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Color Polymorphism and Genetic Structure in the Sea Star *Pisaster ochraceus*

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Abstract. The sea star *Pisaster ochraceus* is one of the more striking species on the rocky shores of the Northeast Pacific, in part due to the dramatic color polymorphism of the adults. Along the open Pacific coast, *Pisaster* populations are 6%–28% orange, with a small percentage of brilliant purple stars and a large percentage of reddish-brown to dull purple ones. However, populations in the San Juan Island Archipelago (Washington, USA) and the southern Strait of Georgia (British Columbia, Canada) are almost entirely brilliant purple. The factors that maintain the color polymorphism, and those that contribute to among-site variation in color frequencies, remain unknown. We examined the relationships between color frequencies and several ecological and morphological variables, and conducted a large-scale phylogeographic survey of *Pisaster* populations. We found very low population genetic structure, suggesting that gene flow is high and geographic variation in color frequencies is not a vestige of Pleistocene glacial refugia. Color frequencies are also unrelated to adult size and to the frequency of injury within a population. However, there are suggestive relationships between color frequency and diet, and with areas of potentially low salinity. We propose that, although the color polymorphism may have an underlying genetic component, the regional-scale variation in color frequency is ecologically controlled.

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Introduction

Many species of marine invertebrates display color polymorphisms in which the frequencies of different morphs vary among habitats, locations, and times (Wicksten, 1989). In some species, the expression of color variation may be environmentally or ontogenetically controlled, and proximally mediated by age, diet, crypsis, predation intensity, predator identity, or pathogens (*e.g.,* Oetinger and Nichol, 1982; Jormalainen and Tuomi, 1989; Bedini, 2002). In such cases, habitat-specific and geographic variation in the distribution of morphs may reflect variation in the distribution of environmental inducers. However, in other species, coloration may be a heritable trait (*e.g.,* Sabbadin and Graziani, 1967; Palmer, 1985; Ekendahl and Johannesson, 1997; Winkler *et al.,* 2001; Hargeby *et al.,* 2004). Although spatial, temporal, or habitat-specific variation in natural selection can maintain such genetic polymorphisms, genetic drift among historically isolated populations can also be important (Owen, 1974; Tarjuelo *et al.,* 2004).

The sea star *Pisaster ochraceus* (Brandt, 1835) is a significant invertebrate predator along exposed rocky shores of the North American Pacific coast, where it plays a dominant role in structuring communities and regulating species richness (Paine, 1966, 1969, 1974; Menge *et al.,* 1994, 2004; Harley, 2003). It also displays a striking example of marine color polymorphism. *Pisaster* individuals within a few meters of each other may be purple, orange, or a range of intermediate colors. At least two carotenoid pigments, mytiloxanthin and astaxanthin, sequestered in the aboral surface, produce these colors in *Pisaster* and other asteroids. Mytiloxanthin, first isolated from *Mytilus californianus,* presumably accumulates in *P. ochraceus* through feeding (Fox and Scheer, 1941). Astaxanthin is the end product of

several distinct metabolic pathways, and the "relative amounts of any one intermediate product are likely to vary with age or state of nutrition" (Fox and Hopkins, 1966). Although the carotenoid evidence points to environmentally driven *P. ochraceus* coloration, much of the earlier pigment biochemistry in asteroids was not explicitly linked with quantitative descriptions of individual color, diet, or geographic variation. Because individuals of *Pisaster* do not express the color polymorphism as juveniles, and because rearing larvae to adulthood may take 4–6 years (Menge, 1975; Strathmann, 1987), the relative contributions of ontogenetic, environmental, and genetic factors to the expression and spatial distribution of this classic color polymorphism remain unexplored. The potential effects of historical isolation and genetic drift on the distribution of color variation in *P. ochraceus* are likewise poorly understood, as only regional-scale genetic studies have been done on this species (Stickle *et al.,* 1992). On the one hand, the extensive dispersal potential of *P. ochraceus* larvae (Strathmann, 1987) suggests that population genetic structure would be unlikely and historical effects minimal. Nonetheless, phylogeographic discontinuities exist in other species with similarly broad dispersal potential (*e.g.,* diatoms, Rynearson and Armbrust, 2004; barnacles, Sotka *et al.,* 2004; reviewed in Palumbi, 1994; Bohonak, 1999; Grosberg and Cunningham, 2001). Because patterns of genetic structure caused by genetic drift can resemble those caused by selection, we attempt to distinguish these two mechanisms by comparing patterns of variation for traits that are presumably neutral (and subject only to drift) to patterns of variation for traits that may be adaptive.

In this paper, we quantitatively document spatial patterns of color variation, along with size, diet, and injury, within and among populations of *Pisaster ochraceus* sampled from British Columbia southward to central California. We also characterize geographic patterns of genetic structure by examining sequence variation in the mitochondrial cytochrome oxidase I gene in populations from southern Alaska to southern California. We then compare patterns of geographic variation in color to those revealed by the genetic analyses.

Materials and Methods

Phenotypic and environmental data

Study organism. Pisaster ochraceus (hereafter, *Pisaster*) occurs in the lower to mid-intertidal on rocky shores from Alaska to Baja California (Morris *et al.,* 1980). It acquires and stores energy over the course of the summer, converts these reserves to gonad during the winter, and spawns in the spring (Mauzey, 1966). The larvae remain in the plankton for 6 to 8 weeks (Strathmann, 1987), and can reproduce asexually during this time (Vickery and McClintock, 2000). The juveniles are highly cryptic, and little is known about their recruitment dynamics (Menge *et al.,* 2004). *Pisaster* has few natural enemies, although gulls (*Larus* spp.; CH and MW, pers. obs.) and sea otters (*Enhydra lutris;* Riedman and Estes, 1990) occasionally prey upon it. Several pathogens infect *Pisaster,* although their impacts on host population dynamics remain poorly understood (Leighton *et al.,* 1991). *Pisaster* individuals may live for several decades (Menge, 1975).

Sample sites and historical data sources. We recorded *Pisaster* color, diet, size, and prevalence of injury at 15 field sites (Fig. 1, Table 1) in the states of Washington (from 1996 to 2000), California (in 1997 and 2003 to 2004), Oregon (in 2004), and the province of British Columbia (in 2005). We conducted all surveys within one hour of low tide on days when low water was below Mean Lower Low Water (note that this chart datum does not apply in Canada). When we surveyed sites multiple times, we used only the sampling date with the largest census to avoid pseudoreplication of color, size, and injury data. However, we deemed *Pisaster* meals to be sufficiently independent through time that we pooled dietary data across sampling dates to increase our sample size.

To assess the long-term stability of color, diet, and size within a *Pisaster* population, we also used three student reports from one site, Hopkins Marine Station in Pacific Grove, California (Howell, 1947; McBlair, 1947; Forbes, 1951). Howell (1947) and McBlair (1947) both reported *Pisaster* color and size, but as it was unclear to us whether their samples were independent, we used only the larger dataset of McBlair (1947). We also used diet data reported by Howell (1947) and color data reported by Forbes (1951).

Color. We binned *Pisaster* color into three categories: orange, brown, and purple (Fig. 2). The orange hue varied only slightly among individuals, making this the easiest color to distinguish. We defined purple as the brilliant purple common in the protected waters of Puget Sound and the Strait of Georgia. Brown included various intergrading shades of brown, brick red, and dull purple. It is important to note that the "purple" of some other publications (*e.g.,* Raimondi *et al.,* 2007) falls into our brown category. We report a few rare colors, including lavender, black, and the mottled brownish-gray of juveniles, as "other." Because we often found it difficult to distinguish between brown and purple, we made all statistical comparisons using percent orange as the response variable. For comparison with historical data, we combined the "orange" and "ochre" categories of McBlair (1947) into orange; this lumping follows that of the related student report by Howell (1947). We considered what Forbes (1951) described as "yellow, from yellow to orange" as equivalent to our orange. Although it is difficult to categorize what is essentially a continuously variable trait (under a number of potential genetic and

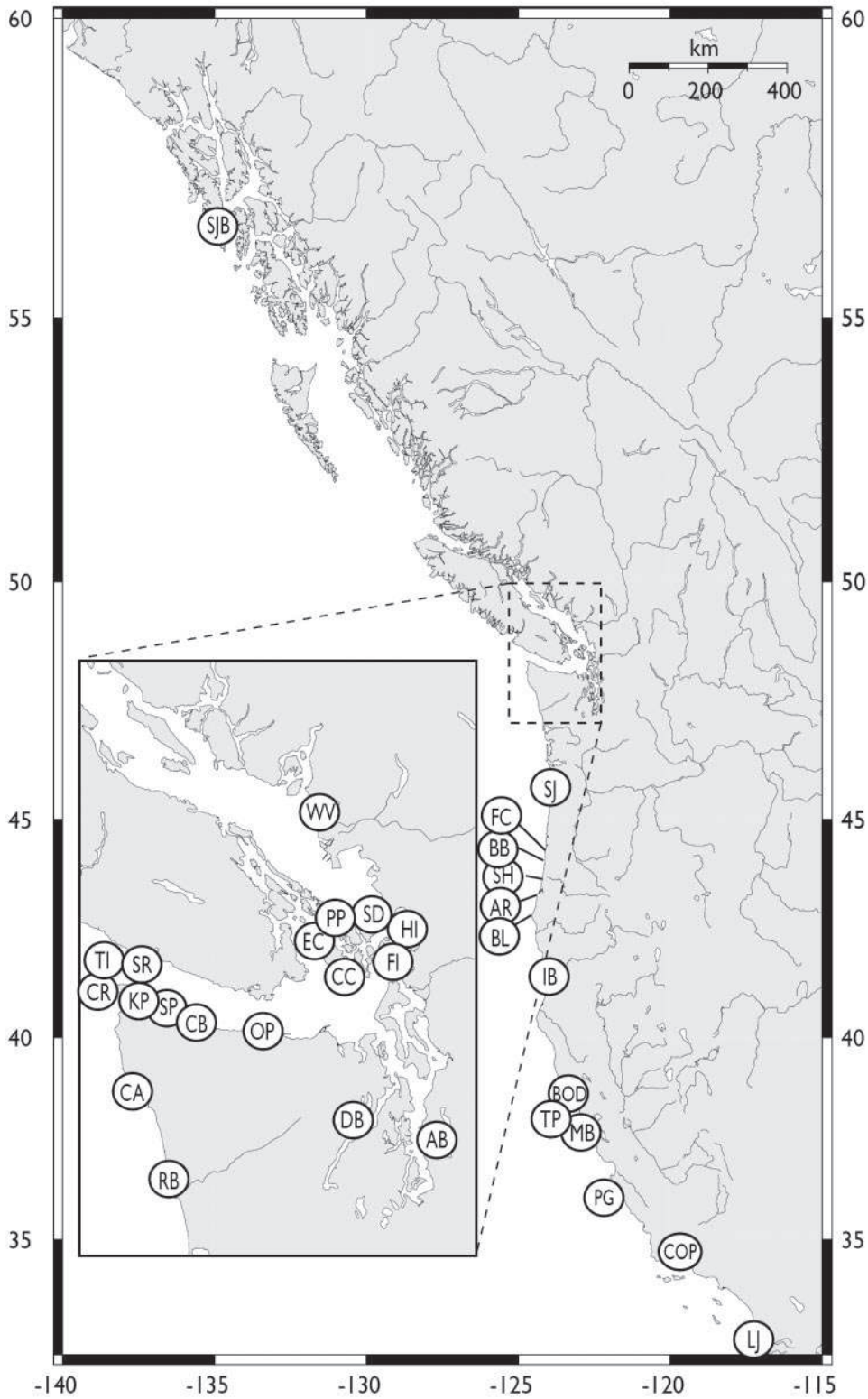

Figure 1. Map of the *Pisaster ochraceus* study region. For site codes and sampling details, see Table 1.

Table 1

Summary of collection data for phenotypic and genetic analysis of Pisaster ochraceus

Collection site	Code	Coordinates	Sample size (*n*)				
			Genetics	Color	Diet	Size	Injury
Saint James Bay, AK	SJB	57.04°N 135.3°W	13	—	—	—	—
West Vancouver, BC	WV	49.26°N 123.2°W	—	59	18	31	31
Fidalgo Island, WA	FI	48.42°N 122.7°W	4	—	—	—	—
Hat Island, WA	HI	48.52°N 122.5°W	26	142	14	140	141
Saddlebag Island, WA	SD	48.54°N 122.6°W	31	44	34	27	36
Shelter Cove (SJA), WA	FHL	48.54°N 123.1°W	12	42	38	42	42
Eagle Cove (SJA), WA	EC	48.49°N 123.2°W	3	—	—	—	—
Pile Pt., WA	PP	48.48°N 123.1°W	—	19	3	19	19
Dabob Bay, WA	DB	47.77°N 122.8°W	23	—	—	—	—
Seattle, WA	AB	47.57°N 122.4°W	21	—	—	—	—
Observatory Pt., WA	OP	48.09°N 123.4°W	—	31	16	27	31
Slip Pt., WA	SP	48.16°N 124.2°W	—	67	40	52	67
Seal Rock, WA	SR	48.36°N 124.5°W	—	45	14	42	45
Chibadehl Rocks, WA	CR	48.39°N 124.7°W	—	163	93	163	159
Tatoosh Island, WA	TI	48.39°N 124.8°W	11	80	21	63	63
Kydaka Pt., WA	KP	48.29°N 124.4°W	8	—	—	—	—
Crescent Bay, WA	CB	48.16°N 123.7°W	11	—	—	—	—
Cape Alava, WA	CA	48.16°N 124.7°W	26	—	—	—	—
Ruby Beach, WA	RB	47.72°N 124.4°W	16	—	—	—	—
Fort Stevens, OR	SJ	46.23°N 124.0°W	26	—	—	—	—
Fogarty Creek, OR	FC	44.51°N 124.0°W	18	—	—	—	—
Boiler Bay, OR	BB	44.50°N 124.0°W	—	157	34	110	111
Strawberry Hill, OR	SH	44.15°N 124.1°W	13	208	23	91	85
Cape Arago, OR	AR	43.34°N 124.4°W	8	—	—	—	—
Cape Blanco, OR	BL	42.83°N 124.6°W	10	—	—	—	—
Indian Beach, CA	IB	41.07°N 124.1°W	11	—	—	—	—
Bodega Bay, CA	BOD	38.36°N 123.1°W	16	157	18	49	78
McClure's Beach, CA	MB	38.18°N 123.0°W	—	151	34	63	63
Hopkins MS, CA	HOP	36.60°N 121.9°W	15	132	39	67	72
Santa Barbara, CA	COP	34.41°N 119.9°W	13	—	—	—	—
La Jolla, CA	LJ	32.85°N 117.3°W	14	—	—	—	—

Dashes (—) indicate no data of a particular type were collected at a site.

environmental controls), cluster analysis using CIE color standards (J. P. Wares, unpubl. data) suggests that our categorization of orange sea stars is robust.

Diet. In field surveys, we scored *Pisaster* diet by overturning sea stars and recording the identity of prey held against the oral disk by the sea star's tube feet. We grouped prey into seven categories: California sea mussels (*Mytilus californianus*); bay mussels (*M. trossulus, M. galloprovincialis,* and their hybrids); goose barnacles (*Pollicipes polymerus*); large acorn barnacles (*Tetraclita rubescens* in Pacific Grove, CA, and *Semibalanus cariosus* elsewhere); small acorn barnacles (*Balanus glandula* and *Chthamalus* spp.); mobile molluscs (limpets, chitons, and whelks); and other, consisting primarily of crabs and annelids.

We scored prey types as present or absent for each sea star, with prevalence of each type expressed as its proportion with respect to all prey records from all sampled sea stars. Since we did not score the abundance of each prey type in each meal, our data represent the proportional occurrence, but not the proportional abundance, of prey types in the diet. Our method thus reflects the behavioral choices made by foraging *Pisaster,* which may, for instance, choose between consuming many barnacles or a single mussel in any given meal. We treated diet data from Howell (1947) and accompanying field notes in a comparable manner. For analysis, we summarized diet in terms of the proportional representation of the mussel *Mytilus californianus, Pisaster's* principal prey. Note that because some sea stars were consuming more than one prey type at a time, our metric slightly underestimates the probability of finding *M. californianus* in any given meal.

Size and injury. We measured *Pisaster* radial size as the distance from the mouth to the tip of the straightest arm, excluding arms that were obviously injured or in the process of regrowing. We recorded size data collected by McBlair (1947) as the average length of the five rays (without the

Figure 2. Purple, brown, and orange morphs of *Pisaster ochraceus* found at Bluestone Point, near the Bamfield Marine Science Centre on Vancouver Island, British Columbia. For statistical analyses, we binned individuals into orange and non-orange (purple + brown + other) categories.

oral disk), plus the radius of the oral disk, both measured to the nearest 2.5 mm. We calculated the radius from McBlair's data by adding one half of the oral disk radius to the mean ray length. Although McBlair's method differed from ours, the complete absence of injured sea stars during our surveys in Pacific Grove, California, suggests that McBlair's averaging technique introduced little bias at this site. We scored individuals as injured if one or more arms was missing or was less than half the length of the longest arm. Because our study sites (with the exception of Hat Island, WA) lacked mobile boulders, injuries likely resulted from partial predation by birds or otters, or damage from drifting debris or from humans.

Statistical analysis. From cluster analysis based on color, diet, size, and injury, three geographic groupings of *Pisaster* field samples emerged (hierarchical clustering using Ward's method in JMP 3.2.6, SAS Institute). We used ANOVA and *post hoc* pairwise comparisons (Tukey-Kramer HSD with alpha = 0.05) to test for differences among geographic clusters for each variable, using sites as replicates for the proportional color, diet, and injury data, and sites nested within region for size. To improve the fit of the residuals to normality, we used an arcsin-square root transformation for all proportional data. To assess historical trends in *Pisaster* phenotype at Hopkins Marine Station, we used contingency table analysis (color, diet) and ANOVA (size) to compare sampling years.

Genetic analysis. We collected about 20–30 podia from individual *Pisaster* at 22 sites from Alaska to central California (Table 1) and preserved tissues in 95% ethanol or a DMSO-enriched buffer (Seutin *et al.,* 1991). To isolate DNA from these tissues, we placed about 5 mg of podia in 600 μl of cell lysis buffer (PureGene DNA Extraction Kit, Gentra) with 2 μl of proteinase-K (Invitrogen, 10 mg/ml), and incubated this mixture at 55 °C for 4 h with agitation. We then extracted genomic DNA following the PureGene

protocol, with final concentrations of 200–600 μg μl^{-1} DNA. We stored isolates at −80 °C.

We amplified a 543-bp portion of the mitochondrial cytochrome *c* oxidase 1 (COI) protein-encoding gene using primers Poch-F(5′-CTAATGATTGGCGCACCAGATA-3′) and Poch-R (5′-GTAGTGAAAGTGGGCAACTACG-3′). We chose this region of COI for its typically high levels of intraspecific variation and its ability to reveal phylogeographic patterns in other species (*e.g.,* Wares and Cunningham, 2005). PCR amplification reactions consisted of 0.02 mmol l^{-1} of each primer, 5 ng DNA, 2.5 mmol l^{-1} of $MgCl_2$, 1 mmol l^{-1} of dNTP, and 0.2 U *Taq* polymerase (Perkin-Elmer), with a 45 °C annealing temperature (35 cycles on a GeneAmp 9700 thermal cycler, Applied Biosystems). We aligned sequences and edited them for ambiguities (low-quality data were considered to have PHRED scores (Ewing and Green, 1998) less than 15 and were scored as ambiguities; most sites had scores >30) with Sequencher 4.2 (GeneCodes Corp., Cambridge, MA).

We first analyzed the COI sequence data for phylogeographic structure using PAUP*4.0*b*10 (Swofford, 2002). Because a large number of individuals shared haplotypes, we transformed the data, using Collapse 1.2 (Posada, 2004), into a set of unique haplotypes. Ignoring ambiguous sites in individual sequences, a heuristic maximum-parsimony search of these data yielded more than 1000 equally parsimonious gene trees, differing only slightly in topology.

We used analysis of molecular variance (AMOVA), implemented with Arlequin 2.001 (Schneider *et al.,* 1997), to characterize the distribution of genetic variation among sites at three scales. First, we calculated standard pairwise F_{ST} among all sites, identifying statistically significant comparisons using the permutation procedure (10,000 replicates) in Arlequin 2.001. Second, we compared values of F_{ST} between pooled sites north and south of Monterey Bay, California, and north and south of Cape Blanco, Oregon. This scale of pooling approximates other studies of genetic differentiation among marine invertebrate populations in the same region (Dawson, 2001). Third, we repeated this analysis, using the geographic clusters identified in our phenotypic analysis as the basis for pooling the genetic data.

We also used the pairwise F_{ST} values to test for equilibrium isolation by distance (Hellberg, 1994; Grosberg and Cunningham, 2001)—a signal of restricted gene flow throughout the species range—by a partial Mantel regression of $\log[\hat{M} = (1-F_{ST})/4F_{ST}]$ *versus* log[distance of separation in kilometers]. In this analysis, a regression slope of −1 is consistent with stepping-stone isolation by distance in a one-dimensional habitat (Slatkin, 1993). Additionally, we calculated for each site two demographic parameters, $\theta(\pi) = 2N_{ef}\mu$ (where N_{ef} is the female effective population size and μ is the substitution rate for the sequenced portion of COI) and Tajima's *D* (Tajima, 1989), using Arlequin. These parameters characterize geographic patterns of diversity; geographical heterogeneity in values of $\theta(\pi)$ would be consistent with a response of *Pisaster* populations to late Pleistocene glaciation, and *D* can be used to assess the potential effects of selection and changes in population size on our estimates of diversity (see Nielsen, 2001).

Results

Phenotypic and environmental patterns

Cluster analysis based on *Pisaster* color, diet, size, and injury revealed three geographic groups of sampling sites: the wave-exposed coasts of California and Oregon; the wave-exposed Olympic Peninsula of Northwest Washington; and the more sheltered waters of the Georgia Strait/Puget Sound, including the San Juan Archipelago and southern British Columbia (Fig. 3; Table 1).

Color frequencies differed among geographic regions: *Pisaster* populations in California, Oregon, and the Olympic Peninsula were 6%–28% orange and 68%–90% brown, whereas those in the Georgia Strait/Puget Sound were approximately 95% purple (Fig. 4). The proportion of orange individuals was lower in the Georgia Strait/Puget Sound than in either of the other two regions (Fig. 4; ANOVA $F_{2.12} = 33.5$, $P < 0.0001$).

Pisaster diet varied among regions, as well (Fig. 5). In California, Oregon, and the Olympic Peninsula, *Mytilus californianus* composed 15%–78% of the diet and was the most prevalent prey item at 8 of 10 sites (Fig. 5). At the remaining two sites (Boiler Bay, OR, and Chibadehl Rocks, WA), small acorn barnacles prevailed. In the Georgia Strait/Puget Sound, where *M. californianus* is absent or rare (Kozloff, 1993), acorn barnacles dominated *Pisaster's* diet (33%–76%), followed by gastropods (9%–39%) (Fig. 5). The one exception to this pattern was West Vancouver, where the bay mussel *M. trossulus* was exceptionally abundant and was the dominant prey. The proportional prevalence of *M. californianus* in *Pisaster's* diet was lower in the Georgia Strait/Puget Sound than in either of the other two regions (ANOVA $F_{2.12} = 29.8$, $P < 0.0001$).

Pisaster individuals were larger in the Olympic Peninsula region than in the Georgia Strait/Puget Sound, and larger in the Georgia Strait/Puget Sound than in coastal California and Oregon (Fig. 6; nested ANOVA, region: $F = 543$, $P < 0.0001$; site(region): $F = 26.9$, $P < 0.0001$). The frequency of injured specimens varied from 0%–14% among sites, but did not differ at the level of regions (Fig. 7; ANOVA $F_{2.12} = 1.34$, $P = 0.30$).

Long-term data from Pacific Grove, California, suggest that *Pisaster* color frequencies, body size, and dietary composition have not appreciably changed over half a century (Table 2). Orange individuals consistently made up 25%–28% of the population, and frequencies did not significantly differ among sampling dates (likelihood ratio $\chi^2 = 0.33$, df = 3,948, $P = 0.95$). Mean individual size ranged from

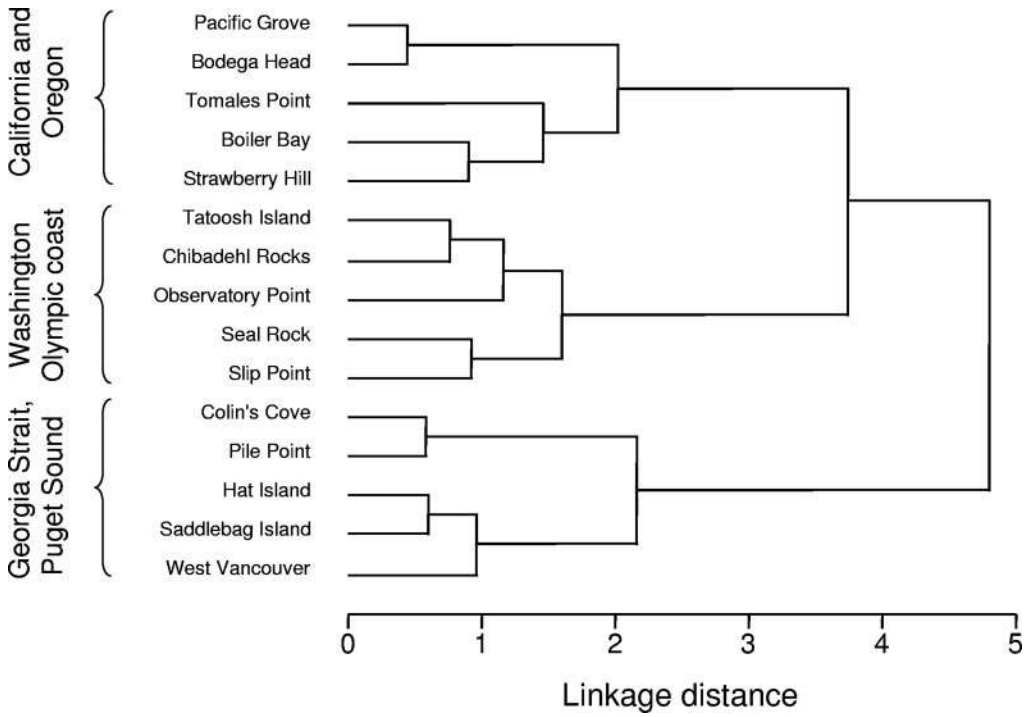

Figure 3. Cluster analysis of *Pisaster ochraceus* color, diet, size, and injury. Three strong clusters emerge: California plus Oregon, wave-exposed sites along Washington's Olympic Peninsula, and wave-protected sites in the San Juan Islands. See Figure 1 and Table 1 for site locations and further details.

8.9 to 9.2 cm, with no statistically detectable differences among sampling dates (ANOVA, $F_{2,216} = 0.44$, $P = 0.64$). *M. californianus* was the most common prey item in all three sampling periods. Its frequency varied almost 2-fold, from 32%–61%, but sample sizes were small and there were no statistical differences among dates (likelihood ratio $\chi^2 = 3.50$, df = 2, 58, $P = 0.17$).

Genetic patterns

From 345 *Pisaster ochraceus* COI haplotypes of 543-bp each (GenBank accessions AY741865–AY742209), only 47 sites were variable (15 parsimony-informative). This variation led to only 43 unique haplotypes, of which one (haplotype A in Fig. 8) was found in 214 individuals across

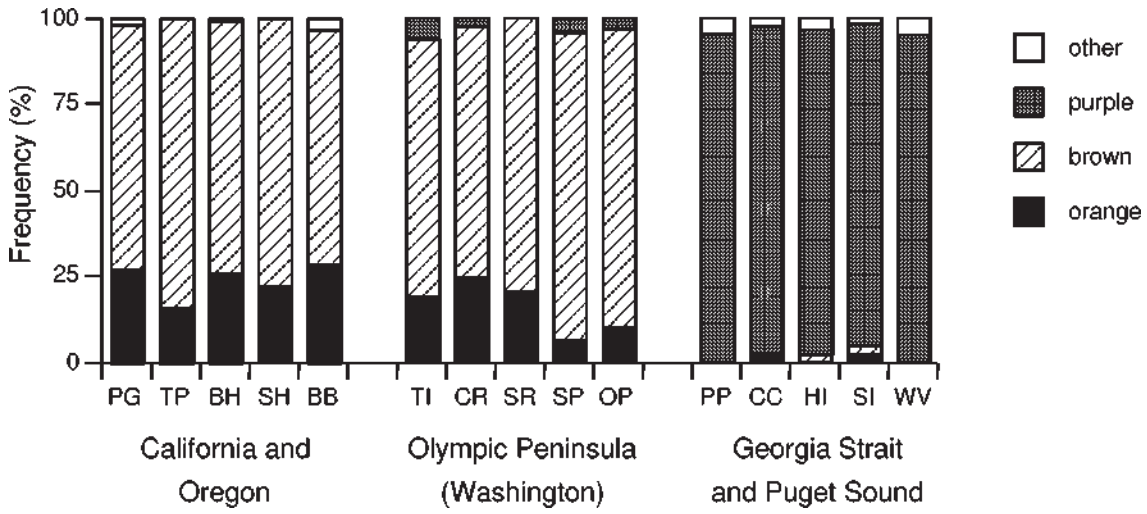

Figure 4. Geographic distribution of *Pisaster ochraceus* color frequencies across sampling sites. See Table 1 for site codes.

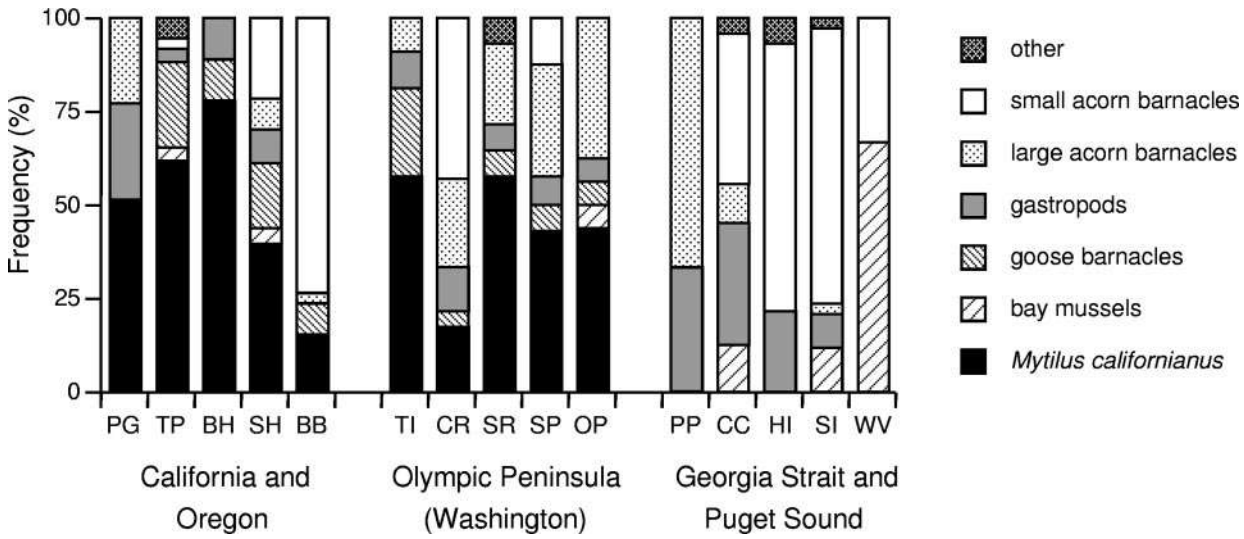

Figure 5. Geographic patterns in *Pisaster ochraceus* dietary composition across sampling sites (not including 1947 data from Pacific Grove). Prey category species are listed in text (Materials and Methods: Diet). See Table 1 for site codes.

all sampled locations. There was no apparent phylogeographic discontinuity in samples from La Jolla, California, to St. James Bay, Alaska, and only the deepest split in the tree (Fig. 8) was supported by bootstrap values higher than 70% (1000 pseudoreplicate fast-heuristic searches). This split did not correspond to the three phenotypically determined geographic regions (Fig. 3) or to phylogeographic and recruitment breaks described for other species spanning Monterey Bay, California, or Cape Blanco, Oregon.

Despite the low level of haplotype diversity (compared to other species sampled across the same range [Sotka *et al.,* 2004]), analyses of molecular variance revealed statistically significant variation in allele frequencies among sites (Table 3). When we grouped sites according to the phenotypically

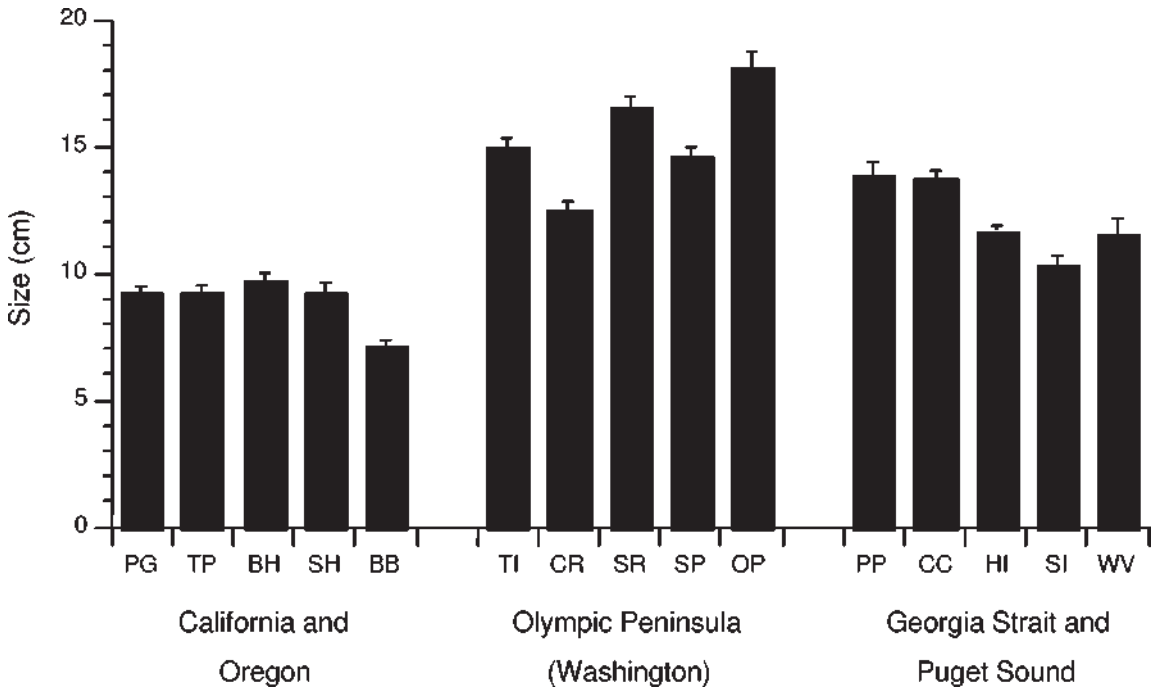

Figure 6. Geographic patterns in *Pisaster ochraceus* size measured from mouth to tip of straightest fully grown arm across sampling sites. Data are means ± 1 standard error. See Table 1 for site codes.

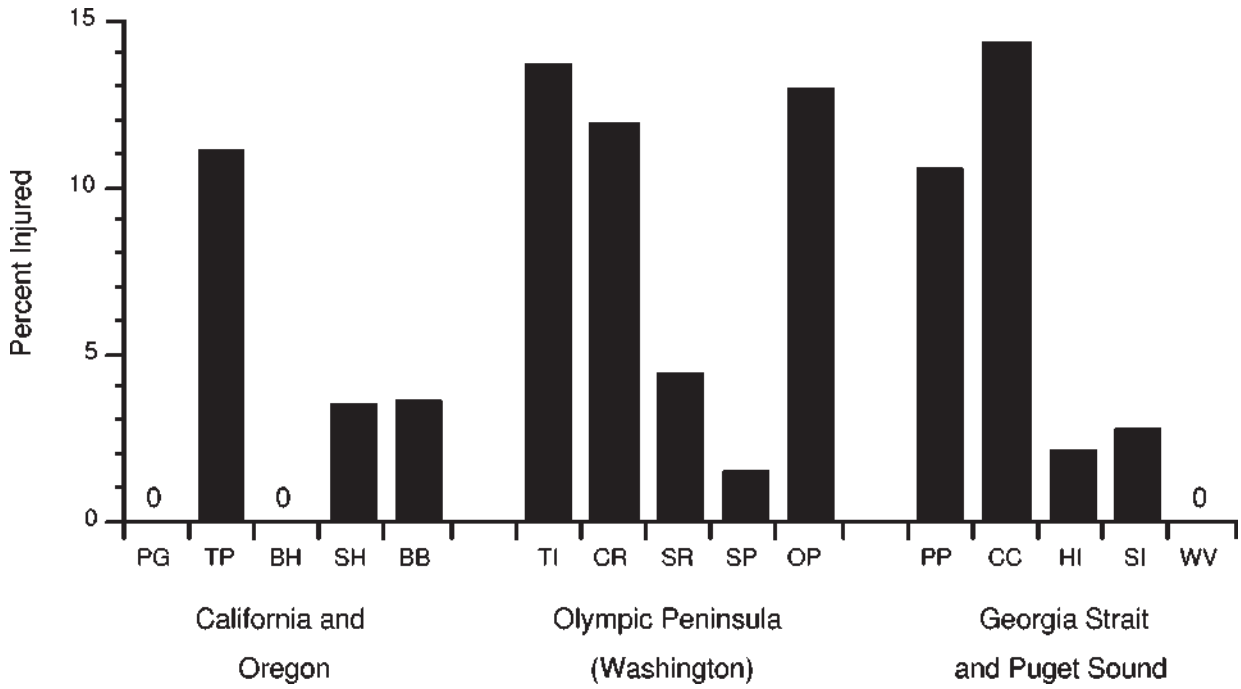

Figure 7. Geographic patterns in *Pisaster ochraceus* injury frequency. Although there were large differences among sites, there were no significant differences among regions. Sites lacking injured sea stars are denoted with "0." See Table 1 for site codes.

defined units, however, the among-group variance components were small and nonsignificant (Table 3). Similarly, when we grouped sites according to whether they were north or south of Monterey Bay and north and south of Cape Blanco, there was no evidence for genetic differentiation. Finally, we found no obvious relationship between individual color and haplotype in these data (results not shown).

The differences among sites appeared to be driven primarily by high pairwise F_{ST} values involving 5 of the 22 sites (Table 4): EC (mean pairwise F_{ST} 0.406), DB (0.335), RB (0.273), BL (0.252), and LJ (0.373, the only one of these five values with $P < 0.05$). Although the EC sample contained only three individuals, the others had representative sample sizes ($n = 10$–23), a broad range of allelic diversity levels ($\theta(\pi) = 0.61$–3.51), and unexceptional values of Tajima's D (none significantly different from zero; Table 5).

Finally, we found no quantitative support for a pattern consistent with equilibrium isolation by distance: the slope of the regression of log $\hat{M}$ *versus* log distance between pairs of sites was slightly positive (0.084), but not different from zero ($P = 0.098$ with 1000 permutations). Molecular diversity indices calculated for all sites showed that 4/7 Georgia Strait/Puget Sound sites had negative (or marginally significant, $P < 0.06$) Tajima's D scores, as did the northern and southernmost sites (St. James Bay and La Jolla, respectively), Cape Arago, and Bodega Bay (Table 5). No clear pattern in haplotype diversity [$\theta(\pi)$] is evident across the sampled sites.

Table 2

Historical stability of Pisaster *color, size, and diet in Pacific Grove, California*

	Color		Size		Diet	
Year	% Orange	*n*	Radius ± S.E.	*n*	% *M. californianus*	*n*
1947	25.0	104	8.88 ± 0.29	104	31.8	22
1951	25.0	667	—	—	—	—
1997	27.7	47	9.02 ± 0.30	47	61.1	18
2004	26.7	134	9.25 ± 0.22	68	42.9	21

Color frequencies, body size, and the representation of *Mytilus californianus* in the diet differed little among sampling dates that spanned 57 years. Statistical comparisons are presented in the text. Dashes (—) indicate that the data were not available.

Discussion

The significance and maintenance of polymorphisms for traits related to fitness is a dominant theme in ecology and evolutionary biology (Hedrick, 1976; Merilaita and Jormalainen, 1997; Merilaita, 2001; Schemske and Bierzychudek, 2001; Toonen and Pawlik, 2001; Ross *et al.,* 2003; Tarjuelo *et al.,* 2004). In many marine organisms that exhibit polychromatism, the relationship between the distribution of color morphs and the patterns of genetic structure is com-

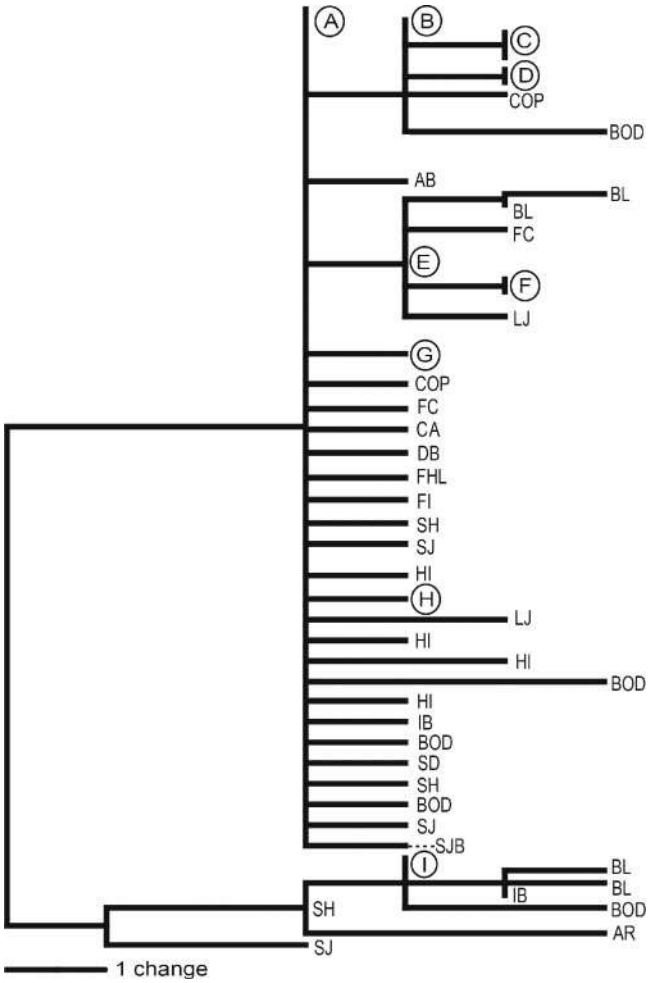

Figure 8. Mitochondrial gene tree (one of >1000 maximum parsimony trees) for *Pisaster ochraceus* sampled at sites in Table 1, Figure 1. Haplotypes shared by multiple individuals are indicated on the phylogram by circled letters, while each unique haplotype is represented by the corresponding location name. For shared haplotypes, the number of individuals (n) and number of populations (P) in which each is found are **A** ($n = 214$, $P = 23$); **B** ($n = 36$, $P = 17$); **C** ($n = 4$, $P = 3$); **D** ($n = 2$, $P = 2$); **E** ($n = 20$, $P = 5$); **F** ($n = 4$, $P = 2$); **G** ($n = 11$, $P = 1$); **H** ($n = 2$, $P = 2$); **I** ($n = 6$, $P = 4$).

plex. In some cases, color morphs are genetically distinct and may even represent separate species (*e.g.,* Tarjuelo *et al.,* 2004). However, in other cases, there may be little association between coloration and genetic structure (*e.g.,* Le Gac *et al.,* 2004; Mackenzie *et al.,* 2004). Beyond this, the effects of environmental and historical factors on the distribution of color variation are even more challenging to distinguish.

The combination of phenotypic, environmental, and genetic data used in our study of *Pisaster ochraceus* allows us to evaluate several plausible explanations for the color polymorphism in this species. Our phenotypic data show that although the various *Pisaster* color morphs are sympatric, their relative frequencies vary geographically, with a predominantly purple population in the San Juan Archipelago and southern Georgia Strait, and brown and orange dominating populations on the Olympic Peninsula of Washington and the outer coasts of Oregon and California. This distribution may reflect genetic differentiation, either through drift or *via* local selection, or environmentally driven plasticity.

The genetic data provide no evidence for historical differentiation among populations, under several geographic scenarios. Mitochondrial sequence data suggest high levels of gene flow among populations, corroborating previous studies of *Pisaster* gene flow along the Alaskan coast (Stickle *et al.,* 1992). Here, mitochondrial sequences failed to reveal any simple correspondence between the distribution of mtDNA haplotypes and the three geographic groups defined on the basis of morphology and ecology. Furthermore, we found little evidence either for differentiation across the previously documented phylogeographic boundary at Monterey Bay (Dawson, 2001; Sotka *et al.,* 2004; Wares and Castañeda, 2005), or for any pattern corresponding to the strong shift in recruitment levels of benthic organisms with planktonic larvae at Cape Blanco, Oregon (Connolly *et al.,* 2001, Menge *et al.,* 2004). Instead, the distribution of genetic variation within regional groups appears to be driven by a few populations with exceptionally low variation, but with distinct mitochondrial haplotypes.

Other genetic data (including sequence data from the elongation factor 1-α and AFLP fragment analysis) are consistent with the absence of geographic structure across much of the range of *P. ochraceus* (M. S. Pankey and J. P. Wares, unpubl. data). The distribution of genetic variation in this species was also inconsistent with a pattern of iso-

Table 3

Analysis of molecular variance in Pisaster ochraceus *among three geographic groups defined by the cluster analysis on ecological patterns (California plus Oregon; Olympic Peninsula; Georgia Strait/ Puget Sound)*

Source of variation	df	SS	Variance components	Percentage of variation
Among groups	2	1.033	−0.02091	−3.35
Among sites within groups	20	46.295	0.12619	20.19
Within sites	316	164.218	0.51968	83.15
Total	338	211.546	0.62496	

Most allelic variation is partitioned among populations (F_{st} 0.168, $P < 0.01$) and within regional groups (F_{sc} 0.195, $P < 0.01$) but not between groups defined by ecological cluster analysis (F_{ct} −0.033, nonsignificant). Other proposed groupings (separating regions on either side of Cape Blanco or on either side of Monterey Bay, or separating individuals by color group) produced similar results. The pattern of variation within regional groups is chaotic and appears to be driven by a few populations within exceptionally low and statistically distinct allelic variation (*e.g.,* Dabob Bay harbors a unique haplotype at high frequency).

Table 4

Significant pairwise F_{st} *between* Pisaster ochraceus *sampling sites, determined by permutation testing in Arlequin*

	SJB	[EC]	FHL	SD	HI	FI	AB	[DB]	CB	KP	TI	CA	[RB]	SJ	FC	SH	AR	[BL]	IB	BOD	HOP	COP
SJB																						
[EC]	−																					
FHL	−	+																				
SD	−	+	−																			
HI	+	+	−	−																		
FI	−	+	−	−	−																	
AB	−	+	−	−	−	−																
[DB]	+	+	+	+	+	+	+															
CB	−	+	−	−	−	−	−	+														
KP	−	+	−	−	−	−	−	+	−													
TI	−	+	−	−	−	−	−	+	−	−												
CA	+	+	−	−	−	−	−	+	−	−	−											
[RB]	−	−	+	+	+	+	+	+	+	+	+	+										
SJ	−	+	−	+	−	−	−	+	−	−	−	+	+									
FC	−	+	−	−	−	−	−	+	−	−	−	−	+	−								
SH	−	−	−	+	+	−	+	+	+	−	+	+	−	−	+							
AR	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	−						
[BL]	−	−	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+					
IB	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+				
BOD	−	−	−	+	+	−	−	+	−	−	−	+	+	−	−	−	−	+	−			
HOP	−	+	−	−	+	−	+	+	−	−	−	+	+	−	−	+	−	+	−	−		
COP	−	+	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	+	−	−	−	
[LJ]	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+

A "+" indicates significant ($P < 0.05$) values of F_{st}, and that populations differ significantly in their mitochondrial COI haplotype frequencies. Populations in brackets appear to be significantly differentiated from >70% of all other populations. These populations are discussed further in Results. See Table 1 for site codes.

lation by distance expected at evolutionary equilibrium, under a linear stepping-stone model of dispersal. Specifically, the regression of log pairwise genetic distances ($\hat{M}$) *versus* log geographic distance between populations did not differ from a slope of zero, suggesting that there is extensive gene flow among the populations we sampled spanning much of the range of this species. The trend toward negative Tajima's *D* scores at Georgia Strait/Puget Sound sites suggests that either differential selection or rapid changes in population size may have influenced the distribution of genetic variation at these sites, relative to the outer coast sites (Nielsen, 2001). In particular, because the Georgia Strait/Puget Sound sites—unlike the others—were glaciated in the late Pleistocene, it might be expected that they should exhibit lower allelic/haplotypic diversity than unglaciated sites, as populations (and genetic diversity) recover either through local recruitment or by immigration from other surviving populations. However, values of Tajima's *D* from sites within the Georgia Strait/Puget Sound are not statistically distinguishable from the values observed at other sites (Table 5), suggesting that if populations were locally exterminated, gene flow from other populations has allowed their recovery since the last glaciation. Thus, historical vicariance and genetic drift are unlikely to have contributed to the formation and maintenance of the color polymorphism. Nevertheless, without data from defined crosses between different color morphs, it is impossible to determine whether polychromatism in *P. ochraceus* is environmentally induced, in which case the distribution of the inducer would have to vary geographically, or genetically based, in which case spatially variable selection would appear to play a role in the distribution of color variants.

Local selection

Although gene flow in *Pisaster* appears to be very high, it remains possible that local variation in selection contributes to *Pisaster's* color polymorphism. If this were the case, the predominantly purple sea stars in Georgia Strait/Puget Sound waters could be a true subpopulation in which bright purple coloration has become very nearly fixed. A number of physical and biotic variables, including thermal stress, salinity, and interspecific interactions, that could influence the selective regime differ between the outer coast and the Georgia Strait/Puget Sound. If coloration is somehow genetically linked with physiological tolerance to any of these variables, such spatial variation in selection could be reflected in color distributions, even in the face of substantial gene flow that limits differentiation among mtDNA markers.

Table 5

Molecular diversity indices $\Theta(\pi)$ *and Tajima's* D *for* Pisaster ochraceus *mitochondrial COI samples*

Site	$\Theta(\pi)$	*D*	*P*
SJB	1.74 ± 1.22	−1.57	<0.06
FI	1.00 ± 0.99	−0.71	NS
HI	0.53 ± 0.51	−1.96	<0.01
SD	0.49 ± 0.48	−1.31	<0.06
FHL	0.76 ± 0.67	−1.51	<0.06
EC	1.33 ± 1.37	0.00	NS
PP	—	—	—
DB	0.61 ± 0.56	0.28	NS
AB	0.86 ± 0.70	−2.23	<0.001
OP	—	—	—
SP	—	—	—
SR	—	—	—
CR	—	—	—
TI	0.33 ± 0.40	−0.10	NS
KP	0.43 ± 0.49	0.33	NS
CB	0.33 ± 0.40	−0.10	NS
CA	0.29 ± 0.35	−1.55	<0.05
RB	2.33 ± 1.51	−1.12	NS
SJ	2.35 ± 1.47	−0.85	NS
FC	0.17 ± 0.27	−1.14	NS
BB	—	—	—
SH	3.55 ± 2.26	−0.15	NS
AR	0.25 ± 0.35	−1.05	<0.06
BL	3.51 ± 2.21	−0.43	NS
IB	0.44 ± 0.48	−1.10	NS
BOD	2.06 ± 1.37	−2.37	<0.001
MB	—	—	—
HOP	0.38 ± 0.43	0.49	NS
COP	0.59 ± 0.57	−1.23	NS
LJ	1.01 ± 0.81	−1.67	<0.05

Significant *P* values for *D* indicate that either neutral molecular evolution or stable population size could be rejected; significantly negative values for *D* suggest either purifying selection or growth in population size (Innan and Stephan, 2000). NS = nonsignificant. Dashes (—) indicate sites at which molecular data were not collected. See Table 1 for site codes.

For example, thermal stress during low tide increases from west to east along the Strait of Juan de Fuca, which may drive variation in the distribution of intertidal invertebrates (Harley and Helmuth, 2003). However, subaerial stress does not appear to affect *Pisaster* foraging behavior in Oregon (Sanford, 2002), and *Pisaster* behaviorally avoids the most severe periods of subaerial stress by retreating into the low intertidal or into sheltered microhabitats. Although salinity in the San Juan Archipelago is typically within about 3 psu of that of the entrance of the Strait of Juan de Fuca, freshwater incursions from the Fraser River plume can substantially reduce salinities for brief periods (Masson and Cummins, 2000). This type of event, even if rare, could impose strong selection on long-lived animals such as *Pisaster.* For instance, an unusual freshwater incursion appears to have killed many *Pisaster* in Southern California (MacGinitie, 1939). Even nonlethal salinity effects (*e.g.,* reduced foraging efficiency) could result in genetic adaptation, although phenotypically plastic responses are also possible (*e.g.*, Sarantchova, 2001).

Geographic variation in the impacts of visual predators such as sea otters could also partially account for the distribution of color morphs of *P. ochraceus.* Sea otters, although currently absent from much of their historic range, were not present in the Georgia Strait/Puget Sound even prior to European colonization (Kenyon, 1969). Finally, *Pisaster's* dietary composition may constitute selective pressure. A feeding strategy used to optimize energy intake from large prey items that can take hours to consume, such as *Mytilus californianus,* may be suboptimal for the smaller prey such as bay mussels, barnacles, and gastropods that make up most of *Pisaster's* diet in the Georgia Strait/Puget Sound. Indeed, genetically determined prey preferences could result in differential color expression among individuals even in the absence of a genetic color polymorphism. However, the role of genotype in *Pisaster* foraging remains unknown.

Environmentally driven plasticity

The absence of a phylogeographic signal in the mtDNA dataset suggests that geographic differences in relative frequencies of *Pisaster* color morphs most likely reflect phenotypic plasticity driven by diet or other environmental factors. Indeed, the distribution of color morphs in our dataset corresponds to spatial variation in patterns of prey consumption. *Pisaster* individuals in outer coastal waters primarily consumed *Mytilus californianus,* whereas the diet of conspecifics in the Georgia Strait/Puget Sound mainly consists of barnacles and bay mussels. In terms of overall biomass, *M. californianus* is one of the most dominant species on wave-exposed shores in the Northeast Pacific, but it is rare along the west side of San Juan Island, Washington, and absent from shorelines in the central and eastern San Juan Islands and southern Strait of Georgia (Kozloff, 1993). Taken together, the paucity of *M. californianus* in the Georgia Strait/Puget Sound, along with the dominance (nearly 100%) of deep purple morphs of *P. ochraceus* in the region, suggest that the expression of orange and brown pigmentation in the predators may depend on the consumption of *M. californianus* (Fox and Scheer, 1941; De Nicola and Goodwin, 1954; Fox and Hopkins, 1966). Where *M. californianus,* and the carotenoid pigments it normally contains, constitute a substantial part of the diet of *P. ochraceus,* the actual color expressed—whether it be brown, orange, or dull purple—may have a genetic component. Under this scenario, brilliant purple is the default color expressed in sea stars lacking access to *M. californianus* or some other critical dietary resource. If genetic and dietary studies substantiate this hypothesis, then

polychromatism in *Pisaster* would exemplify an interaction between diet and intrinsic genetic control.

Additional observational and anecdotal evidence supports the hypothesis that environmentally modulated plasticity is the simplest plausible explanation for the distribution of color variants in *P. ochraceus.* Notably, small fractions of orange stars have purple coloration at the tips and along the undersides of their rays, especially if a ray is actively growing (*e.g.,* following injury; CH, pers. obs.). Even smaller fractions have a tracework of purple coloration on their aboral surface (CH, pers. obs.). Very small *Pisaster* are not chromatically differentiated, and some orange adults may turn purple when held for long periods under laboratory conditions (J. Pearse, Long Marine Laboratory, University of California Santa Cruz, pers. comm.). These observations imply that individuals express different pigments as they grow or age; however, the effects of age and diet remain confounded for the time being.

Whatever the forces controlling polychromatism in *Pisaster* may be, these forces apparently persist over relatively long ecological time scales. *Pisaster* size, color frequency, and diet have not detectably changed in over half a century in Pacific Grove, California (Table 2). Because the mean size of *Pisaster* populations can change over much shorter time scales *via* growth or migration (Paine, 1976), long-term stasis in mean size suggests that the Pacific Grove population has not undergone any substantial changes in dietary composition or prey abundance over the past 57 years. Paine (1976) reported similar stasis in mean size for *Pisaster* on the outer coast of Washington.

Conclusions

Spatial variation in the distribution of color morphs in *Pisaster ochraceus,* especially the predominance of purple sea stars in the Georgia Strait/Puget Sound, has been noted by others (*e.g.,* Kozloff, 1993; Raimondi *et al.* 2007), but explanations for this pattern remain untested. In the absence of information from genetically defined crosses, we do not yet know the extent to which polychromatism in *P. ochraceus* is genetically based. Indirect evidence, based on the correspondence between the spatial distribution of neutral genetic variation and color morph frequencies, has provided insight into this question in other organisms such as *Botryllus schlosseri* (Sabbadin and Graziani, 1967); *Idotea balthica* (Merilaita and Jormalainen, 1997); *Littorina mariae* (Reimchen, 1989); *Nucella lapillus* (Etter, 1988); and *Argopecten purpuratus* (Winkler *et al.,* 2001). At this point, however, the data reported in the present study suggest that historical isolation of populations and limited gene flow are unlikely to be the primary forces governing spatial polychromatism in *P. ochraceus.* It is important to understand the underlying mechanism causing such variation because if this color variation is heritable, then the polymorphism should be transient unless it is adaptive. If it is not heritable, then it raises the question of what maintains steep gradients of change over short distances (*e.g.,* the rapid shift toward "purple" populations in Puget Sound despite homogeneous color frequencies along the entire outer coast from Alaska to La Jolla). Although there may be an underlying genetic component to the coloration of *P. ochraceus,* regional-scale variation in color appears to be under ecological control.

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