and molecular data (both mitochondrial and nuclear) reveal a sharp break at 29.35° N, a region with no obvious barriers to dispersal, with no evidence for mixing across this narrow transition zone. Results also showed a milder break at the Guerrero Negro Lagoon (28° N), a location where breaks have been reported for other taxa.

Keywords

Copepoda, incipient species, mitochondrial DNA, phylogeography, postzygotic isolation, reproductive asymmetry, *Tigriopus*, western North America.

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INTRODUCTION

Studies of reproductive and phylogenetic divergence in incipient species can provide insight into the genetic and evolutionary mechanisms underlying phylogeographical patterns. These incompletely isolated taxa often illustrate a particular aspect of 'the species problem' (*sensu* Mayr, 1942) in which reproductive compatibility is non-transitive; that is, group A may be compatible with group B, and group B may be compatible with group C, but group A is incompatible with group C. Classic examples of this pattern are 'ring species' such as the *Ensatina* salamanders encircling California's

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Central Valley (e.g. Kuchta *et al.*, 2009) or greenish warblers surrounding the Himalayas (e.g. Irwin *et al.*, 2005). In these examples, a series of populations form a ring that overlaps at the ends – neighbouring populations can interbreed, with the exception of the secondary contact zone between incompatible 'end' populations. Non-transitive reproductive compatibility is also found in other geographical configurations of incompletely isolated taxa, such as the largely allopatric subspecies of *Drosophila bipectinata* (Kopp & Frank, 2005). Such cases are useful for studying speciation in progress and understanding phylogeographical patterns.

The copepod *Tigriopus californicus* (Baker, 1912) provides a different example of non-transitive reproductive compatibility. It inhabits supralittoral tidepools ranging from central Baja California (Mexico) to Alaska (USA). While all life stages of the species are free-swimming, genetic data show extremely low effective dispersal, with fixed genetic differences between populations separated by only a few kilometres of sandy beach (e.g. Burton & Lee, 1994; Edmands & Harrison, 2003). *Tigriopus californicus* has not been reported from the Gulf of California or the southern Pacific coast of Baja California, even where seemingly appropriate habitat exists. The southern range limit may be controlled by the species' upper thermal tolerance, as southern populations apparently possess limited scope for the evolution of increased tolerance (Kelly *et al.*, 2012). Crosses between populations from California and further north result in first-generation hybrids with fitness similar to or greater than that of their parents, and second-generation hybrids with some degree of fitness reduction (e.g. Edmands, 1999).

When Ganz & Burton (1995) extended sampling of *T. californicus* southwards to Baja California, they found very different patterns of reproductive compatibility that fell into three groups: 'northern', 'southern' and 'intermediate' (Fig. 1). The southernmost population they sampled, Playa Altamira, showed a 'southern' pattern, in which females crossed with males from California or the other three Baja California populations (including both northern and intermediate populations) produced no offspring, whereas the reciprocal crosses produced at least F1 hybrids. One population, Punta Banda, showed a 'northern' pattern, in which females crossed with Playa Altamira males produced sterile F1 hybrids, the reciprocal cross produced no offspring, and crosses with the other three populations (San Diego, Punta Morro and Punta Baja) produced both F1 and F2 hybrids. Finally, the two remaining Baja California populations (Punta Morro and Punta Baja) showed an 'intermediate' pattern, with females producing F1 and F2 hybrids when crossed with either 'northern' or 'southern' males, whereas males produced no offspring when crossed with 'southern' females but were fully compatible with 'northern' females. In cases where F1 hybrids appeared to be unable to produce offspring, backcrosses with parentals confirmed that the F1 hybrids were sterile. The southernmost population is thus reproductively isolated from most *T. californicus* populations, yet genetic exchange is still possible through 'intermediate' populations. The asymmetrical incompatibility means that gene flow for maternally inherited mitochondrial genes goes only from north to south (because 'southern' females are incompatible with both 'northern' and 'intermediate' males), while biparentally inherited nuclear genes can move in both directions. Notably, this isolation is purely postzygotic, as there is no indication of prezygotic isolation in the species, even in crosses between 'southern' and 'northern' populations (Ganz & Burton, 1995; Palmer & Edmands, 2000).

The previous work on *T. californicus* by Ganz & Burton (1995) thus revealed an intriguing example of apparent incipient speciation, with a gap of > 200 km between the 'southern' population (Playa Altamira) and the nearest sampled 'intermediate' population (Punta Baja), leading to questions about the precise geographical location of the reproductive boundary. The southernmost sampled population, Playa Altamira, occurs well north of the established marine biogeographical boundary of Punta Eugenia (Briggs, 1974), prompting questions of whether the species extends further south. The present study therefore expands sampling of *T. californicus* along the Baja California peninsula, both in the gap region and south of Playa Altamira. Previous phylogenetic work in *T. californicus* has included only sparse samples from Baja California (Burton & Lee, 1994; Burton, 1998; Edmands, 2001). This previous work revealed deep mitochondrial breaks coincident with some previously described marine biogeographical boundaries (Puget Sound, Cape Mendocino and Point Conception) but not others (Dixon Entrance) and showed additional breaks of equal or greater magnitude in the absence of recognized provincial boundaries (e.g. Valentine, 1966). Here, we expand on phylogenetic work in the Baja California peninsula, including tests for concordance with reproductive isolation, in order to better understand the processes driving the incipient speciation at the southern end of the range of *T. californicus*.

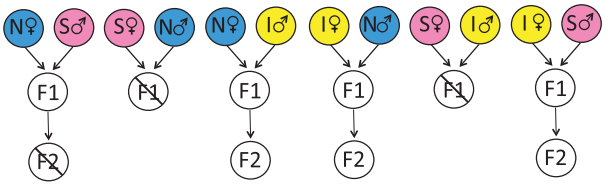

Figure 1 Patterns of reproductive compatibility for northern (N), intermediate (I) and southern (S) compatibility groups of *Tigriopus californicus* in western North America. The three reproductive groups were originally reported by Ganz & Burton (1995) and were corroborated by patterns in the larger set of populations used in the current study (Table 1).

MATERIALS AND METHODS

Sample collection and culture maintenance

Populations of *T. californicus* were sampled at 18 locations ranging from Baja California Sur, Mexico, to Washington, USA (see Appendix S1 in Supporting Information). For phylogenetic comparison, populations presumed to be *T. japonicus* were also sampled at three locations in Taiwan (Appendix S1). Samples for DNA sequencing were collected from 1999 to 2004, while those for single nucleotide polymorphism (SNP) assays were collected in 2011. Copepod samples and their natural seawater were transferred to 400-mL beakers and fed finely ground *Spirulina* flakes. Beakers were housed in an incubator set at 20 °C with a 12 h light: 12 h dark cycle.

Mitochondrial phylogeny

Genomic DNA was extracted from individual copepods by incubating them in 10% 'Chelex' chelating resin for 20min at 100 °C, chilling them on ice for 5 min, and then spinning them down for 5 min. A 713-bp segment of the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) was amplified by PCR using primers modified from Folmer *et al.* (1994): CO1VLel (5′-GAGGGGCTACGAACCACAAAGATA-3′) and CO1VHel (5′-TACACCTCAGGATGTCCAAAAAATCA-3′). In addition, the external primer DLPVH (5′-AAAAACT GAGCCTTAGCTCAAA-3′) was added at 20% of the concentration of each of the other two primers to increase product yield (Palumbi *et al.*, 2002). A 25-μL reaction was used with a final $MgCl_2$ concentration of 2.5 mM. Reactions consisted of a 5-min denaturation step at 94 °C, followed by 45 cycles of 60 s at 94 °C, 60 s at 50 °C, and 90 s at 72 °C, followed by a 5-min extension at 72 °C. The PCR products were then directly sequenced using a Beckman Coulter CEQ 8000 automated sequencer (Brea, CA, USA). Because previous studies showed little if any mitochondrial variation within geographical sites (Burton & Lee, 1994; Edmands, 2001), we sequenced only 1–4 individuals per site.

Sequences were aligned and edited using SEQUENCHER 4.6 (Gene Codes Corporation, Ann Arbor, MI, USA). The 86 sequences we used include 24 new sequences and 62 sequences obtained from GenBank (all GenBank accession numbers are listed in Appendix S1). This data set includes 41 populations of *T. californicus*, plus samples of the congeners *Tigriopus japonicus* and *Tigriopus fulvus*, with the harpacticoid copepod *Coullana* sp. used as an outgroup. Phylogeny reconstruction was based on a 505-bp aligned region corresponding to positions 64 to 564 in the *T. californicus COI* sequence (L43049; Lee, 2003). The best-fit model of nucleotide substitution was assessed using JMODELTEST 0.1.1 (Posada, 2008), which incorporates the program PHYML (Guindon & Gascuel, 2003). Eighty-eight candidate models were assessed on a fixed topology (fixed BioNJ–JC tree) using the Bayesian information criterion (BIC), the Akaike information criterion (AIC) and the corrected AIC (AIC_c). Both BIC and AIC_c found the most appropriate model to be HKY+I+G (weights 0.7009 and 0.4487, respectively), while AIC favoured TIM3+I+G, albeit with a lower weight (0.2820). Model HKY+I+G was therefore used for subsequent analyses.

Phylogenies were constructed using four different methods. Bayesian analyses were performed with MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001), beginning with the random default tree and using values nst = 2 and rates = invgamma. Analyses were run for ten million generations with sampling every thousand generations. The burn-in value was set at 2500 (25% of samples) and appropriate convergence was indicated by an average standard deviation of split frequencies below 0.006 and potential scale reduction factors (PSRFs) close to 1.0 for all parameters (range: 1.000–1.004). Maximum parsimony analyses were performed in MEGA 5 using the closest neighbour interchange (CNI) option with search level 1 and 100 random additions of sequences. Nodal support was assessed by 500 bootstrap replicates. Maximum likelihood analyses were also conducted in MEGA 5 (Tamura *et al.*, 2011) using the HKY+I+G model (all sites) and the nearest neighbour interchange method (NNI) with the initial tree determined automatically. Five hundred bootstrap replicates were run. Maximum likelihood analyses were also run on GARLI (Zwickl, 2006) implemented on the CIPRES portal (Miller *et al.*, 2010) using the same model (HKY+I+G) and 500 bootstrap replicates. Mean sequence divergence (HKY distance) between clades A–F was calculated using PAUP* 4.0b10 (Swofford, 2003).

Nuclear single nucleotide polymorphisms (SNPs)

To further investigate the phylogenetic breaks detected in the mitochondrial data, a small number of individuals from six populations (not the same individuals used for mitochondrial DNA sequencing) were screened for 190 mapped SNPs, using the methods in Foley *et al.* (2011). Briefly, DNA was extracted from individual copepods in 50 μL proteinase *K* lysis buffer, dried down at 65 °C and sent to Roswell Park Cancer Institute (Buffalo, NY, USA) for SNP genotyping using the iPlex Gold Assay on a MassARRAY Compact (Sequenom, San Diego, CA, USA) mass spectrometer. The genotyped samples came from populations 24 ($n = 5$), 27 ($n = 18$), 28 ($n = 5$), 30 ($n = 4$), 31 ($n = 5$) and 34 ($n = 4$). Because several of these populations are quite divergent from those for which the SNP assays were designed, only a subset of the loci could be scored consistently. The data set was therefore reduced to 42 loci that were scored in every individual tested. This included loci on each of the species' 12 chromosomes (chromosome linkage assignment is shown in Appendix S2, with mapping details in Foley *et al.*, 2011). GENETIX 4.05 (Belkhir *et al.*, 2004) was used to calculate differentiation between populations (using Weir and Cockerham's θ) and to visualize differentiation among individual genotypes using factorial correspondence analysis.

Mating experiments

In *T. californicus*, males can mate repeatedly, but females mate only once and use stored sperm to fertilize multiple clutches of eggs (e.g. Egloff, 1966; Burton, 1985). Mature males use their antennules to clasp immature, virgin females and mate-guard them until the female completes her terminal moult. The male then fertilizes the female before releasing her. To set up crosses, we placed clasped pairs on a piece of filter paper under a dissecting microscope and used a fine probe to tease immature females away from mature males.

Tests of reproductive compatibility (Table 1) involved > 4000 male–female crosses, concentrating on population 24 (northern group), population 29 (intermediate group) and population 34 (southern group). For each population pair, both reciprocal crosses and both parental crosses were set up, with an average of 34.8 male–female pairs per cross. No individuals were used for more than one cross. Crosses were set up in Petri dishes containing filtered seawater and *Spirulina* cyanobacteria, with up to five males and five females in each dish. Dishes were checked every 2–3 days. When females formed egg sacs, they were isolated in a new dish. When F1 larvae hatched, they were pooled into a designated 500-mL beaker for each type of F2 cross. When F1 females formed egg sacs, they were again isolated in a Petri dish and monitored for the production of F2 larvae. Reproductive success was scored as 'yes' or 'no', based on the presence or absence of larvae in the first clutch for both the F1 and F2 offspring.

In some types of crosses, F1 females (f) mated with F1 males (m) produced egg sacs, but those egg sacs generally failed to hatch. For two of these cross types (24f × 34m and 30f × 31m), we measured egg sac area and diameter in F1 females mated with F1 males for both the hybrid crosses (e.g. 24f × 34m) and the parental control crosses (e.g. 24f × 24m and 34f × 34m). Egg sacs were photographed using a Leica MZ12 dissecting microscope at 32× magnification and measurements were made with Optimas 5.2 image analysis software (Optimas Corp., Bothell, WA, USA). Between 6 and 50 egg sacs were measured for each of the six cohorts. For each of the two cross types, one-way ANOVA (Statistica 7.1, StatSoft, Tulsa, OK, USA) was used to compare the three cohorts (e.g. 24f × 34m, 24f × 24m and 34f × 34m). If significant differences were found, Tukey's HSD (honestly significant difference) tests for unequal *n* (Spjøtvoll/Stoline tests) were used to assess whether the hybrid cohort differed from one or both parental control cohorts.

One possible cause of reproductive asymmetry, such as that seen in the current study, is cytoplasmic incompatibility driven by *Wolbachia* or other intracellular parasites (e.g. Turelli, 1994). To determine whether incompatibility could be cured by antibiotics (as has been found in other taxa, e.g. Breeuwer, 1997), we set up 20 replicates of four types of crosses (24f × 34m, 24m × 34f, 24 × 24 and 34 × 34) under three conditions: (1) 30 mg tetracycline per litre of seawater; (2) 30 mg oxytetracycline per litre of seawater; and (3) untreated seawater. These antibiotic concentrations were chosen based on pilot experiments – higher concentrations were found to cause unacceptably low survivorship. We also used PCR to screen populations 24 and 34 using *Wolbachia*-specific *ftsZ* gene primers (Holden *et al.*, 1993).

In some cases (denoted by asterisks in Table 1), interpopulation crosses that failed to produce offspring in previous assays (based on at least 22 mated females) unexpectedly produced substantial numbers of viable offspring. To determine whether the offspring were truly hybrids, a subset of animals from these crosses was genotyped for microsatellite loci showing fixed allelic differences between the populations in question (Edmands & Harrison, 2003; Harrison *et al.*, 2004; Hwang *et al.*, 2012; S. Edmands, D.L. Peterson & K.B. Kubow, unpublished data). DNA was extracted from individual copepods by incubating them in 50 μL proteinase-K cell lysis buffer for 1 h at 65 °C followed by 15 min at 99 °C. Microsatellite loci 197, 228, 558, 1202, 1203 and 62J8 were amplified using methods described in Harrison *et al.* (2004). The PCR products were then analysed for fragment length using a Beckman Coulter CEQ 8000 automated sequencer.

While *T. californicus* males have been reported to clasp immature, virgin females (Egloff, 1966), they may also 'rarely' clasp adult females (Burton, 1985). If these adult females have already been inseminated by males from their own population, this may lead to false conclusions of successful hybridization. To quantify the frequency of male clasping mistakes, we separated 504 clasped pairs and isolated each of the putative virgin females in a single well of a 24-well plate. Plates were checked every 7 days until day 21 and were scored as female, female with offspring (indicating a non-virgin female), male, or dead before maturity. The study was set up in two blocks: (1) freshly collected copepods from population 24; and (2) population 28 copepods maintained in the lab for over a year.

RESULTS

Sample collection

Population sampling (Fig. 2, Appendix S1) filled in the gap between the 'intermediate' population 29 and the 'southern' population 34 with four new populations. In addition, six new populations were collected south of population 35, extending the known range of *T. californicus* to population 41, 140 km south of the established biogeographical boundary of Punta Eugenia. The gap between populations 35 and 36 is coincident with largely unsuitable habitat in the Guerrero Negro Lagoon region. *Tigriopus californicus* populations were not found immediately south of population 41, even where suitable habitat occurred.

Mitochondrial phylogeny

The four phylogenetic methods (Bayesian, parsimony, and maximum likelihood using mega and garli) confirm the

Table 1 The proportion of mated females of *Tigriopus californicus* that produced live offspring (total number of mated females). F1 crosses are listed first, followed by F2 crosses, with the last generation of crosses that successfully produced offspring underlined. Intrapopulation crosses are shown in bold. Dashes indicate crosses that were not tested and asterisks indicate putative hybrids disproven by microsatellite analyses (34f × 24m produced F1 that were disproven in all individuals tested, 30f × 31m produced F2 that were confirmed in only 2 of the 23 individuals tested, 24f × 34m produced F2 that were disproven in all individuals tested). Data cells are colour-coded by crosses that produced no offspring (light grey), F1 offspring only (stippled grey) or F1 and F2 offspring (dark grey). Parental populations are colour-coded by inferred reproductive compatibility group (northern, N = blue; intermediate, I = yellow; southern, S = pink; N/I = green).

Male parent population	Female parent population														
	24	27	28	29	30	31	32	33	34	36	37	38	39	40	41
24	**0.85 (93)**	0.80 (10)	0.85 (26)	0.70 (10)	1.00 (10)	0.00 (10)	0.00 (16)	0.00 (19)	0.02 (133)*	0.00 (10)	0.00 (9)	0.00 (4)	0.00 (11)	0.00 (11)	0.00 (14)
	0.77 (91)	0.83 (6)	0.74 (12)	0.57 (7)	1.00 (21)										
27	0.88 (8)	**0.90 (10)**	0.80 (10)	0.38 (8)	—	—	—	—	0.00 (19)	—	—	—	—	—	—
	0.80 (5)	**0.88 (8)**	0.83 (6)	0.40 (5)											
28	0.70 (10)	0.90 (10)	**0.73 (114)**	0.83 (12)	—	—	—	—	0.00 (84)	—	—	—	—	—	—
	0.60 (10)	0.56 (9)	**0.98 (102)**	0.63 (8)											
29	0.57 (7)	0.63 (8)	0.50 (8)	**1.00 (22)**	0.80 (15)	0.00 (15)	0.00 (19)	0.00 (12)	0.00 (22)	—	—	—	—	—	—
	0.25 (8)	0.43 (7)	0.67 (6)	**1.00 (22)**	1.00 (30)										
30	0.63 (16)	—	—	—	**0.70 (98)**	0.00 (79)	—	—	0.00 (101)	—	—	—	—	—	—
	0.91 (11)				**0.89 (82)**										
31	0.95 (30)	—	—	—	0.62 (103)	**0.77 (83)**	—	—	1.00 (14)	—	—	—	—	—	—
	0.00 (4)				0.42 (43)*	**0.92 (89)**			0.98 (42)						
32	0.78 (41)	—	—	—	—	—	**0.88 (25)**	—	0.81 (16)	—	—	—	—	—	—
	0.00 (32)						**0.73 (33)**		1.00 (37)						
33	0.86 (21)	—	—	0.74 (19)	—	—	—	**0.96 (24)**	0.91 (22)	—	—	—	—	—	—
	0.00 (4)			0.97 (32)				**0.80 (30)**	1.00 (30)						
34	0.75 (118)	0.64 (44)	0.65 (176)	0.53 (15)	0.50 (40)	1.00 (10)	0.86 (21)	0.81 (16)	**0.93 (213)**	0.63 (8)	0.40 (5)	0.60 (5)	0.70 (10)	0.62 (13)	1.00 (13)
	0.32 (114)*	0.00 (2)	0.07 (30)	1.00 (8)	0.00 (3)	1.00 (34)	1.00 (34)	1.00 (31)	**0.95 (182)**	0.67 (6)	1.00 (9)	0.71 (7)	0.84 (19)	0.80 (25)	0.94 (90)
36	0.50 (12)	0.67 (6)	—	—	—	—	—	—	0.71 (7)	**0.86 (7)**	—	—	—	—	—
	0.00 (1)	0.00 (3)							0.80 (5)	**0.96 (26)**					
37	0.62 (13)	0.60 (5)	—	—	—	—	—	—	1.00 (2)	—	**0.91 (11)**	—	—	—	—
	0.00 (13)	0.00 (2)							0.93 (15)		**0.79 (42)**				
38	0.38 (8)	0.40 (5)	—	—	—	—	—	—	0.88 (8)	—	—	**1.00 (6)**	—	—	—
	0.00 (10)	0.00 (4)							0.40 (6)			**0.75 (8)**			
39	0.50 (12)	0.60 (5)	—	—	—	—	—	—	0.90 (10)	—	—	—	**0.86 (14)**	—	—
	0.00 (37)	0.00 (3)							0.63 (41)				**0.79 (28)**		
40	0.77 (13)	0.50 (6)	—	—	—	—	—	—	0.80 (10)	—	—	—	—	**1.00 (9)**	—
	0.00 (23)	0.00 (4)							1.00 (24)					**1.00 (34)**	
41	0.92 (13)	0.50 (6)	—	—	—	—	—	—	0.71 (7)	—	—	—	—	—	**0.86 (14)**
	0.00 (49)	0.00 (4)							0.54 (24)						**0.65 (62)**

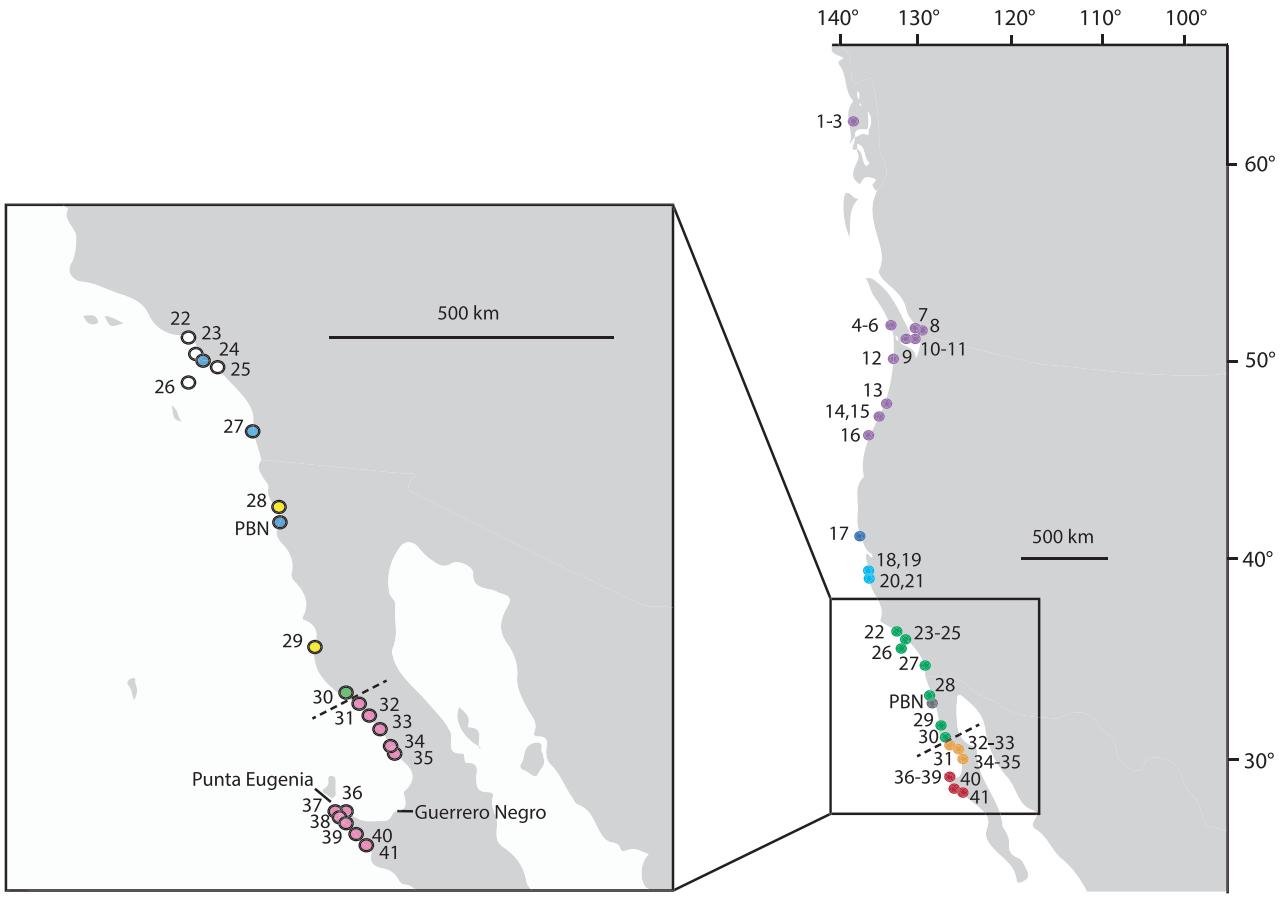

Figure 2 Map of North America (Lambert azimuthal equal-area projection) showing sampling locations for 41 *Tigriopus californicus* populations. Location names are listed in Appendix S1. For the full map on the right, locations are colour-coded by mitochondrial clade as in Fig. 3 (grey = not tested). For the inset map on the left, locations are colour-coded by reproductive group as in Figs 1 and 2 (white = not tested). Also shown on the map is the location of Punta Banda (PBN), which was not included in the current study but was found by Ganz & Burton (1995) to fall into the N compatibility group. The dashed line indicates the location of the sharp reproductive and phylogenetic break between populations 30 and 31.

same six *T. californicus* clades (A–F), albeit with variable levels of support (Fig. 3). Within clade C, support for monophyly of the intermediate (I) and northern/intermediate (N/I) reproductive groups (populations 28–30) is strong. There is also moderate support for clade (E, F) and weak support for clades (A, B) and (A, B, C). Phylogenetic analysis did not support the monophyly of *T. californicus*, *T. japonicus* or the genus *Tigriopus*, although resolution of deeper relationships was weak due to the highly distant outgroup and saturation for *COI*.

The six clades were well differentiated (Table 2), with mean HKY distances ranging from 0.175 (between clades A and B) to 0.368 (between clades A and E). The expansion of sampling at the southern end of the range provided fine resolution of the geographical limit between clades C and E (between populations 30 and 31, see Fig. 2). These two clades are separated by a geographical distance of only *c.* 12 km, yet they differ by an HKY distance of 0.341, a genetic distance on a par with that between the most geographically divergent clades. This 12-km area is a largely unexplored region that is difficult to access (due to steep cliffs) and includes both sandy beach and rocky intertidal habitats. Finally, clades E and F, separated by the Guerrero Negro Lagoon, have an HKY distance of 0.191.

Nuclear SNPs

Divergence between populations 31 and 34 was moderate ($\theta = 0.078$), possibly suggesting a recent expansion, while divergence between all remaining population pairs was high, with θ ranging from 0.685 to 1.000 (Table 3). Notably, populations 30 and 31 differed by a θ of 0.911 and had fixed differences at 11 loci, spanning eight chromosomes. A two-dimensional factorial correspondence analysis (Fig. 4) shows individuals falling into three widely separated clusters: (1) population 27; (2) populations 24, 28 and 30; and (3) populations 31 and 34. All SNP data are available in convertible GENEPOP format (Raymond & Rousset, 1995; Rousset, 2008) in Appendix S2.

Mating experiments

Crossing experiments (Table 1) showed patterns that were generally consistent with one of the three compatibility

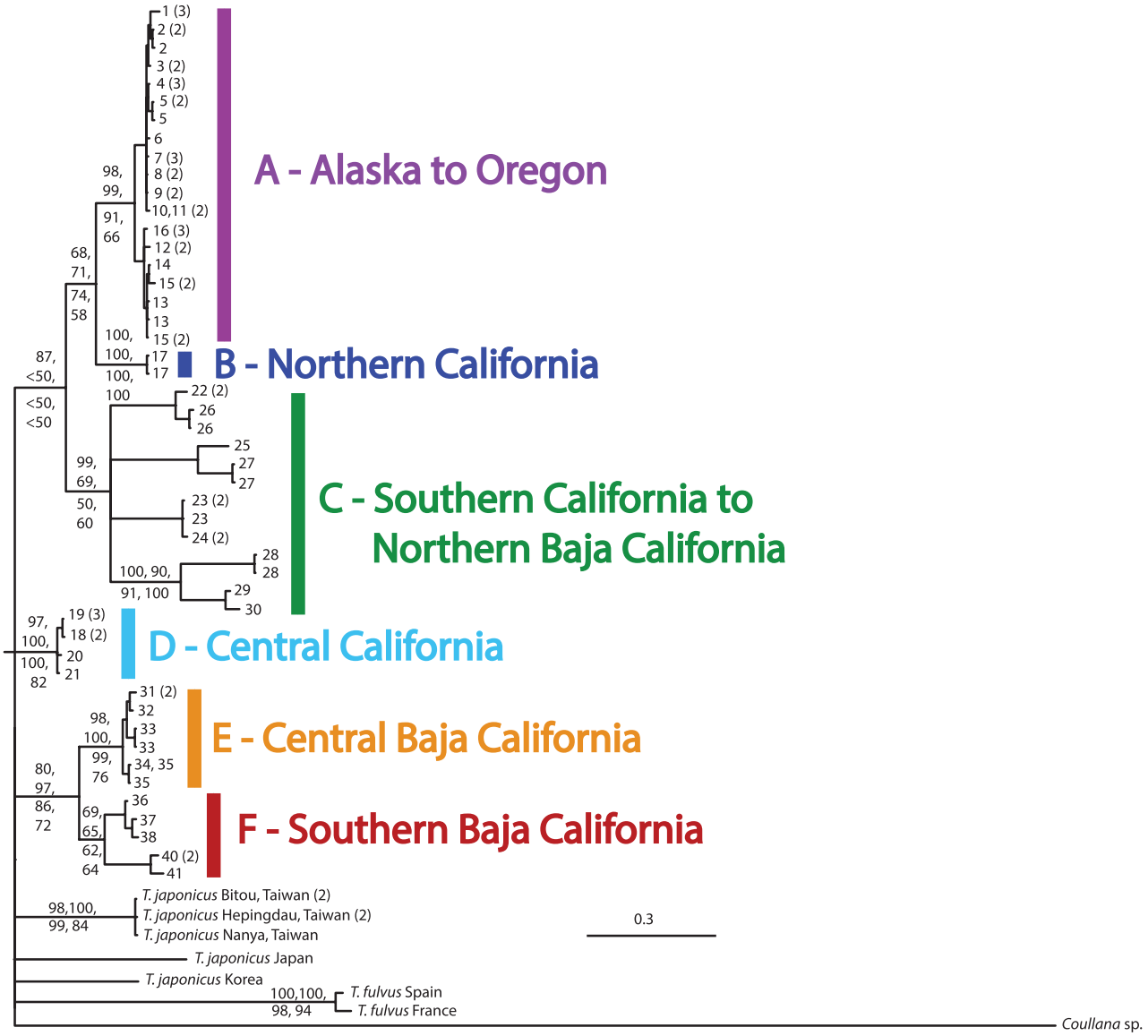

Figure 3 Bayesian majority rule consensus phylogram (unconstrained) for a 505-bp segment of mitochondrial *COI* from 41 *Tigriopus californicus* populations (numbered from north to south) and samples from congeners *T. japonicus* and *T. fulvus*, rooted with outgroup *Coullana* sp. Location names and sequence accession numbers are listed in Appendix S1. When more than one individual of each haplotype was found, the number is given in parentheses. Support for major nodes is given above branches for Bayesian posterior probability values and maximum parsimony bootstrap values, and below branches for maximum likelihood bootstrap values (MEGA and GARLI). The scale bar shows HKY distance.

Table 2 Mean sequence divergence (HKY) between clades of *Tigriopus californicus* in western North America.

	Clade 1				
Clade 2	B	C	D	E	F
A	0.175	0.269	0.239	0.368	0.344
B	—	0.285	0.272	0.349	0.333
C		—	0.303	0.341	0.329
D			—	0.310	0.306
E				—	0.191

groups shown in Fig. 1. Populations 24 and 27 fell into the northern group (N), populations 28 and 29 fell into the intermediate group (I), and population 31 and all populations further south fell into the southern group (S). It was not clear whether population 30 belongs to the N or I group, because 30f × 34m crosses did not proceed beyond the F1, as expected for N females, but a small subset of the 30f × 31m crosses produced F2, as expected for I females. F2 egg sac size was measured for one cross in which F2 egg sacs were produced but failed to hatch (24f × 34m) and one cross in which F2 egg sacs occasionally hatched (30f × 31m). In both crosses, hybrid egg sac area and diameter was smaller than that of both parental controls, and ANOVA post-hoc tests showed these differences to be significant in seven of eight cases (Table 4). Thus, even in cases like 30f × 31m, where F2

Table 3 Pairwise divergence between populations of *Tigriopus californicus* in western North America, based on 42 nuclear SNPs. Above the diagonal: Weir and Cockerham's θ. Below the diagonal: percentage of permuted θ values that are above/below the observed value, based on 1000 permutations.

	Population no. (sample size)					
Population no.	24 (n = 5)	27 (n = 18)	28 (n = 5)	30 (n = 4)	31 (n = 5)	34 (n = 4)
24		0.965	1.000	0.849	0.963	0.939
27	0.00/99.5		0.958	0.947	0.965	0.961
28	0.00/93.8	0.00/99.9		0.685	0.969	0.951
30	0.00/93.6	0.00/99.3	0.00/92.1		0.911	0.878
31	0.00/94.0	0.00/100.0	0.00/94.1	0.00/95.8		0.078
34	0.00/93.6	0.00/99.1	0.00/93.7	0.00/85.3	7.20/80.2	

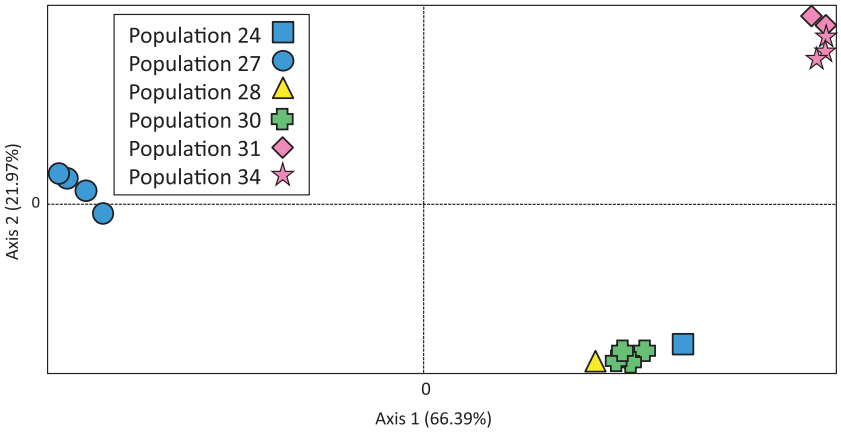

Figure 4 Factorial correspondence analysis for individuals from six populations of *Tigriopus californicus* from western North America based on 42 nuclear SNPs. Populations are colour-coded by reproductive compatibility group: N (blue), I (yellow), N/I (green) and S (pink).

Table 4 One-way ANOVA for F2 egg sac area and diameter for three groups (female parent control, male parent control and hybrid) in each of two crosses between populations of *Tigriopus californicus* from western North America. Significant post-hoc assays (Tukey's HSD for unequal n) are shown in bold.

		ANOVA			Hybrid vs. female parent		Hybrid vs. male parent	
Cross	Phenotype	d.f.	F	P-value	Proportional deviation	P-value	Proportional deviation	P-value
24f × 34m	Egg sac area	2, 63	20.55	0.000	−0.321	0.070	**−0.529**	**0.000**
24f × 34m	Egg sac diameter	2, 63	19.99	0.000	**−0.225**	**0.037**	**−0.385**	**0.000**
30f × 31m	Egg sac area	2, 62	10.16	0.000	**−0.124**	**0.014**	**−0.168**	**0.000**
30f × 31m	Egg sac diameter	2, 62	9.40	0.000	**−0.110**	**0.023**	**−0.155**	**0.000**

hybrids were successfully produced, there is evidence for partial reproductive incompatibility. Neither the antibiotic treatments nor the PCR assays provided evidence for the presence of *Wolbachia* or other incompatibility-inducing bacteria.

Tests of male clasping mistakes were assessed separately for each of the two blocks. In the first block (freshly collected population 24, 288 pairs), 0.8% of males were found to have clasped previously inseminated females and 3.5% of males were clasping other males. In the second block (lab population 28 culture, 216 pairs), no males were found to be clasping previously inseminated females and 0.6% were clasping other males.

DISCUSSION

Genetic patterns

The mitochondrial phylogeny helped determine the geographical limit between the highly divergent (HKY distance 0.341) clades C and E, which are separated by a geographical distance of less than 12 km between populations 30 and 31, encompassing no apparent barriers to dispersal. Nuclear SNP data for a small set of samples corroborated this break, with substantial divergence (θ = 0.911) between populations 30 and 31, including fixed differences at 11 loci. In addition, a phylogeographical break of 0.191 (HKY distance) was found

between clades E and F coincident with Guerrero Negro. Although genetic patterns in this study are based on a small number of individuals at each geographical location, this study and previous studies (e.g. Burton & Lee, 1994; Edmands & Harrison, 2003) show extremely low genetic variation within locations.

Integrative analyses of genetic and reproductive discontinuities, such as the current study, contribute to the ongoing debate concerning the complex geological history of the Baja California Peninsula (reviewed in Hurtado *et al.*, 2010; Ledesma-Vázquez & Carreño, 2010). Previous phylogeographical studies have reported mid-peninsula breaks ranging from latitude 27° to 30° N and have attributed these breaks to factors such as abrupt changes in climate and habitat, or vicariant events including transpeninsular seaways (e.g. Upton & Murphy, 1997; Riddle *et al.*, 2000; Crews & Hedin, 2006). In the current study, the major break between clades C and E (*c.* 29.35° N), as well as the minor break between clades E and F (*c.* 28° N), are both well north of a proposed Miocene mid-peninsular seaway that may have occurred near the present-day location of Bahia San Ignacio at about 27° N on the Pacific side (Ledesma-Vázquez & Carreño, 2010). The break at 28° N is concordant with several previous studies (e.g. Lindell *et al.*, 2008; Hurtado *et al.*, 2010; Markow & Pfeiler, 2010) and appears to be associated with the Guerrero Negro Lagoon. The stronger break at *c.* 29.35° N has not, to our knowledge, been reported in other taxa.

Highly relevant work on phylogenetic patterns in the Baja California intertidal zone comes from recent studies of supralittoral *Ligia* isopods (Hurtado *et al.*, 2010; Markow & Pfeiler, 2010). Like *Tigriopus*, coastal *Ligia* are crustaceans restricted to the high rocky intertidal zone, lack a dispersive larval stage and show extreme levels of allopatric differentiation, with estuaries and stretches of sandy beach acting as effective dispersal barriers. Mitochondrial phylogenies for both *Tigriopus* and *Ligia* show northern Baja California samples grouping with a subset of California samples. Both taxa also show breaks coincident with Guerrero Negro, with the level of mitochondrial sequence divergence between clades on either side of this break being higher for *T. californicus* (HKY distance 19.1%; K2P distance 18.6%) than for *Ligia* (K2P distance 12–14%; Hurtado *et al.*, 2010). The *Ligia* studies do not show a break in the region around 29.35° N, although this region coincides with a sampling gap.

It is possible that the break we found at 29.35° N was originally caused by a temporary seaway, with subsequent expansion to the north. The small number of DNA sequences for each population does not allow effective demographical tests for such a population expansion. An alternative to this vicariant explanation is that the phylogenetic break is not the result of a barrier to gene flow, but instead results from limited dispersal and genetic drift. Drift is particularly likely for mitochondrial DNA, which has one quarter of the effective population size of nuclear markers (Irwin, 2002). The species does show a number of deep mitochondrial breaks further north, some of which do not correspond to known geographical barriers (Burton & Lee, 1994; Burton, 1998; Edmands, 2001), but these breaks involve lesser genetic divergence, and none is coincident with a major reproductive barrier.

Reproductive patterns

Reproductive patterns were generally consistent with genetic patterns, and showed a particularly dramatic discontinuity near 29.35° N. Our reproductive categories (N, I, S and N/I) were purely qualitative; that is, a cross was scored as producing F2 if only one live F2 larva was observed. It should be noted that separate quantitative studies (e.g. Hwang *et al.*, 2012) show that some compatible crosses still show extremely low hybrid viability. For example, in the 28f × 34m cross (an I × S cross), the mean number of hybrid larvae, relative to the midparent, was 23% in the F1, 1% in the F2 and 0% for both backcrosses.

Distinguishing reproductive categories was complicated by cases in which crosses that had consistently produced no offspring suddenly produced large numbers of offspring. Some of these variable results could be real. That is, our inability to definitively assign population 30 to either the N or I compatibility group may be because the location is a hybrid zone between N and I types. However, the limited molecular data do not support this possibility, in that the population 30 mitochondrial haplotype was distinct from all other haplotypes surveyed and the nuclear SNP data showed numerous fixed differences from other sampled locations. Alternatively, location 30 may be a distinct population that harbours genetic variation for reproductive compatibility. Indeed, we would expect that incipient species would go through phases in which genes responsible for reproductive isolation might not be fixed within local populations (e.g. Kopp & Frank, 2005). However, in some of the unexpectedly successful hybridizations the putative hybrids were disproven by genotyping (Table 1). A likely source of such errors is males clasping individuals that are not actually virgin females. Ours is the first study to quantify male clasping mistakes, and the frequency of males found to be clasping immature males (0.6–3.5%) was particularly surprising.

The genetic mechanisms underlying the observed patterns of reproductive compatibility must involve a large degree of asymmetry and may involve separate mechanisms for the F1 and F2 patterns. Our results, like those of Voordouw *et al.* (2008), do not support *Wolbachia* or other intracellular bacteria as the cause of reproductive asymmetry. A second potential cause of extreme reproductive asymmetry is transposable-element-mediated hybrid dysgenesis such as that commonly found between strains of *Drosophila* (Kidwell, 1983). Finally, a third possible cause of reproductive asymmetry, not mutually exclusive with either intracellular bacteria or transposable elements, is the presence of deleterious interactions between nuclear and mitochondrial genes, a subject of much study in *T. californicus* (e.g. Burton & Barreto, 2012).

The species problem

Do populations south of 29.35° N qualify as a different species? Under the biological species concept, the answer is ambiguous. The N and S populations are essentially isolated because reproduction cannot proceed beyond the F1. However, the two groups retain the potential for gene flow via the I populations, which are reproductively, but not always geographically, intermediate. If the I populations were to perish, the N and S groups would then be *bona fide* species. Phylogenetic analysis of congeneric samples further complicates the issue of species boundaries, and the resolution of higher-order relationships is poor due to the saturation of *COI* at deeper nodes. Putative *T. japonicus* samples from Japan, Korea and Taiwan do not form a monophyletic group. Similarly, Ki *et al.* (2009) used ITS–5.8S rDNA sequences and did not find monophyly of Asian *Tigriopus* relative to *T. californicus*. More comprehensive data are needed to better define taxonomic boundaries in both *T. californicus* and *T. japonicus*.

No evidence for mixing across the break at 29.35° N

Within clade C, there is some geographical 'hopscotching' of reproductive groups, with one N population (Punta Banda or PBN, studied by Ganz & Burton, 1995) being found south of an I population (no. 28). However, no geographical overlap was found between the N/I populations (clade C) and the S populations (clade E + F), which are separated by a distance of less than 12 km in a region featuring no obvious barriers to dispersal. The lack of mixing between clades C and E + F was seen not just in the modest numbers of samples used for mitochondrial and nuclear assays, but also in the mating experiments. Here, clade C showed a mix of N, I and N/I reproductive types, but not a single individual showed the S reproductive type, as none of the 588 crosses between clade C males and clade (E, F) females produced offspring.

Dispersal capacity in *T. californicus* is a conundrum. On the one hand, gene flow is clearly low, with strong and stable differentiation between populations separated by only a few kilometres (Burton, 1997). This isolation is possibly maintained by heavy predation on animals transported to lower tidal levels (e.g. Dethier, 1980), and yet long-distance colonization clearly occurs, as populations have apparently accomplished rapid post-glacial expansion (Edmands, 2001), and colonized remote outcrops and offshore islands (Burton, 1998; Edmands, 2001). The congener *T. brevicornis* shows similar patterns of restricted dispersal and yet has apparently colonized large geographical areas within the past 12,000–15,000 years and has even traversed the Atlantic (Handschumacher *et al.*, 2010). It seems unlikely that limited dispersal capacity is preventing geographical overlap between the N/I and S compatibility groups in *T. californicus*.

Alternatively, copepods may be dispersing across the boundary and they may even be mating with residents, because no prezygotic isolation has been found between reproductive groups (Ganz & Burton, 1995). Note, however, that prezygotic barriers between the specific populations that are most likely to come into contact (populations 30 and 31) have not yet been quantified, so the possibility of reinforcement persists. Even if mating between the two divergent clades does occur in nature, hybrid viability may be so low that migrant alleles cannot become effectively established. This seems plausible because even in a 'successful' cross of intermediate females with southern males the viability of second-generation hybrids was found to be extremely low (e.g. 28f × 34m; Hwang *et al.*, 2012). Hybrid inviability in the offspring of migrants could also explain why there is apparent 'hopscotching' among N and I populations (which are fully compatible) but not between N/I and S populations (which are partially incompatible). A final possibility is that low levels of gene flow across the boundary have occurred, but this was not detected due to our limited geographical sampling. A more fine-scale phylogenetic study, focusing on the boundary zone, could help tease out any ongoing contact between these incipient species.

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REFERENCES

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. & Bonhomme, F. (2004) *GENETIX 4.05, logiciel sous Windows pour la génétique des populations*. Laboratoire Génome, Populations, Interactions, CNRS, UMR 5000, Université de Montpellier II, Montpellier.

Breeuwer, J.A.J. (1997) *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestani*. *Heredity*, **79**, 41–47.

Briggs, J.C. (1974) *Marine zoogeography*. McGraw Hill, New York.

Burton, R.S. (1985) Mating systems of the intertidal copepod *Tigriopus californicus*. *Marine Biology*, **86**, 247–252.

Burton, R.S. (1997) Genetic evidence for long term persistence of marine invertebrate populations in an ephemeral environment. *Evolution*, **51**, 993–998.

Burton, R.S. (1998) Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution*, **52**, 734–745.

Burton, R.S. & Barreto, F.S. (2012) A disproportionate role for mtDNA in Dobzhansky–Muller incompatibilities? *Molecular Ecology*, **21**, 4942–4957.

Burton, R.S. & Lee, B.N. (1994) Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proceedings of the National Academy of Sciences USA*, **91**, 5197–5201.

Crews, S.C. & Hedin, M. (2006) Studies of morphological and molecular phylogenetic divergence in spiders (Araneae: *Homalonychus)* from the American southwest, including divergence along the Baja California Peninsula. *Molecular Phylogenetics and Evolution*, **38**, 470–487.

Dethier, M.N. (1980) Tidepools as refuges: predation and the limits of the harpacticoid copepod *Tigriopus californicus* (Baker). *Journal of Experimental Marine Biology and Ecology*, **42**, 99–111.

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. *Molecular Ecology*, **10**, 1743–1750.

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

Egloff, D.A. (1966) *Ecological aspects of sex ratio and reproduction in experimental and field populations of the marine copepod* Tigriopus californicus. PhD Thesis, Stanford University, Palo Alto, CA.

Foley, B.R., Rose, C.G., Rundle, D.E., Leong, W., Moy, G.W., Burton, R.S. & Edmands, S. (2011) A gene-based SNP resource and linkage map for the copepod *Tigriopus californicus*. *BMC Genomics*, **12**, 568.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Ganz, H.H. & Burton, R.S. (1995) Genetic differentiation and reproductive incompatibility among Baja California populations of the copepod *Tigriopus californicus*. *Marine Biology*, **123**, 821–827.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704.

Handschumacher, L., Steinarsdóttir, M.B., Edmands, S. & Ingólfsson, A. (2010) Phylogeography of the rock-pool copepod *Tigriopus brevicornis* (Harpacticoida) in the northern North Atlantic, and its relationship to other species of the genus. *Marine Biology*, **157**, 1357–1366.

Harrison, J.S., Peterson, D.L., Swain, J.R. & Edmands, S. (2004) Microsatellite DNA markers for the intertidal copepod *Tigriopus californicus*. *Molecular Ecology Notes*, **4**, 736–738.

Holden, P.R., Brookfield, J.F.Y. & Jones, P. (1993) Cloning and characterization of an *ftsZ* homologue from a bacterial symbiont of *Drosophila melanogaster*. *Molecular and General Genetics*, **240**, 213–220.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Hurtado, L.A., Mateos, M. & Santamaria, C.A. (2010) Phylogeography of supralittoral rocky intertidal *Ligia* isopods in the Pacific region from central California to central Mexico. *PLoS ONE*, **5**, e11633.

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. *Conservation Genetics*, **13**, 567–579.

Irwin, D.E. (2002) Phylogeographic breaks without geographic barriers to gene flow. *Evolution*, **56**, 2383–2394.

Irwin, D.E., Bensch, S., Irwin, J.H. & Price, T.D. (2005) Speciation by distance in a ring species. *Science*, **307**, 414–416.

Kelly, M.W., Sanford, E. & Grosberg, R.K. (2012) Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 349–356.

Ki, J-S., Lee, K-W., Park, H.G., Chullasorn, S., Dahms, H.-U. & Lee, J-S. (2009) Phylogegraphy of the copepod *Tigriopus japonicus* along the Northwest Pacific rim. *Journal of Plankton Research*, **31**, 209–221.

Kidwell, M.G. (1983) Hybrid dysgenesis in *Drosophila melanogaster*: factors affecting chromosomal contamination in the P-M system. *Genetics*, **104**, 317–341.

Kopp, A. & Frank, A.K. (2005) Speciation in progress? A continuum of reproductive isolation in *Drosophila bipectinata*. *Genetica*, **125**, 55–68.

Kuchta, S.R., Parks, D.S., Mueller, R.L. & Wake, D.B. (2009) Closing the ring: historical biogeography of the salamander ring species *Ensatina eschscholtzii*. *Journal of Biogeography*, **36**, 982–995.

Ledesma-Vázquez, J. & Carreño, A.L. (2010) Origin, age, and geological evolution of the Gulf of California. *Gulf of California: biodiversity and conservation* (ed. by R. Brusca), pp. 7–23. University of Arizona Press, Tucson, AZ.

Lee, B-N. (2003) *Genetic structure of* Tigriopus californicus *populations inferred from mitochondrial cytochrome oxidase I sequences*. PhD Thesis, University of Houston, TX.

Lindell, J., Méndez-de la Cruz, F.R. & Murphy, R.W. (2008) Deep biogeographical history and cytonuclear discordance in the black-tailed brush lizard (*Urosaurus nigricaudus*) of Baja California. *Biological Journal of the Linnean Society*, **94**, 89–104.

Markow, T.A. & Pfeiler, E. (2010) Mitochondrial DNA evidence for deep genetic divergences in allopatric populations of the rocky intertidal isopod *Ligia occidentalis* from the eastern Pacific. *Molecular Phylogenetics and Evolution*, **56**, 468–473.

Mayr, E. (1942) *Systematics and the origin of species, from the viewpoint of a zoologist*. Columbia University Press, New York.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, LA*, pp. 1–8. Institute of Electrical and Electronics Engineers (IEEE), Washington, D.C.

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

Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L. & Grabowski, G. (2002) *The simple fool's guide to PCR, version 2.0*. University of Hawaii, Honolulu, HI.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Raymond, M. & Rousset, F. (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Riddle, B.R., Hafner, D.J., Alexander, L.F. & Jaeger, J.R. (2000) Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. *Proceedings of the National Academy of Sciences USA*, **97**, 14438–14443.

Rousset, F. (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Swofford, D.L. (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Turelli, M. (1994) Evolution of incompatibility-inducing microbes and their hosts. *Evolution*, **48**, 1500–1513.

Upton, D.E. & Murphy, R.W. (1997) Phylogeny of the side-blotched lizards (Phrynosomatidae: *Uta*) based on mtDNA sequences: support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution*, **8**, 104–113.

Valentine, J.W. (1966) Numerical analysis of marine molluscan ranges on the extratropical northeastern Pacific shelf. *Limnology and Oceanography*, **11**, 198–211.

Voordouw, M.J., Stebbins, G., Robinson, H.E., Perrot-Minnot, M.-J., Rigaud, T. & Anholt, B.R. (2008) Genetic variation in the primary sex ratio in populations of the intertidal copepod, *Tigriopus californicus*, is widespread on Vancouver Island. *Evolutionary Ecology Research*, **10**, 1007–1023.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, University of Texas, Austin, TX.

SUPPORTING INFORMATION

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

Appendix S1 Populations of *Tigriopus* used for phylogeny and/or reproductive assays.

Appendix S2 SNP genotype data for 42 loci in six populations, formatted for GENEPOP.

BIOSKETCH

Dennis Peterson is broadly interested in evolution and conservation of marine organisms. He began this study as part of his doctoral dissertation in the Edmands Lab at the University of Southern California.

Author contributions: D.L.P. designed the study and performed much of the initial research. K.B.K. conducted mating experiments and DNA sequencing. M.J.C. tested for incompatibility-inducing bacteria. L.R.K. performed crosses and morphometric analyses. M.M.W. tested for male clasping mistakes. W.L. and B.C.P. completed the SNP assays. S.E. conducted data analyses and drafted the manuscript, which was approved by all authors.

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