

SOUTHERN HOSPITALITY: A LATITUDINAL GRADIENT IN GENE FLOW IN THE MARINE ENVIRONMENT

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In recent years population genetics and phylogeographic studies have become increasingly valuable tools for inferring both historical and present-day genetic patterns within marine species. Here, we take a comparative approach to population-level study, analyzing original mitochondrial DNA data from 969 individuals representing 28 chiton (Mollusca: Polyplacophora) species to uncover large-scale genetic patterns along the Pacific coast of North America. The data reveal a distinct latitudinal connectivity gradient among chitons: species that exist at lower latitudes tend to have more isolated populations. This trend appears to be a product of between-species differences; within species, no significant gradient in connectivity is observed. Lower average annual sea surface temperatures are hypothesized to contribute to longer larval duration (and by extension, greater connectivity) among lecithotrophic species, providing a mechanism for the observed positive correlation between gene flow and latitude. Because increased isolation among populations may lead to speciation, a latitudinal trend in gene flow may contribute to the increased species diversity observed at lower latitudes.

KEY WORDS: Chiton, gene flow, latitude, marine, Polyplacophora.

Population genetics and phylogeographic studies, which have multiplied in the past decade as a result of more easily accessible DNA sequence data, have largely described the geographic distribution of genetic markers for single species. While providing improved resolution and insight into the interplay of geography and genetics, this approach suffers from a myopia: single species patterns say little about the generalities of past and present forces shaping intraspecific variation. The present challenge is to

move beyond individual species patterns and into the realm of comparative population genetics and phylogeography to link specific biological, ecological, and environmental processes (cause) to observed levels of divergence among populations (effect). One response is to sample as many species as possible across a broad geographic area and search for correlations between inferred gene flow and biological or physical factors (Dawson 2001; Wares and Cunningham 2001; Wares 2002).

Recent genetic work suggests that much more biological variation exists in the ocean than was previously believed: “species” once thought cosmopolitan have been revealed as

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genetically diverse but cryptic groups (Knowlton 2000; Dawson and Jacobs 2001; Baker 2003), planktonic larval stages do not necessarily result in panmictic populations (Buroker 1983; Benzie and Stoddart 1992; Kyle and Boulding 2000), selective clines have become apparent in areas with little obvious environmental gradation (Koehn et al. 1980; Sotka et al. 2004), and historically limited gene flow has left patterns apparent long after its cause has disappeared (Benzie and Williams 1997). However, despite many interesting insights into genetic differentiation in the sea, the extent to which these observations reveal generalities—for example, do the findings apply only to a single species, specific taxonomic group, or ecological guild?—remains unclear.

Chitons (Mollusca: Polyplacophora), which are common and distributed widely, provide a useful study system for comparative population genetics and phylogeography. The 28 species analyzed here (see the Appendix) are all intertidal grazers that have lecithotrophic larvae and, as far as is known, free-spawn gametes. Because of their ecological similarity, geography and environmental factors are the primary variables in comparing population structure among these species. The essential question then becomes, *how are genetic discontinuities distributed in space?*

The relative roles of different structuring (i.e., gene-flow-limiting) factors vary for each species: pattern is a result of an amalgam of processes (historical and present, stochastic and deterministic, intrinsic [biological/ecological] and extrinsic [physical/environmental]). Biologists seek to disentangle these to link the causes of population divergence with their effects: intraspecific diversity and ultimately, speciation. Understanding contemporary gene flow is a critical step toward detailing the processes that have resulted in the species present today.

Here, we test the null hypothesis that chiton species with significant population structure are distributed randomly between Alaska and Baja California. The data reveal a distinct positive correlation between species latitude and gene flow. This trend is due to differences between species, rather than among populations within species. We then discuss the possible link between the observed latitudinal gradient and coincident changes in sea surface temperature (SST) and other ecological variables.

Methods

Twenty-eight chiton species (see the Appendix) were sampled along the Pacific coast at varying intensities. A total of 969 individuals in 130 populations was analyzed at the cytochrome *c* oxidase subunit I (COI) mtDNA locus. Sampling intensity averaged 4.6 populations per species ($SD = 2.32$) and 7.52 individuals per population ($SD = 3.54$). To avoid artificially inflating population divergences in the analysis, deeply divided reciprocally monophyletic clades within morphologically identified taxa were

treated as separate species. This was necessary in the cases of *Chaetopleura lanuginosa* clades 1 and 2, and in *Leptochiton rugatus* and *L.* species (see the Appendix).

Collection permits were obtained from the relevant agencies in Mexico, California, Oregon, Washington, British Columbia, and Alaska. Voucher specimens corresponding to each DNA sequence were deposited in the Invertebrate Zoology collection of the Santa Barbara Museum of Natural History and tissue samples of each individual are maintained in nitrogen vapor at -110°C in the Monell Cryo collection at the American Museum of Natural History in New York City.

Genomic DNA was extracted from specimens collected from the field between 2001 and 2005 and kept in 95%–100% ethanol at room temperature. Extractions were carried out using Qiagen Dneasy kits, eluted in water and kept at -20°C for short-term use. In two species, older tissues (two populations of *Nuttallina* spp. Collected and frozen at -80°C by D. Eernisse) were used to supplement recent collections, but the inclusions of these individuals did not affect the outcome of the analyses.

Polymerase chain reaction (PCR) was carried out on the DNA extracts, using standard reagents and the universal COI primers HCO2198 and LCO1491 (Folmer et al. 1994), annealing at 51°C , resulting in approximately 650 bp. This mtDNA locus is a convenient and appropriate way of estimating gene flow among populations and has been widely used for this purpose (Avice 2000, and references therein). In cases of degraded or low-concentration DNA extract, ready-made PCR beads (GE Healthcare, Piscataway, NJ) were used in place of batch-mixed reagents to amplify product. PCR products were cleaned using either 96-well filter plates (Millipore Corp., Billerica, MA) or AmPure beads (Agencourt Corp. Beverly, MA, protocols available from manufacturer).

DNA sequencing was carried out using ABI BigDye Terminator reactions (Applied Biosystems), with an annealing temperature of 50° . Cycle sequence products were cleaned with 70% isopropanol, 70% ethanol, resuspended in formamide, and read on an ABI 3730xl automated sequencer (Applied Biosystems, Inc.). The resulting DNA sequences were verified by aligning reads from both 5' and 3' directions for the majority of individuals, using Sequencher software (GeneCodes Corp.), and managed with MacClade (Maddison and Maddison 2003). Distinct mitochondrial variants for COI were calculated with Collapse 1.2 (Posada 2004) and these are listed in the documentation with separate source sequences including 902 newly deposited in GenBank under accession numbers EF200703–EF201604 and 45 sequences deposited previously (EF159577–88, 94–96, 602–04, 06–09, 17–18, 23, 28–31, 34, 45, 54, 72–74, 82–83, 85–92). Some sequences were shorter than others and were assigned to two or more haplotypes due to collapsing paradoxes.

The degree of subdivision within species was compared using F_{ST} values calculated in an analysis of molecular variance

(AMOVA) framework. This method derives the fixation index from the genetic covariance components among populations, using the number of mutations that distinguish haplotypes (Excoffier et al. 1992). For this analysis, each collection location was treated as a separate population. Under an AMOVA framework, more genetic variance within a population than between populations will produce a negative F_{ST} value. In the context of free-spawning species, these values are biologically meaningless, and were considered to be zero for purposes of data interpretation. The significance of F_{ST} values was determined by permutation testing using 1000 iterations with the level of significance set at $\alpha = 0.05$. All F_{ST} analyses were carried out using Arlequin software (Schneider et al. 2000), based on pairwise genetic distances.

Under the island model of gene flow (Wright 1931), F_{ST} values are directly proportional to the number of migrants exchanged among populations according to the equation: $F_{ST} = 1/(2N_e m + 1)$ for haploid genomes, where N_e = the effective population size and m = the fraction of migrants in a population (Wright 1951; Hudson et al. 1992). However, the assumptions underlying the island model (e.g., an infinite number of populations of equal size, each giving and receiving the same fraction of migrants each generation) are not met by natural populations, and as such the estimation of gene flow according to Wright's equation is problematic (Whitlock and McCauley 1999). The estimates of gene flow among chiton populations provided here are therefore intended to be "ballpark" figures that illustrate the magnitude of differences between species, rather than precise measures of genetically effective migration.

To ensure that no systematic bias was introduced into the analysis as a result of comparing species with different range sizes, a Mantel test (which tests for correlation between two independent matrices, Smouse et al. 1986) was performed on each species to compare genetic versus geographic distance among population pairs using average pairwise distance, π (Tajima 1983; Excoffier 2000), at the COI locus. For all species but one, geographically distant populations are no more likely to be divergent than more proximate population pairs, and thus there is no bias inherent in comparing their genetic patterns among species with different range sizes. The inclusion of the one species that showed a significant relationship between geographic and genetic distance (*Cyanoplax hartwegii*, $r^2 = 0.48$, $P = 0.016$) in the analysis does not substantially change its outcome (see Results). However, to further ensure that sampled latitudinal range is not a confounding factor in the dataset, a regression of species F_{ST} corrected for sampling effort is also presented in Results (Fig. 1B).

Regression analyses, Wilcoxon rank sum tests, and an unpaired t -test were also employed to evaluate the dataset. A regression analysis is used to describe the relationship between an independent and a dependent variable, assuming independence of normally distributed y -values and a fixed set of x -values (Hampton

1994). The coefficient of determination, r^2 , describes the percentage of variance in the y -variable attributable to the x -variable, and is assigned a confidence value from a distribution table of r -values (Hampton 1994). A Wilcoxon rank sum test is a non-parametric method of comparing the magnitude of differences between two paired samples, and assumes that each observation being compared is independent and that both samples are distributed about the same median (Goldstein 1964; Conover 1980); a Mann–Whitney test is the unpaired equivalent of the Wilcoxon rank sum. Finally, an unpaired t -test assumes that the observed variables are independent and approximately normally distributed, and its confidence value assigned from a distribution of t -value probabilities (Hampton 1994).

Results

A marked latitudinal gradient in population connectivity is apparent among the sampled species (Fig. 1). Species with significant F_{ST} values exist at disproportionately low latitudes (Wilcoxon rank sum test; 2-sided $P < 0.005$), whereas all northern species are panmictic. A regression of significant F_{ST} values against average latitude sampled is significant ($r^2 = 0.4963$, $P = 0.016$; corrected for variable latitudinal sampling as in Fig. 1B, $r^2 = 0.37$, $P = 0.04$).

An analysis of the dataset excluding small (two population, $n = 5$) or large ($> five$ populations, $n = 9$) sample sizes does not change the observed trend, though the degree of correlation varies (species with two sampled populations excluded, $r^2 = 0.7637$, $P = 0.005$; species with $> five$ populations sampled excluded, $r^2 = 0.62$, $P = 0.1$). Therefore, the latitudinal gradient inferred from chiton genetics data is not sampling dependent; however more extensive sampling shows the correlation more strongly.

Though accurate estimation of gene flow is difficult to derive from F_{ST} data (Whitlock and McCauley 1999), Wright's approximation is a useful heuristic for comparing levels of connectivity within species. The chiton data suggest that gene flow levels vary over nearly two orders of magnitude between species with significant F_{ST} values, from $N_e m = 4.25$ (*Nuttallina fluxa*) to $N_e m = 0.048$ (*Chaetopleura lanuginosa*, clade 1), where $N_e m$ is the absolute number of migrants between population per generation.

To examine the relationship between latitude and connectivity within species, species having populations both with and without significant pairwise F_{ST} values were evaluated by comparing the normalized latitudinal rank of significant versus non-significant populations. This test revealed that isolated populations within species are not significantly more likely to exist in the southern half of the sampled ranges, though some nonsignificant difference is observed (Mann–Whitney test; $P = 0.12$). Similarly, an unpaired t -test revealed that northern and southern populations within species are equally diverse (π , $P > 0.05$; northern

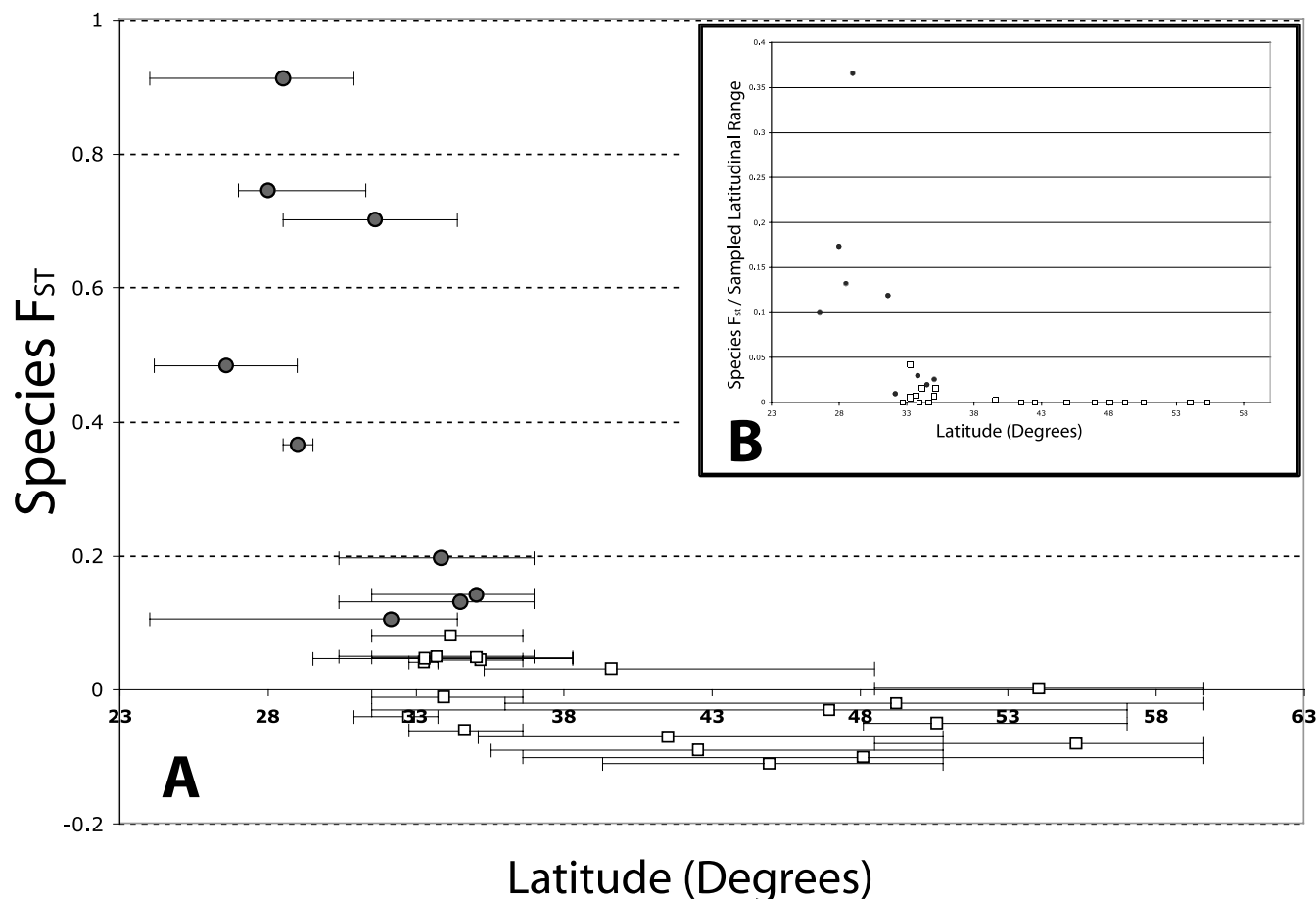


Figure 1. (A) Scatter plot of overall species F_{ST} values against average latitude of sampled populations for 28 species of northeastern Pacific chitons. Values significantly different from zero ($P < 0.05$) are shown as filled-in circles; nonsignificant values are empty squares. Bars indicate the maximal and minimal latitudinal extent of sampling for each species; asymmetrical error bars are the result of unequal sampling effort. F_{ST} values of zero are arbitrarily set to small negative values to show sampling extent. 1B (inset): the same regression, corrected for sampled latitudinal range, shows a similar trend.

half of populations versus southern; species with odd numbers of populations sampled omitted central-most population). Thus the latitudinal connectivity gradient apparent between species is not observed among populations within species.

Although there is no systematic bias introduced into the analysis by comparing species with different ranges (see Methods), there is a correlation between species' sampled range and degree of population structure. Perhaps counterintuitively, species with larger sampled ranges have significantly *fewer* populations with significant F_{ST} values (Wilcoxon rank sum; $P < 0.0005$). Species were sampled approximately proportionally to their ranges: more widespread species were sampled over a greater latitudinal range, and as such, the observed correlation is unlikely to be a sampling artifact. This finding further justifies the comparison of species with varying range sizes: if species with large ranges are *less* likely to be structured, their inclusion would tend to dilute any signal of a trend in population structure, rather than amplifying it.

Discussion

Chiton species are increasingly subdivided along a latitudinal gradient in southern California and on both sides of the Baja California peninsula, whereas species with distributions restricted to central California and the Pacific Northwest appear panmictic. Within species, however, southern populations are neither more isolated nor more diverse than northern populations at the COI mtDNA locus.

From a biogeographic perspective, these results indicate a clear distinction between species with distributions primarily south of Point Conception (34.5°N) and those restricted to the colder waters of the north. Only species with a sampled range extending into Baja California were observed to have significant F_{ST} values; conversely, no species that exists north of San Francisco showed any significant subdivision. However, Point Conception itself appears not to be the primary cause of restricted gene flow in southern species: only three of 11 species sampled on either side of

the putative biogeographic barrier had a significant species F_{ST} value, whereas species predominantly sampled along the Baja California peninsula were much more structured. This pattern of genetic differentiation in southern California and northwestern Mexico has been little noted elsewhere for nearshore marine organisms. Dawson's (2001) review of phylogeographic studies along the west coast did not report a southern California–Baja California break, though some fish species were deserved to have restricted gene flow along the Pacific coast of the Baja peninsula. Edmands (2001) demonstrated unremarkable divergence across the same range in a small number of copepods, though earlier allozyme work suggested high population structure in the same species (Ganz and Burton 1995). Recently, workers on the Pacific coast of the Baja Peninsula have described panmixia in the spiny lobster (García-Rodríguez and Perez-Enriquez 2006) and very low gene flow across the same latitudinal range in a marine plant (Muñiz-Salazar et al. 2005), though no effort to synthesize individual species patterns in the region has been undertaken.

Though other environmental variables vary predictably with latitude, average annual SST is a good candidate to impact population connectivity. Significantly restricted gene flow among chiton populations and SST are negatively correlated ($r^2 = 0.54$, $P = 0.018$). Mitochondrial DNA mutation rates have been shown to vary with temperature and body size (Gillooly et al. 2005 and references therein); however, there is no correlation in the chiton dataset between π and body size (reported length; $P = 0.477$) or temperature (SST; $P = 0.308$). As a result, the observed correlation among population connectivity, latitude, and SST may be due to larval developmental and dispersal differences, though historical climatology, sea surface currents, or selection also might contribute to the trend.

It has long been noted that larval development occurs faster in warmer waters across many taxa (McLaren et al. 1969; Strathmann 1974; Hoegh-Guldberg and Pearse 1995), though much of this type of data derive from echinoderms alone. Although the slopes of the curves describing the temperature–development relationship vary between species even within a group (Hoegh-Guldberg and Pearse 1995), they are uniform in direction. There is relatively little specific information available for the effect of temperature on chiton development, but there is no reason to believe they are an exception to this rule. Faster larval development would result in decreased larval duration, lower dispersal potential, and decreased connectivity among populations.

It is not clear if climatological history, a considerable factor in the genetic patterns of many intertidal invertebrates (Dahlgren et al. 2000; Hellberg et al. 2001; Hughes et al. 2003; Marko 2004), has left a measurable footprint on chiton populations. In general, the habitat destruction brought on by northern glaciers would have resulted in lower genetic diversity in northern populations relative to southern ones (e.g., Govindarajan et al. 2005; Wilson 2006),

yet within species, southern and northern populations are equally diverse. However, because all of the sampled species north of 45°–48°N, the maximum extent of glaciation in the Pleistocene (Dyke and Prest 1987; Blaise et al. 1990; Bernatchez and Dodson 1991), are panmictic, any signal of Pleistocene habitat loss would have since been swamped out by unrestricted gene flow. A diverse founding population upon postglacial reinvasion would also produce the genetic pattern observed in northern populations.

Other chiton genetic data suggest that a seasonal sea surface current pattern may drive genetic discontinuity between southern California and northern Baja and increase F_{ST} values toward the southern end of the sampled range (Kelly 2006). A bifurcation in the dominant current pattern—as part of the California current turns northward to form the southern California countercurrent, the remainder continues south (Hickey 1993; Strub and James 2000)—could disrupt gene flow between southern California and northern Baja among marine animals with planktonic larvae. However, a seasonal collapse of the countercurrent would mollify the current's effect and other such disruptive currents would be necessary throughout Baja California to explain fully the observed genetic pattern.

Selection could also drive the observed trend. If predation on planktonic larvae increases at lower latitudes or if selection opposes free spawning due to prevalent offshore current regimes that sweep larvae out to sea, gene flow among populations would decline independent of temperature or oceanographic mechanisms. Brooding development is unlikely to have been missed in southern species, but because these species are in general poorly known, it is possible that some lay benthic egg masses (see Eernisse 1988) and consequently have limited planktonic duration.

Even a slight change in larval duration can have profound effects on speciation rate in marine invertebrates (Meyer 2003). If chiton species restricted to lower latitudes had consistently shorter larval duration (and by extension, smaller dispersal potential), this would sponsor an increased rate of divergence among populations and ultimately speciation. If extinction did not rise concomitantly so as to negate the increased speciation rate, such a scenario would describe a positive feedback loop in which an increasing number of species in the tropics would result from shorter planktonic larval periods. Moreover, Reitzel et al. (2004) note that water temperature would have a direct selective effect on lecithotrophic larvae, ubiquitous among chitons, because they are nonfeeding.

However, the lack of evidence for a latitudinal effect on gene flow *within* species complicates an argument for a connectivity gradient driven by water temperature and changes in development time. It is not obvious why the level of connectivity should be relatively uniform within species; perhaps larval duration is consistently shorter in species occupying southern latitudes despite a level of temperature-driven environmental plasticity among species at all latitudes. Hoegh-Guldberg and Pearse (1995) argue

that “one of the consequences of attaining the ability to develop at low temperature is an overall slower rate of development,” which is consistent with the idea that larval duration and development is only so plastic.

Notably, the observed pattern differs from the one discussed by Martin and McKay (2004), in which increased genetic isolation among low-latitude populations was observed *within* species and cited as a potential mechanism for increasing species number in the tropics.

Several testable hypotheses arise from the above observations. As data on chiton larval development become available, they should show faster development in warmer waters, though this relationship is not expected to be of equal magnitude across species. If sea surface currents play a major role in structuring chiton populations, those currents are expected to be increasingly less continuous along the southern coast of Baja California and increasingly more continuous to the north of the continent. The relative roles of temperature versus sea surface current in driving the latitudinal connectivity gradient may be parsed by surveying species that brood their young or lay eggs, as these do not disperse planktonically and therefore may be less affected by sea surface currents. Finally, if selection were driving the observed pattern one would expect populations with greater numbers of predators on planktonic larvae and intense offshore current regimes to be more genetically isolated.

Conclusion

Genetic discontinuities in chiton species are not distributed randomly along the Pacific coast. Instead, there is a marked latitudinal gradient in population connectivity among these taxa: southern species tend to be more subdivided. This trend is not driven by within-species differences in connectivity, but rather by differences between species: species with less genetic differentiation among populations tend to live further north, whereas those with greater differentiation among populations are more likely to live further south. This finding is in contrast to earlier work on latitudinal gene flow patterns, which reported within-species variation (Martin and McKay 2004; Sotka et al. 2004). The absence of such a gradient among conspecific populations suggests that, at a broad scale, connectivity is driven by species' characteristics rather than by population-level differences or local geography. Though sea surface currents or climatic changes may also play a role, it seems probable that warmer waters contribute to the observed trend, decreasing connectivity by speeding up larval development, and thus reducing larval dispersal time and distance. If extinction rates were equal across latitudes, such a scenario would contribute to the observed latitudinal species gradient (Roy et al. 1998) as restricted gene flow results in divergence among incipient species. However further tests are necessary to eliminate the possibility the observed latitudinal trend is due to a correlated

trend in selection promoting greater larval retention. Finally it is important to note that, as demonstrated here, a synthetic approach to population genetics can reveal trends that are not apparent from single-species analyses.

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Appendix. Chiton species, individuals, and populations sampled, including the latitudinal range of sampled populations. The overall species F_{ST} is given, with significant values in bold. Gene flow ($N_e m$) is estimated using the equation $F_{ST} = 1/(2N_e m + 1)$, where N_e is the effective population size and m is the proportion of migrants in a population. Taxonomy follows Eernisse et al. (In Press).

Species	Total indivs.	Sampled pops.	Latitudinal sampling (degrees)	Overall F_{ST}	Estimated average $N_e m$
<i>Callistochiton crassicosatus</i>	15	2	36.6–33.75	0.04516	10.57174491
<i>Callistochiton palmulatus</i>	19	3	36.6–31.5	0	N/A
<i>Chaetopleura lanuginosa, Clade 1</i>	37	6	30.9–27.75	0.91322	0.047513195
<i>Chaetopleura lanuginosa, Clade 2</i>	10	2	29.5–28.5	0.36594	0.866344209
<i>Cyanoplax berryana</i>	17	2	37–30.4	0.0504	9.420634921
<i>Cyanoplax dentiens</i>	52	5	48.5–35.3	0.03184	15.20351759
<i>Cyanoplax hartwegii</i>	87	8	37–30.4	0.19709	2.036912071
<i>Cyanoplax keepiana</i>	55	7	34.4–28.5	0.70208	0.212169553
<i>Ischnochiton tridentatus</i>	20	2	29–24.15	0.48463	0.531714917
<i>Katharina tunicata</i>	54	9	59.6–36	0	N/A
<i>Lepidozona cooperi</i>	21	3	36.6–31.5	0.0809	5.680469716
<i>Lepidozona mertensii</i>	25	4	57–31.5	0	N/A
<i>Lepidozona pectinulata</i>	45	6	33.75–30.9	0	N/A
<i>Leptochiton rugatus</i>	29	4	36.6–32.75	0	N/A
<i>Leptochiton sp.</i>	23	4	57–48.1	0	N/A
<i>Mopalia ciliata</i>	25	4	38.3–31.5	0.04877	9.752204224
<i>Mopalia hindsii</i>	35	5	50.8–35.1	0	N/A
<i>Mopalia kennerleyi</i>	30	5	59.6–48.5	0	N/A
<i>Mopalia lignosa</i>	33	4	50.8–35.5	0	N/A
<i>Mopalia muscosa</i>	44	6	38.3–29.5	0.04751	10.02410019
<i>Mopalia plumosa</i>	19	4	37–30.4	0.13165	3.297949107
<i>Mopalia spectabilis/ferreirai</i>	22	2	59.6–36.6	0	N/A
<i>Mopalia swanii</i>	26	2	59.6–48.5	0.00251	198.7031873
<i>Nuttallina californica</i>	44	6	37–31.5	0.14192	3.023111612
<i>Nuttallina fluxa</i>	99	12	33.75–24	0.10534	4.246535029
<i>Stenoplax conspicua</i>	28	4	33.75–29	0.04193	11.4246363
<i>Stenoplax mariposa</i>	33	5	31.3–24.15	0.746	0.170241287
<i>Tonicella lineata</i>	22	4	50.8–39.3	0	N/A