Climate-Driven Range Expansion and Morphological Evolution in a Marine Gastropod

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Little is known about the phenotypic consequences of global climate change, despite the excellent Pleistocene fossil record of many taxa. We used morphological measurements from extant and Pleistocene populations of a marine gastropod (*Acanthinucella spirata*) in conjunction with mitochondrial DNA sequence variation from living populations to determine how populations responded phenotypically to Pleistocene climatic changes. Northern populations show little sequence variation as compared to southern populations, a pattern consistent with a recent northward range expansion. These recently recolonized northern populations also contain shell morphologies that are absent in extant southern populations and throughout the Pleistocene fossil record. Thus, contrary to traditional expectations that morphological evolution should occur largely within Pleistocene refugia, our data show that geographical range shifts in response to climatic change can lead to significant morphological evolution.

Pleistocene [1.8 million to 10,000 years before the present (yr B.P.)] climatic fluctuations caused dramatic shifts in the geographic distributions of many species, both terrestrial and marine (*1*, *2*). The effects of these range fluctuations on genetic population structure and speciation have received much attention (*3*–*6*), but little is known about their phenotypic consequences. Here we investigate the effects of Pleistocene climatic changes on evolution in a Californian marine gastropod, *Acanthinucella spirata* (Blainville, 1832).

Fossiliferous late Pleistocene terraces are common along the coast of California and preserve over 77% of the shallow-water molluscan species still living in the region (*7*). This fossil record shows that the geographic distributions of many shallow-water Californian mollusks changed in response to late Pleistocene climatic shifts (*2*, *8*, *9*). The excellent preservation of mollusks in these terraces provides a unique opportunity for directly measuring morphological changes associated with the Pleistocene range shifts.

A. spirata is a common intertidal carnivorous gastropod that presently ranges from Tomales Bay, California (38.2°N), to Punta Baja, Baja California (29.9°N). Juveniles emerge directly from egg capsules attached to rocks (*10*), without an intervening planktonic larval stage; hence the dispersal ability of *A. spirata* is low and the potential for population differentiation is high. Previous work revealed a difference in shell morphology between Pleistocene and Recent populations, suggesting a climatically driven late Pleistocene recolonization of the northern part of the species' range from a southern refugium (*11*).

To test this phylogeographic prediction, we collected individuals from 14 populations of *A. spirata* between San Diego and Tomales Bay, a distance of over 1050 km (*12*). A 660–base pair fragment of the mitochondrial gene encoding cytochrome c oxidase subunit I (COI) was amplified and sequenced from 117 individuals (*13*). The 33 unique haplotypes (GenBank accession numbers AY017492-AY017524, AY027511-AY027516, and AY027687-AY027694) define three well-supported (parsimony and neighbor-joining bootstrap support >95%) clades: one to the north of Point Fermin (in southern Los Angeles County) and two to the south (Fig. 1). Only the Point Fermin population contains haplotypes from all three clades, supporting the hypothesis that the Los Angeles region may have served as a refugium during late Pleistocene cooling episodes (*11*). Genetic diversity (as measured by the mean number of pairwise differences between individuals) in the Point Fermin population is also more than twice as high as in any other population. The phylogeographic break between northern and southern clades (Fig. 1) coincides geographically with one previously reported in a teleost fish (*14*) but lies well south of the major biogeographic boundary at Point Conception (*15*–*18*).

The most striking pattern in the genetic data is a steep decline in genetic diversity from south to north (Fig. 1). Each of the six southernmost populations has a different

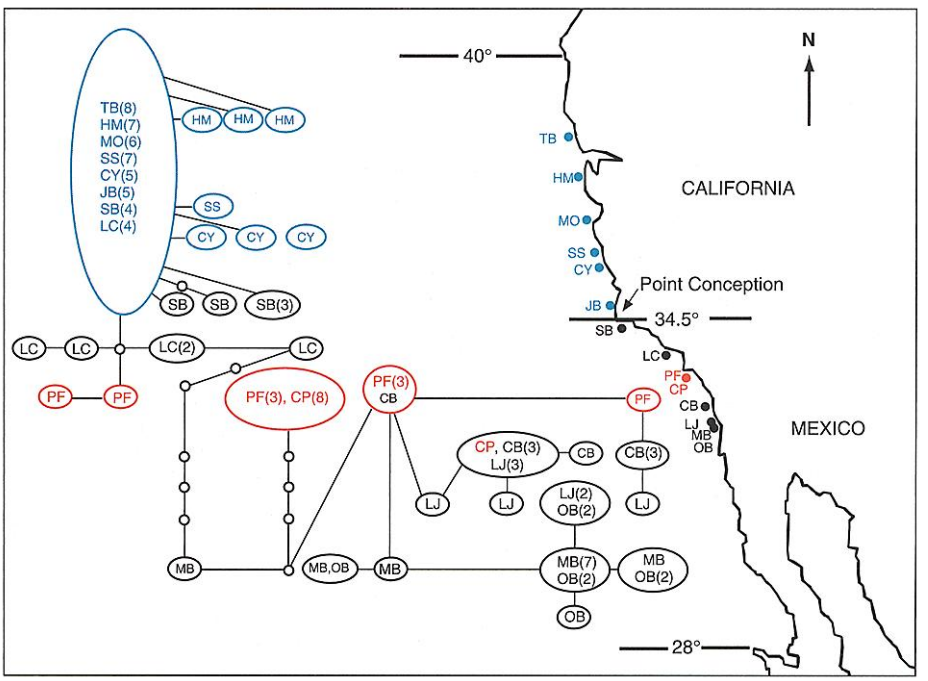

Fig. 1. Parsimony network for 33 unique mitochondrial COI haplotypes obtained from sampling 117 *A. spirata* from 14 populations from along the coast of California. Numbers in parentheses indicate the number of identical haplotypes from a locality. Open circles indicate inferred mutational steps between sampled haplotypes. Populations are as follows. North of Point Conception (shown in blue): Tomales Bay (TB), Half Moon Bay (HM), Monterey Bay (MO), San Simeon (SS), Cayucos (CY), and Jalama Bay (JB). South of Point Conception: El Capitan State Beach, Santa Barbara (SB); Leo Carillo State Beach (LC); Point Fermin (PF) and Cabrillo Beach (CP), both in San Pedro (shown in red); Carlsbad (CB); La Jolla (LJ); Mission Bay (MB); and Ocean Beach (OB).

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most-frequent haplotype, but a single most-common haplotype is shared by all eight populations north of Point Fermin, which together span a geographic distance over four times greater than that covered by the six southernmost populations (Fig. 1). The decline in genetic diversity is even more dramatic across Point Conception. In populations of *A. spirata* north of this boundary, no haplotype other than the most common one occurs more than once (Fig. 1). Results from an analysis of molecular variance (AMOVA) (*19*) show that populations north of Point Conception are not subdivided, whereas among-population variation accounts for a large proportion (72%) of the total variance in populations south of Point Conception ($P < 0.001$, Table 1).

The decline in nucleotide diversity north of Point Conception could result from either a recolonization of that part of the range from a southern refugium or from a selective sweep. For *A. spirata*, the range expansion hypothesis is supported by multiple lines of evidence as follows. (i) Phylogenetic rooting of *A. spirata* haplotypes using two closely related species (*20*), *A. paucilirata* (GenBank accession number AY017490) and *A. punctulata* (GenBank accession number AY017491), as outgroups shows that the southern clades are ancestral to the northern clade (*21*). (ii) Pairwise haplotype differences in the populations north of Point Conception show a unimodal decline from a peak at zero differences, whereas the southern populations show a more ragged distribution (Fig. 2), a difference consistent with a recent and rapid population expansion north of Point Conception (*22*). (iii) Several other temperate marine and terrestrial species found in habitats restricted to the coast of western North America show the same pattern of reduced genetic variation toward the northern end of their ranges (*23*–*25*). Such parallel patterns in different genetic markers in taxa as disparate as birds and mollusks are more likely to stem from similar responses to past climatic changes in the region rather than from multiple independent selective sweeps. (iv) The sharp change in haplotype diversity occurs at Point Conception, a major biogeographic boundary marked by large changes in oceanographic conditions that have a strong influence on present-day species range limits (*15*, *16*, *18*). (v) Specimens of *A. spirata* are present in middle Pleistocene deposits throughout California and are common in late Pleistocene deposits (oxygen isotopic stage 5e, about 125,000 yr B.P.) south of Point Conception (*9*), but they are either absent or extremely rare in late Pleistocene assemblages from the north (*26*). Taken together, these different lines of evidence support the range expansion hypothesis rather than a selective sweep. Furthermore, judging from the extreme homogeneity of northern populations ($\pi = 0.45$, no transversions), this expansion took place quite recently, around 12,000 to 31,000 yr B.P. (*27*).

To investigate the phenotypic consequences of rapid northward range expansion, we measured the shape and size of shells from *A. spirata* individuals from living populations and from middle and late Pleistocene terrace assemblages (*28*). Individuals from all populations north of Point Conception combined differ significantly from southern individuals in both shell shape and size (Fig. 3 and Table 2). In

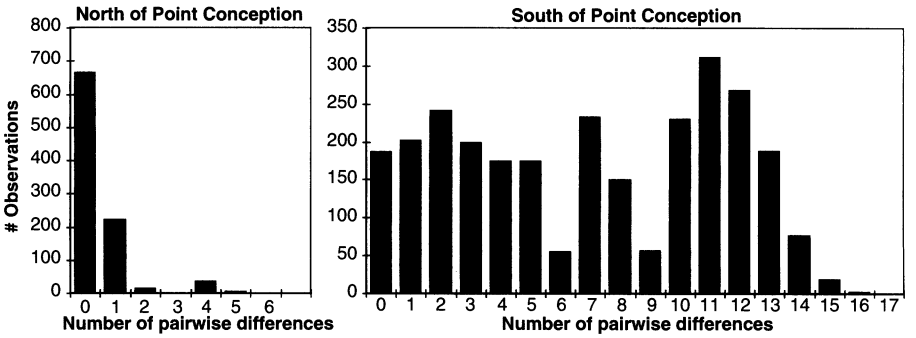

Fig. 2. Distributions of pairwise base pair differences in COI sequences between sampled *A. spirata* individuals north (**left**) and south (**right**) of Point Conception.

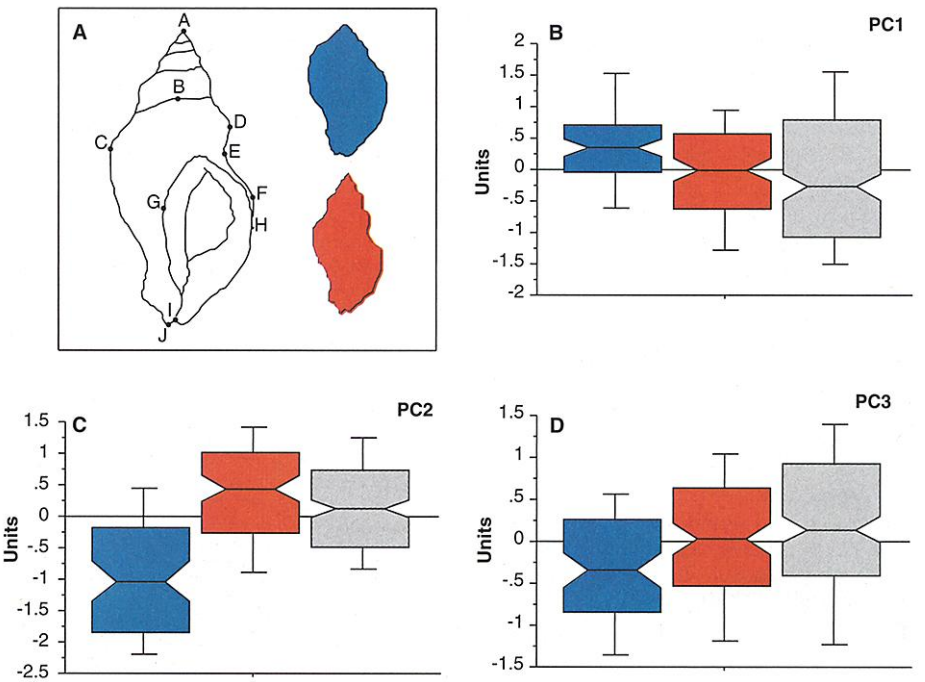

Fig. 3. Spatial and temporal trends in shell shape and size in *A. spirata*. (**A**) The line drawing on the left shows the 10 positional landmarks used in this study. On the right, the blue silhouette is for a northern specimen and the red is for a southern specimen. Drawings are not to scale. Note changes in the relative spire height as well as in the angularity of the whorls. (**B** through **D**) Box plots of the scores on the first three principal component axes. The notches in each box show the 95% confidence interval around the median, and the top and bottom lines show the 90th and 10th percentiles, respectively. The blue boxes represent individuals from all populations north of Point Conception, the red boxes represent all individuals from populations to the south of this point, and the gray boxes represent all Pleistocene individuals. PC1 corresponds primarily to shell size; PC2 and PC3 correspond to various aspects of shape.

Table 1. Genetic variation and population subdivision north and south of Point Conception.

Population	Haplotype diversity (*H*) (*43*)	Nucleotide diversity (π) (*44*)	Variation among populations (%)	Variation within populations (%)
North of Point Conception	0.30 ± 0.09	0.45 ± 0.41	−2.2	102.2
South of Point Conception	0.93 ± 0.01	7.98 ± 3.32	72.0	28.0

terms of shape, the largest north-south difference is along the second principal component axis (PC2), which reflects differences in the relative height of the spire (Fig. 3 and Table 2). Shell shapes and sizes of Pleistocene individuals differ significantly from Recent northern populations combined but not from those to the south of Point Conception (Fig. 3 and Table 2). Most notably, the median PC2 scores for Recent northern populations fall outside the 95% confidence intervals for Pleistocene and for Recent southern populations combined (Fig. 3).

In combination, these genetic and morphological data not only indicate that there has been significant phenotypic evolution of *A. spirata* since the late Pleistocene, with changes concentrated in populations north of Point Conception, but also show that the genetically depauperate recolonized portion of the range is inhabited by morphotypes that are absent or rare in the fossil record of this species (Fig. 3). This significant difference in morphology between the Recent northern populations and the Pleistocene assemblages is unlikely to be due to sampling because (i) sample sizes for the Pleistocene and Recent populations are comparable (179 individuals versus 161 individuals, respectively), and (ii) we collected living individuals only from high intertidal habitats to control for the possibility of depth-related phenotypic variation, whereas the fossil assemblages are time-averaged and presumably drawn from a larger range of depths and microhabitats (*2*) that should inflate morphological variation. Thus, the novel northern morphotypes have either evolved since the expansion of this species' range or alternatively were rare in the past (and hence not preserved in the record) but are currently being favored in the northern part of the range of *A. spirata*. Thus, our data show that extinction-recolonization dynamics associated with climatic change have resulted in significant morphological changes in this species.

A species' response to climatic change can involve shifts in geographic range limits as well as adaptation to new conditions (*2*, *29*, *30*). Phenotypic responses to Pleistocene and Holocene climatic fluctuations have been documented in rodent populations (*31*, *32*) as well as in insular land snails (*33*). The present results demonstrate that phenotypic changes can occur not only as an alternative to a geographical range shift but also in association with a range expansion.

Pronounced morphological change in a recolonized region contradicts the expectation that such changes should largely occur within Pleistocene refugia (*34*). In fact, *A. spirata* from the refugium (the San Pedro region) are unremarkable in terms of morphology. Instead, the picture revealed here accords with ecological studies in which rapid morphological evolution followed experimental (*35*) or anthropogenic (*36*) introductions into unoccupied habitat. Our data suggest that rapid morphological evolution may also be common in nature when changing climatic conditions alter the geographic ranges of species. Such a conclusion is further supported by data from freshwater fishes, in which post-Pleistocene colonization of new habitats has also led to evolutionary divergences (*37*, *38*).

Ongoing studies of biotic responses to global change have either focused on ecological aspects such as changes in community compositions or species associations (*1–4*, *8*) or on genetic changes (*5*, *6*). The phenotypic consequences of global climate change have remained surprisingly neglected despite the excellent Pleistocene fossil record of many vertebrate and invertebrate taxa. Our data show that depending exclusively on genetic markers to judge evolutionary responses to environmental change may provide only an incomplete picture of biotic response. A better understanding of how species respond to climatic changes requires that we integrate ecological, genetic, morphological, and paleontological data.

Table 2. MANOVA of the scores on the first three principal components axes summarizing morphological patterns.

Comparison	Mean difference PC1	Mean difference PC2	Mean difference PC3
Recent north to south	0.407*	−1.26*	−0.37†
Pleistocene to Recent north	0.491*	−1.07*	−0.59*
Pleistocene to Recent south	0.084	0.191	−0.22

*Significant at 5% level or better using both Bonferroni/Dunn and Games-Howell post hoc tests. †Significant using Games-Howell test only.

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12. Adult *A. spirata* were collected from high intertidal habitats between December 1999 and April 2000 by K.R. and/or D.P.B. at localities indicated in Fig. 1. *A. paucilirata* and *A. punctulata* were included as outgroups (*20*). Specimens were preserved in ≥70% ethanol and photographed for morphometric analyses, then crushed to extract tissue.
13. DNA was extracted from muscle tissue using cetyltrimethyl ammonium bromide (CTAB) methods, removing polysaccharide polymerase inhibitors with PhytoPure resin (Amersham). We amplified the DNA using primers HCOI (*39*) and LCOI+ (5'-GTCAACAAAATCATAAAGATATTGGAAC-3') and standard polymerase chain reaction profiles with an annealing temperature of 50°C. Products were directly sequenced in both directions on an ABI 377 sequencer, using the amplification primers. There were no indels and only three nonsynonymous substitutions within *A. spirata* (singletons in the Half Moon Bay, Leo Carillo, and Carlsbad populations). Parsimony networks were obtained with the software TCS 1.01 (D. Posada). Relationships among the three clades revealed by networks were polarized by means of neighbor-joining and parsimony methods (*40*), with *A. paucilirata* and *A. punctulata* (GenBank accession numbers AY017490 and AY017491) as outgroups. Various weighting schemes produced the same results. Analysis of molecular variation (*19*) and pairwise mismatch analyses (*22*) were performed with Arlequin 2.000 (*41*) based on simple pairwise distances.
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28. For living populations, morphological analyses were based on the same specimens as for sequence analysis; in a few localities, these were supplemented by additional specimens collected at the same time. Pleistocene specimens came from collections in the Natural History Museum of Los Angeles County and the California Academy of Sciences. The total number of Pleistocene specimens (179) used was similar to the total of living specimens (161) in order to minimize sample size effects. Ten positional landmarks on each photographed shell (Fig. 3) were used to obtain eight measurements with the image analysis software ImagePro Plus. All measurements were collected by one of us (D.P.B.) to maintain consistency. Morphological measurements were log-transformed, and a principal components analysis (PCA) using a correlation matrix was used to generate the shape morphospace (*42*). Only specimens >1.4 cm in total length were analyzed to control for ontogenetic changes in shell shape. Measurements were not size-standardized, and all data were combined into a single PCA. The first four principal components explained 95.8% of the total variance (PC1 = 80.1%, PC2 = 9.7%, PC3 = 3.9%, and PC4 = 2.1%). A second PCA using a covariance matrix produced similar results. A multivariate analysis of variance (MANOVA) of the principal component scores was used to test for spatial and temporal differences in morphology (Table 2). Further details about the measurements and PCA results are available from the authors.

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Parent-Offspring Coadaptation and the Dual Genetic Control of Maternal Care

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In many animal species, the amount of care provided by parents is determined through a complex interaction of offspring signals and responses by parents to those signals. As predicted by honest signaling theory, we show that in the burrower bug, *Sehirus cinctus*, maternal provisioning responds to experimental manipulations of offspring condition. Despite this predicted environmental influence, we find evidence from two cross-foster experiments that variation in maternal care also stems from two distinct genetic sources: variation among offspring in their ability to elicit care and variation among parents in their response to offspring signals. Furthermore, as predicted by maternal-offspring coadaptation theory, offspring signaling is negatively genetically correlated with maternal provisioning.

Parent-offspring conflict occurs because the fitness of parents and the fitness of individual offspring are maximized at different levels of parental investment (*1*, *2*). A variety of models predict that the evolutionarily stable (ESS) level of parental care lies somewhere between the values that maximize parent and offspring fitness (*2*, *3*). Offspring influence the ESS because parents respond to signals produced by offspring. In most circumstances, parents are expected to respond to offspring signals, either active or passive, that reliably indicate condition (*4*–*7*). Differences among offspring in the signals they produce are typically thought to result from environmental influences on condition. The nonexclusive possibility that signaling differences among offspring are genetically based has been relatively unexplored (*8*).

The behavioral interaction between parents and offspring generates a complex form of inheritance wherein genes in two individuals can influence the phenotype of a single trait, parental care (*9*–*11*). Parent-offspring conflict theory has focused on evolutionary endpoints rather than processes primarily because of ". . .the immense difficulty in understanding the genetics underlying parent-offspring conflict" (*2*). Consequently, even the most fundamental genetic assumptions, such as the existence of genetic variance for both parental and offspring components, remain largely untested (*8*). Furthermore, important theoretical predictions cannot be evaluated without measuring other aspects of the genetic architecture of parental care. When selection favors an intermediate level of parental care, many combinations of parent and offspring genotypes are equally fit (*10*, *11*). This selection for maternal-offspring coadaptation is expected to generate a negative correlation between the genetic components expressed by parents and offspring (*12*).

Burrower bugs (*13*) (*Sehirus cinctus*, Hemiptera: Cydnidae) exhibit maternal care (*14*–*16*). Females lay clutches of approximately 40 to 150 eggs in shallow burrows in the soil. A female guards her clutch for about 10 days until the eggs hatch. At that time, she begins collecting small mint nutlets (*Lamium* spp.) that she deposits in the burrow to provision her offspring (Fig. 1). Provisioning is not directed to individual offspring, but rather to the clutch as a whole, and continues through the end of the second stadium (about 10 days after hatching). Care is obligate; unprovisioned clutches do not survive (*17*). Although specific offspring signals have not been identified, the influence of offspring on provisioning can be observed. Because a female can produce multiple clutches within a season, it is possible that maternal care expended on one clutch reduces a female's residual reproductive value (*18*).

Parental and offspring influences on provisioning are difficult to disentangle because of covariances that are expected to exist between parents and their offspring (*9*, *10*, *19*). Cross-fostering [Experiments I and II (*20*, *21*)] eliminates genetic and phenotypic sources of covariance between parents and the offspring for which they care (*22*, *23*). We cross-fostered clutches after egg deposition, so our design may not completely eliminate potential pre-hatching sources of covariance. By splitting clutches [Experiment II (*21*)], we could detect the effect of offspring signaling on maternal provisioning averaged over multiple unrelated maternal genotypes. Using these techniques, we show that burrower bug (i) females respond to offspring signals, (ii) females respond differently to offspring whose condition has been experimentally reduced, (iii) offspring vary genetically in their ability to elicit provisioning, and (iv) maternal and offspring genetic components of provisioning are negatively correlated.

If females respond to offspring signals and if signaling intensity increases with offspring number, then females rearing larger clutches should provision more than females rearing smaller clutches (*24*). However, a relation between clutch size and provisioning could exist simply because females that produce large clutches are also better providers. We tested these alternatives by evaluating the influence of biological and foster clutch size on provisioning in 138 cross-fostered mother-offspring pairs (*20*, *25*). Consistent with the offspring signaling hypothesis, foster clutch size had a significant effect on provisioning (t test: $t = 6.78$, df = 135, $P < 0.0001$), whereas biological clutch size did not ($t = -0.01$, df = 135).

We performed a separate experiment to determine if offspring condition influenced maternal provisioning. We manipulated offspring condition by removing provisioned

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