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Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes

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Abstract

Previous studies of the intertidal copepod *Tigriopus californicus* revealed one of the highest levels of mitochondrial DNA differentiation ever reported among conspecific populations. The present study extends the geographical sampling northward, adding populations from northern California to south-east Alaska. The mitochondrial phylogeny for the entire species range, based on cytochrome oxidase I sequences for a total of 49 individuals from 27 populations, again shows extreme differentiation among populations (up to 23%). However, populations from Oregon northwards appear to be derived and have interpopulation divergences five times lower than those between southern populations. Furthermore, although few individuals were sequenced from each locality, populations from Puget Sound northward had significantly reduced levels of within-population variation. These patterns are hypothesized to result from the contraction and expansion of populations driven by recent ice ages.

Keywords: biogeography, isolation-by-distance, mitochondrial DNA, range expansion, refugia, stepping stone.

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Introduction

There was once a time when speciation was considered 'a line of peril below which phylogeneticists must not tread' (Avise 2000) due to the anastomosing nature of conspecific lineages. This line has now been enthusiastically breached, due in part to the popularity of mitochondrial markers whose asexual nature results in hierarchical branching patterns even within sexual species. The recent explosion in studies of intraspecific phylogeny, and more specifically phylogeography, has shed much light on our knowledge of biogeography, patterns of colonization, rates of gene flow and the process of speciation itself (Avise 2000 and references therein).

This explosion of studies allows us to begin comparing phylogeographic patterns in marine vs. terrestrial groups. For many marine species, high dispersal capacity and a lack of strong physical barriers may blur phylogeographic patterns. However, for species with limited dispersal capacity, prevailing current directions may accentuate clinal biogeographic patterns. This is particularly true for species that are restricted to the essentially linear habitat of shallow continental margins. In these habitats, speciation is expected to be most likely at the ends of species ranges (Valentine 1984; Reid 1990; Ganz & Burton 1995).

The intertidal copepod *Tigriopus californicus* is a particularly interesting subject for phylogeographic study because its populations span a vast range of genetic divergence, allowing a glimpse of the interface between intraspecific and interspecific differentiation. Extensive genetic work has been done on the species and every marker surveyed [allozymes, nuclear and mitochondrial DNA (mtDNA)] has revealed extreme genetic differentiation, even over short geographical distances (Burton *et al*. 1979; Burton 1994, 1998; Burton & Lee 1994; Ganz & Burton 1995). The species has been reported from Torch Bay in south-east Alaska (Dethier 1980) south to Playa Altamira in central Baja California, Mexico (Ganz & Burton 1995). The southernmost populations showed exceptionally high genetic divergence, with one population also exhibiting nearly complete reproductive isolation, prompting the suggestion that it might be

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Table 1 Collection locations and sample sizes

Species/Collection location	Abbreviation	Latitude	Longitude	Individuals sequenced
Tigriopus californicus				
(A) Starrigavan Campground, Baranof I., AK	STA	57°08′ N	135°22′ W	3
(B) Halibut Point, Baranof I., AK	HAL	57°06′ N	135°24′ W	3
(C) Japonski I., AK	JAP	57°03′ N	135°21′ W	2
(D) Aguilar Point, Vancouver I., B.C.	AP	48°51′ N	125°08′ W	3
(E) Grappler Point, Vancouver I., B.C.	GP	48°50′ N	125°08′ W	3
(F) Pachena Bay, Vancouver I., B.C.	PAC	48°46′ N	125°08′ W	1
(G) Friday Harbor Laboratories, San Juan I., WA	FHL	48°33′ N	123°00′ W	3
(H) Victoria, Vancouver I., B.C.	VIC	48°28′ N	123°17′ W	2
(I) Eagle Cove, San Juan I., WA	EC	48°28′ N	123°02′ W	1
(J) Cattle Point, San Juan I., WA	CP	48°27′ N	122°58′ W	2
(K) Fourth Beach, Olympic National Park, WA	FB	47°37′ N	124°23′ W	2
(L) Yaquina Head, OR	YAQ	44°50′ N	124°04′ W	2
(M) Strawberry Hill Wayside, OR	SH	44°15′ N	124°07′ W	1
(N) Bob Creek Wayside, OR	BCW	44°15′ N	124°07′ W	4
(O) Cape Arago (tidepool 1), OR	CA1	43°18′ N	124°24′ W	2
(P) Cape Arago (tidepool 3), OR	CA3	43°18′ N	124°24′ W	1
(Q) Bodega Bay Marine Laboratory, CA	BOD	38°19′ N	123°04′ W	2
(R) Natural Bridges State Park, CA	NB	36°57′ N	122°04′ W	2
(S) Santa Cruz (end of Swift Street), CA	SC	36°57′ N	122°03′ W	3
(T) Pacific Grove, west side of Point Cabrillo, CA	PG	36°37′ N	121°54′ W	1
(U) Carmel, CA*	CAR	36°33′ N	121°55′ W	1
(V) Point Dume, CA	PD	34°00′ N	118°48′ W	2
(W) Abalone Cove, Palos Verdes Peninsula, CA	AB	33°44′ N	118°22′ W	3
(X) Royal Palms, Palos Verdes Peninsula, CA	RP	33°43′ N	118°19′ W	2
(Y) San Diego (south of Ocean Beach Pier), CA	SD	32°45′ N	117°15′ W	2
(Z) Punta Morro, Baja California, Mex.	PMO	31°52′ N	116°40′ W	2
(ZZ) Playa Altamira South, Baja California, Mex.	PAS	28°32′ N	114°05′ W	2
Tigriopus fulvus				
Banyuls Sur Mer, France	BAN	42°29′ N	3°08′ E	1
Blanes, Spain	BLA	41°40′ N	2°48′ E	1

*DNA sequence obtained from GenBank.
I. = Island; AK = Alaska, USA; B.C. = British Columbia, Canada; WA = Washington, USA; OR = Oregon, USA; CA = California, USA; Mex. = Mexico.

considered a semispecies or subspecies (Ganz & Burton 1995).

Nuclear and mitochondrial gene genealogies for *T. californicus* have revealed striking, and largely concordant, patterns. While initial studies showed a strong phylogeographic break coincident with the Point Conception biogeographic boundary (Burton & Lee 1994), further geographical sampling revealed breaks of equal or greater magnitude at locations not consistent with biogeographic boundaries (Burton 1998). To date, genetic work on *T. californicus* has focused on the southern part of the species range, from Baja California to Central California. The present study uses an overlapping segment of the same mitochondrial gene used in previous studies to extend the sampling northwards, past several recognized biogeographic boundaries, to the end of the species range in southeast Alaska.

Materials and methods

Sample collection

Samples of *Tigriopus californicus* were collected from high intertidal and supralittoral pools at 26 locations ranging from southeast Alaska, USA to Baja California, Mexico (Table 1). Samples from 24 locations were collected in July and August 1996, and tidepools from the same rock outcrop were combined because previous work has shown high gene flow among pools within outcrops (Burton & Swisher 1984). To test this assumption, two tidepools within the same outcrop at Cape Arago, Oregon were collected in January 1997 and analysed separately. Samples of the Mediterranean congener *Tigriopus fulvus* were collected in August 1999 for use as an outgroup. A subset of the animals from each location was frozen at –80 °C within 2 weeks of collection.

DNA extraction, polymerase chain reaction and sequencing

DNA was extracted by boiling single copepods (either frozen or live) for 8 min in 50 μL of 10% chelating resin (Walsh *et al*. 1991). The polymerase chain reaction (PCR) was used to amplify a 710-base pair (bp) fragment of the mitochondrial gene cytochrome oxidase I (COI) from one to three individual copepods per population (Table 1) using primers LCO1490 and HCO2198 in Folmer *et al*. (1994). This strategy of sequencing a few individuals from many populations was based on previous work that has repeatedly shown the vast majority of genetic variation in this species to be distributed among populations (e.g. Burton *et al*. 1979; Burton & Lee 1994; Burton 1998). Amplification followed standard procedures (Saiki *et al*. 1988), using a reaction volume of 50 μL and a magnesium chloride concentration of 2 mM. The following thermocycle profile was used: 3 min at 94 °C followed by 35 cycles of (60 s at 94 °C, 60 s at 45 °C and 90 s at 72 °C), followed by 5 min at 72 °C.

PCR products were electrophoresed on a low-melting-point agarose gel, excised from the gel using sterile razor blades and purified using either QIAquick spin columns (Quiagen) or Geneclean II silica matrix (Bio 101). Purified products were sequenced using Taq-DyeDeoxy Terminator Cycle sequencing (Applied Biosystems) and electrophoresed on an ABI Prism 377 DNA sequencer. All samples were sequenced using primer HCO2198 and ambiguous sites were resolved by also sequencing the opposite strand using primer LCO1490. As has previously been reported (Burton 1998), these 'universal' primers occasionally amplified what appeared to be a pseudogene, identifiable by apparently heterozygous nucleotide sites and high levels of amino acid substitution (including frame-shifting indels). These sequences were removed from the present analyses. Sequences used for this study have been deposited in GenBank under accession numbers AF096931–AF096941, AF096943–AF096975 and AF315360–AF0315373.

Analyses

DNA sequences were aligned and edited using SEQUENCHER 3.0 (Gene Codes Corporation). Phylogeny reconstruction was based on a 552-bp region corresponding to positions 64–615 in the *T. californicus* COI sequence (Lee 1993; GenBank accession number L43049). This GenBank sequence was also included in the phylogeny, bringing the total number of *T. californicus* populations to 27. Translations from DNA to protein were done with MACCLADE (version 3.0, Maddison & Maddison 1992). Trees were constructed with PAUP* (version 4b1, Swofford 1999) by heuristic searches, using both maximum parsimony and distance methods (minimum evolution, uncorrected 'p' distance). Nodal support was assessed by 500 bootstrap replicates. ARLEQUIN (version 2.0, Schneider *et al*. 2000) was used to calculate F_{ST} and the level of gene flow (*Nm*) between each pair of populations (e.g. Slatkin 1991). Levels of within- and between-population sequence divergence in different clades were compared by unpaired, two-tailed *t*-tests.

Geographic divergence between populations was measured as the shortest straight line distance between localities. Latitude and longitude for each locality was determined either with a geographical positioning system or by inspection of 24 000 : 1 or 60 000 : 1 scale maps. Distance between pairs of latitude/longitude co-ordinates was calculated by a computer program available at http://jan.ucc.nau.edu/~cvm/latlongdist.html. Correspondence between genetic and geographical distance is typically measured by regressing log (geographical distance) against log (pairwise *Nm*) (e.g. Slatkin 1993). In this study, log(*Nm* + 1) was used instead because this transformation is recommended when observed values are small (Zar 1999) and was necessary in this case because gene flow between many population pairs was 0.

Results

When nucleotide data were translated into amino acids, this produced a very poorly resolved tree with short branch lengths, reflecting interpopulation divergences of 0–3%. In contrast, nucleotide data produced fairly well-resolved trees with substantial branch lengths. Figure 1 presents one of many equally parsimonious trees in order to illustrate the very large branch lengths, reflecting interpopulation divergences of 0–23%. While there are over 200 equally parsimonious trees for the nucleotide data, the major groupings are well-supported, as indicated by the many bold lines showing nodes found in the strict consensus parsimony tree. Analysis by a distance method (minimum evolution) showed the same crown groups, but differed as to the branching order of the southernmost clades. A more conservative tree is therefore shown in Fig. 2, including only groupings with at least 50% bootstrap support by both parsimony and distance methods. This tree shows the branching order of clades from central California south to Mexico as unresolved, but supports northern clade A-Q (Alaska to northern California) from which clades A-J (Alaska to Puget Sound) and K-P (outer coast of Washington to Oregon) are derived.

Breakpoints in this mitochondrial phylogeny were only partially concordant with well-recognized biogeographic breakpoints (shown by asterisks in Figs 1 and 2). Of the six major provincial boundaries described by Valentine (1966), four (Point Conception, Monterey Bay, Cape Mendocino and Puget Sound) are consistent with genetic discontinuities, and one (Dixon Entrance) is not consistent with a genetic discontinuity. In addition, many of the deepest

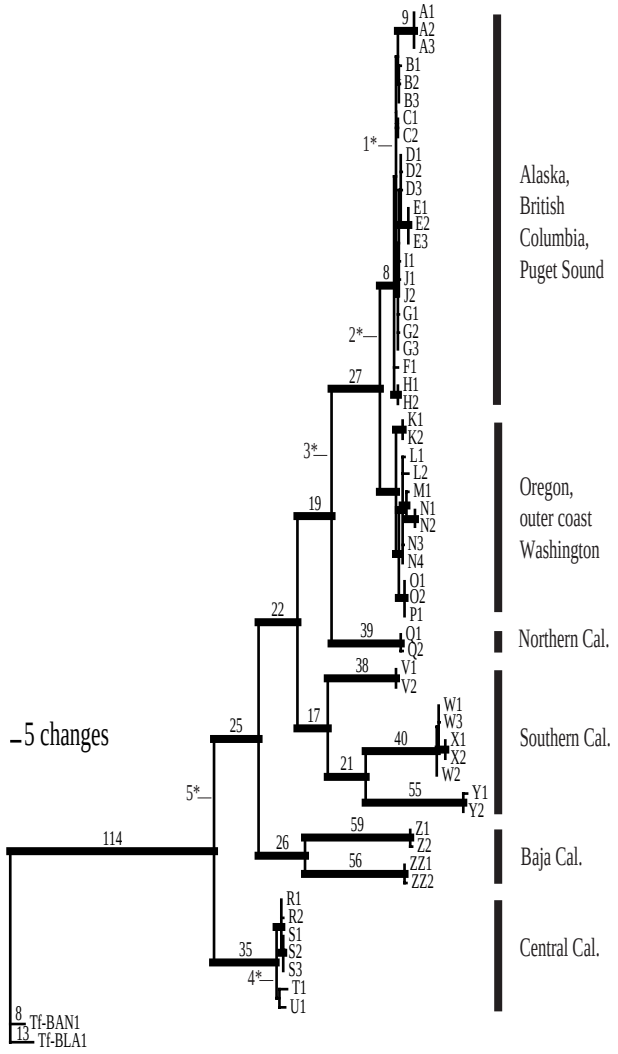

Fig. 1 Phylogeny constructed with maximum parsimony using a 552-base segment of cytochrome oxidase I. The tree shown is one of 216 equally parsimonious trees of length 704 and consistency index 0.565. Numbers along branches indicate the absolute number of substitutions (numbers less than six were omitted for clearer presentation). See Table 1 for locality information. Numbered asterisks correspond to biogeographic breakpoints shown in Fig. 2. Cal. = California.

phylogenetic breaks (for example, between Z and ZZ or between WX and Y in Fig. 1) occur in the absence of any recognized provincial boundary. The sixth provincial boundary, Punta Eugenia, is just south of the species' published range. However, copepods that appear to be *Tigriopus californicus* have more recently been found south of this biogeographic breakpoint (R.S. Burton personal communication, 1996; D. Peterson personal communication, 2000).

Populations from Alaska to northern California showed considerably lower interpopulation differentiation than populations from central California to Baja California. While there was a rough correspondence between genetic and geographical distance over the whole species range (Fig. 3a), sequence divergence per kilometer was five times lower within the northern group (A-Q; mean 0.0002; SE 5×10^{-5}) than within the southern group (R-ZZ; mean 0.001; SE 9×10^{-5}), and this difference was highly significant by an unpaired, two-tailed *t*-test ($t = -4.73$, $P = 0.0001$, d.f. = 187). Including population Q in the low divergence group is something of an arbitrary decision—its inclusion is favoured by its low sequence divergence (mean 0.0001/km) from other northern populations, but the lack of sampling in the region around population Q makes this a weak test. Genetic variation within populations also varied among regions. For locations in which more than one individual was assayed, mean within-population sequence divergence was 0.030% in clade A-J, 0.390% in clade K-P, 0.180% in population Q and 0.196% in group R-ZZ. An unpaired, two-tailed *t*-test shows that this variation was significantly lower in A-J than in either K-P ($P = 0.042$) or R-ZZ ($P = 0.027$).

Genetic distance is expected to correspond with geographical distance if gene flow occurs only between adjacent populations and if gene flow and genetic drift have equilibrated (Slatkin 1993). Significant correspondence (Fig. 3) between gene flow [$\log(Nm + 1)$] and geographical distance [log(km between populations)] was found over all segments of the tree tested (entire in-group, R-ZZ, A-Q and K-P) with the exception of the northernmost clade, A-J. No gene flow was detected between many population pairs ($Nm = 0$). Removing these points (which are saturated by drift) strengthened the regression in four out of the five cases tested. Regressions in Fig. 3 used log ($Nm + 1$) instead of the more standard log (Nm) because of the large number of population pairs for which Nm was 0; however, this reduced the strength of the regressions. For example, using data for the entire in-group and excluding population pairs for which $Nm = 0$, regression of log(geographical distance) on the x axis against log (Nm) on the y axis gives an r^2 of 0.290, while regression against log ($Nm + 1$) on the y axis gives an r^2 of only 0.192.

Discussion

The extreme mitochondrial sequence divergence (up to 23%) found in this study among conspecific populations is remarkable, but it was not unexpected for this species. Over a smaller geographical scale, interpopulation allozyme divergence has long been known to be about an order of magnitude higher than in most species (e.g. Burton *et al*. 1979; Burton 1994; Ganz & Burton 1995) and mitochondrial differentiation has more recently been shown to exceed 18% (Burton & Lee 1994; Burton 1994, 1998). What is novel about the present study is that when geographical sampling was extended northwards, these new populations exhibited

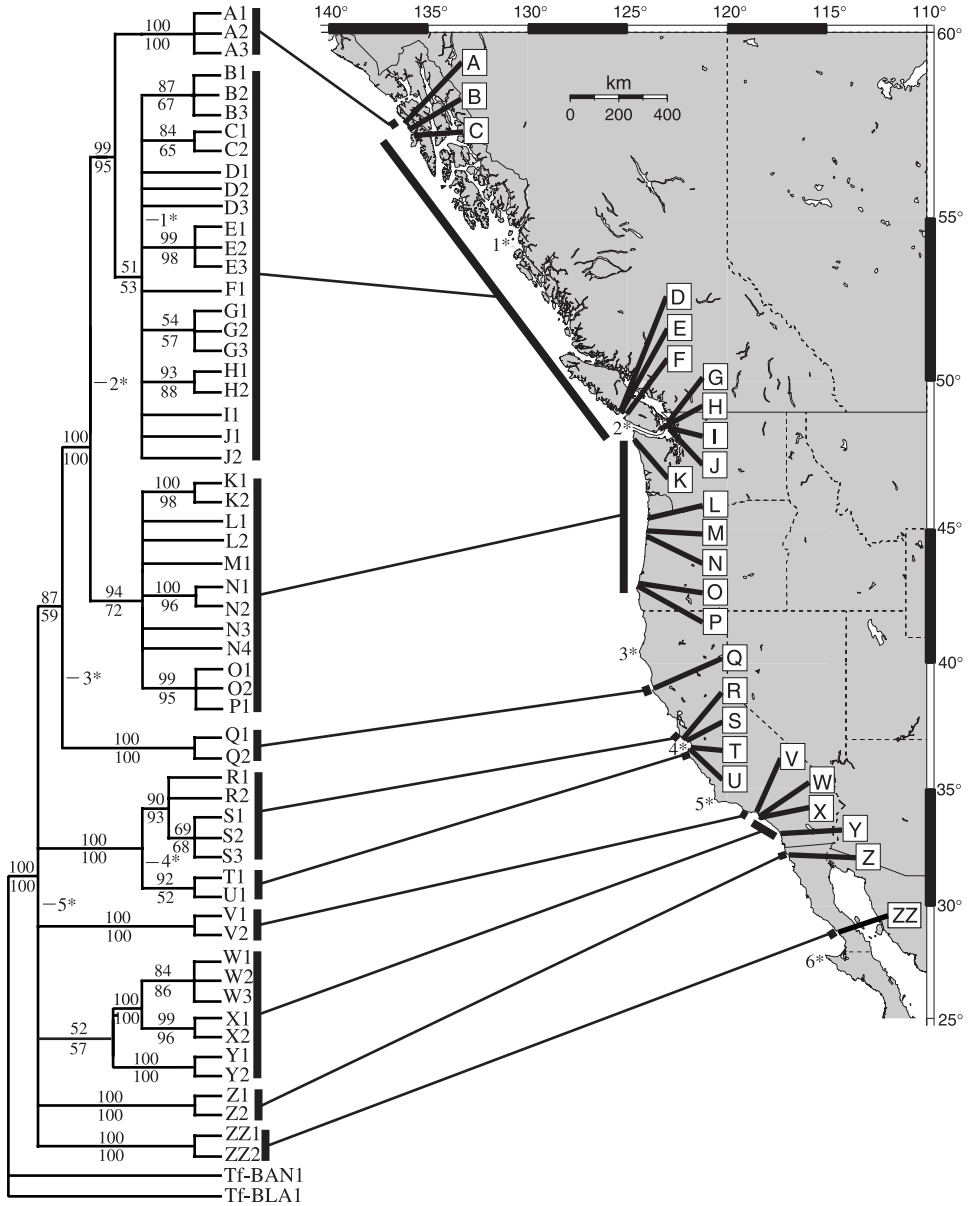

Fig. 2 Phylogeny based on cytochrome oxidase I DNA sequence data (552 bases) in *Tigriopus californicus* individuals from 27 localities. Five hundred bootstrap replicates were performed by PAUP* 4b1 using both parsimony and distance (minimum evolution) methods. Only groupings with at least 50% bootstrap support by both methods are shown. Parsimony bootstrap numbers are below branches; distance bootstrap numbers are above branches. The asterisks mark biogeographic boundaries recognized by Valentine (1966): (1) Dixon Entrance (2) Puget Sound (3) Cape Mendocino (4) Monterey Bay (5) Point Conception (6) Punta Eugenia.

a very different pattern. Sequence divergence per kilometer was five times lower in populations from northern California to Alaska than in previously sampled populations from central California to Baja California. This pattern is reminiscent of that found in other coastal marine invertebrates in the same region. For example, a study of two sibling species of *Nucella* snails (Marko 1998) showed that one species (*Nucella emarginata*) had considerably higher phylogeographic structure in southern California and Baja California than in central California, while the other species (*Nucella ostrina*) had greater population structure in central California than in northern California to Alaska.

There are several possible (not mutually exclusive) explanations for reduced population differentiation at northern latitudes. One is that higher exposure to UV radiation may elevate mutation rates in southern populations. Initial support for this hypothesis comes from the finding that full-sibling mating caused greater inbreeding depression in two southern populations than in one northern population (Palmer & Edmands 2000), a pattern consistent

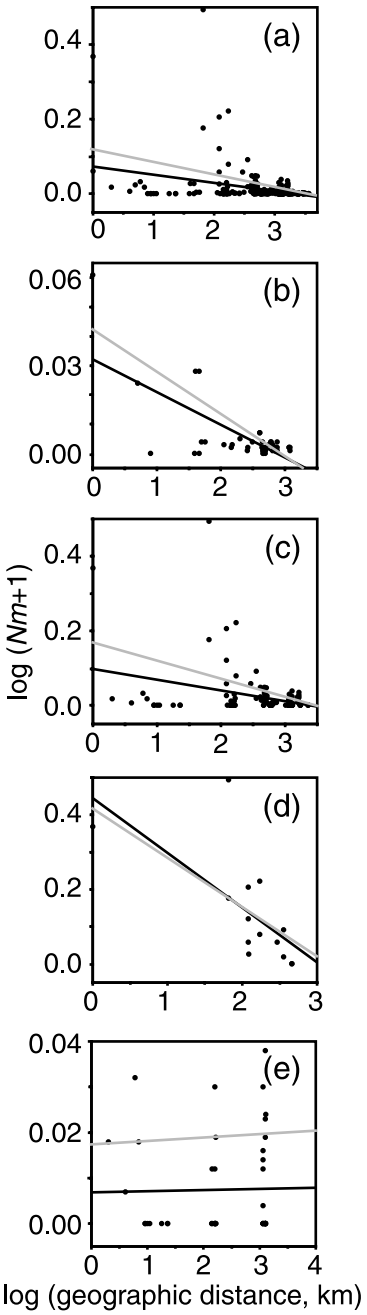

Fig. 3 Relationships between distance of separation (log geographical distance) and gene flow [log (*Nm* + 1)] over different spatial scales. Regression lines are shown for all pairs of populations ('all': solid lines) and after removing populations pairs for which *Nm* = 0 ('rem': stippled lines). (a) Entire in-group, all: $y = 0.073 - 0.022x$, $r^2 = 0.112$, $P = 0.0001$, $n = 349$; rem: $y = 0.116 - 0.035x$, $r^2 = 0.192$, $P = 0.0001$, $n = 246$. (b) Populations R-ZZ, all: $y = 0.032 - 0.011x$, $r^2 = 0.486$, $P = 0.0001$, $n = 45$; rem: $y = 0.042 - 0.015x$, $r^2 = 0.709$, $P = 0.0001$, $n = 39$. (c) Populations A-Q, all: $y = 0.097 - 0.029x$, $r^2 = 0.1$, $P = 0.0002$, $n = 134$; rem: $y = 0.174 - 0.052x$, $r^2 = 0.213$, $P = 0.0001$, $n = 81$. (d) Populations K-P, $y = 0.445 - 0.147x$, $r^2 = 0.452$, $P = 0.0085$, $n = 14$; rem: $y = 0.425 - 0.133x$, $r^2 = 0.381$, $P = 0.0324$, $n = 12$. (e) Populations A-J, $y = 0.007 + 2.607 \times 10^{-4}x$, $r^2 = 0.0004$, $P = 0.8922$, $n = 44$; rem: $y = 0.018 + 0.001x$, $r^2 = 0.008$, $P = 0.7386$, $n = 17$.

with higher mutational loads in the south. A second possible explanation for reduced differentiation at high latitudes is that northern populations have had fewer generations to diverge due to slower development times. In *Tigriopus californicus*, as in many ectotherms, generation time is inversely correlated with temperature (Vittor 1971).

A third potential explanation is that postglacial expansions and contractions depleted genetic differentiation among northern populations. This is a common and much-discussed pattern in marine, freshwater and terrestrial organisms (e.g. Reid 1990; Hewitt 1996; Avise & Walker 1998; Bernatchez & Wilson 1998). During the Pleistocene, glaciers retreated and advanced up to 20 times (Martinson *et al*. 1987). At the glacial maxima (~18 000 years ago), sea levels decreased by as much as 100 m and North American ice sheets extended down as far as 40° N (Avise & Walker 1998; Bernatchez & Wilson 1998). On the Pacific coast, extensive and continuous glaciation extended down to at least the southern limit of Puget Sound (Pielou 1991; Lindstrom *et al*. 1997). While less is known about marine species, terrestrial species are generally thought to have been forced into refugia to the north in Beringia and to the south in Cascadia and California, with a few species persisting in refugia within the ice sheets (Pielou 1991; Lindstrom *et al*. 1997).

In *T. californicus*, postglacial recolonization may have involved three events: colonization of northern California (population Q), followed by expansion into Oregon through the outer coast of Washington (clade K-P) and into Puget Sound through southeast Alaska (clade A-J). If the phylogeographic patterns in the north were driven by recent glaciations, then the separation date for the two northernmost clades (A-J and K-P) might be expected to fall within the Pleistocene (about 2 million to 10 000 years ago, Thompson & Turk 1997). Because there is no fossil record for *Tigriopus*, the best available means of calibrating its molecular clock is arguably Knowlton *et al*. (1993) data for geminate species of shrimp on either side of the Isthmus of Panama. This gives an estimated mitochondrial substitution rate of 2.2–2.6% per million years for species spanning a similar range of divergence (6–20%). Using the same distance measure (Kimura's corrected percent sequence divergence, Kimura 1980), the separation time between *T. californicus* groups A-J and K-P is estimated to be between 1.8 and 2.1 million years. This places the separation date near the beginning of the Pleistocene. It has been argued, however, that divergence times should be corrected for variation within clades. This can be done using the procedure described by Avise & Walker (1998), in which net sequence divergence between phylogroups A and B is estimated as $p_{AB(net)} = p_{AB} - 0.5(p_A + p_B)$, where p_A and p_B are the mean sequence divergence among individuals within groups A and B, respectively, and p_{AB} is the mean sequence divergence between individuals in the two groups. This correction reduces the

separation time for groups A-J and K-P to between 1.3 and 1.6 million years.

Estimating separation times requires invoking a molecular clock, a practice that is fraught with peril under even the best of circumstances due to variable evolutionary rates within and among genes and lineages (e.g. Ayala 1997). The situation is particularly perilous in *Tigriopus* because there is reason to believe its clock may be ticking at an unusually high rate. For example, *T. californicus* populations only about 10 km apart on Santa Cruz Island have greater than 17% mitochondrial divergence (Burton 1998), which translates into between 6 and 8 million years of divergence according to a standard molecular clock (Knowlton *et al*. 1993). It is very difficult to imagine these and other geographically proximal *T. californicus* populations remaining isolated over these time scales in the face of large fluctuations in sea level. An alternative explanation is that *T. californicus* populations have elevated divergence rates, due perhaps either to high mutation rates (caused by deficient proofreading enzymes?) or to frequent bottlenecks in their ephemeral pool habitats which may accelerate the process of genetic drift. In any case, the estimated separation date should be treated with more than the usual dose of scepticism, but still presents a benchmark for further investigation. However, even if the *T. californicus* clock is ticking an order of magnitude faster than the standard clock, and geographically proximal southern populations have remained isolated for only hundreds of thousands of years, it is not easy to explain how such an organism could have rapidly colonized over 2000 km of northern coastline since the last glacial retreat approximately 12 000 years ago (Pielou 1991). One hypothesis is that dispersal is more frequent than it appears, but that gene flow between divergent (southern) populations is thwarted by reduced fitness in hybrid offspring (e.g. Burton 1990; Edmands 1999).

Range contractions and expansions typically deplete within-population variation, in addition to between-population variation. For example, postglacial expansions are thought to have caused the reduction in allozyme heterozygosities found in northern populations of cup corals (Hellberg 1994) and marine snails (Marko 1998). Because the present study focused on screening few individuals from many populations, and because the mitochondrial gene used showed almost no variation within populations (overall F_{ST} was 0.98), there is little power to detect patterns in within-population variation. Still, even with this limited data set the northernmost clade (A-J) showed significantly lower intrapopulation divergence than either the next clade to the south (K-P) or the southernmost group (R-ZZ). Stronger tests of the hypothesis that northern populations have reduced variation will require molecular markers that segregate within populations, and efforts are underway to develop such markers (microsatellites and amplified fragment length polymorphisms).

The genetic discontinuities found in this study correspond only very roughly with established biogeographic boundaries (e.g. Valentine 1966). While four out of the five biogeographic boundaries within the species range were concordant with genetic breaks, a number of genetic breaks of equal or greater magnitude occurred in the absence of provincial boundaries. Burton (1998) warned of the dangers of attempting to test concordance with limited geographical sampling. Initial studies suggested that *T. californicus* was one of the only organisms shown to exhibit clear genetic differentiation across the Point Conception Boundary (Burton & Lee 1994). An attempt to hone in on the boundary point revealed that geographically intermediate sites grouped neither with the southern clade nor the northern clade, but instead formed three new clades (Burton 1998). Further work is needed to determine if the genetic breaks found at Monterey Bay, Cape Mendocino and Puget Sound are artefacts of limited sampling.

For four out of the five spatial scales tested, gene flow between populations showed a significant negative correlation with geographical distance, as expected if dispersal is primarily between adjacent localities, and gene flow and genetic drift have equilibrated (Slatkin 1993). This relationship however, explains only 10–49% of the variation. The lack of perfect correspondence can be attributed to a number of factors (e.g. Hellberg 1994) including natural selection, limits to the power of the genetic markers or the regression model, violation of the assumptions regarding mating system or linear distribution of organisms, long distance dispersal, or lack of equilibrium between gene flow and drift. Perhaps most interesting is the one case in which there was no correspondence whatsoever between gene flow and distance—the northernmost clade which includes populations spanning over 1200 km from Puget Sound to Alaska. The most obvious potential explanation for this lack of pattern is that recolonization of this northern region has occurred too recently to allow gene flow and genetic drift to equilibrate. Surveys of more rapidly evolving molecules, currently under development, may provide further fuel for the hypothesis that the patterns observed in the mitochondrial phylogeny are driven by recent climatic change.

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This work is part of an ongoing project focusing on the genetic basis of outbreeding depression and incipient speciation in the copepod *Tigriopus californicus*. The phylogeny study was begun while the author was a postdoctoral fellow in Michael Lynch's laboratory at the University of Oregon, and completed while the author was an Assistant Professor at the University of Southern California.
