# Testing hypotheses for chaotic genetic structure: patterns of spatial and temporal genetic heterogeneity among recruits, subadults and adults of the intertidal anomuran *Petrolisthes cinctipes*.

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**ABSTRACT**

Hypotheses to explain chaotic genetic structure (i.e. a surprising degree of non-geographic temporal or spatial population differentiation) include: 1) variation in source of larval recruits, 2) self-recruitment and local subdivision, 3) variance in reproductive success (sweepstakes reproduction), and 4) pre- or post-settlement natural selection. We evaluated the relative contribution of each of these four processes to the observed patterns of population differentiation among geographic populations of the porcelain crab, *Petrolisthes cinctipes*. Approximately 50 individuals of each size class (new recruits, sub-adults, adults) were collected at each of nine sites across northern California in each of three consecutive years for genotyping (N= 3602). Significant and consistent population structure ($\theta \sim 0.08$) was detected among sites from each replicate year of sampling (1997-1999). Significant population structure was also detected both among years and among sites, but the pattern of population structuring differed by size class. Among years, the differentiation of new recruits was highest ($\theta = 0.12$) year-to-year, followed by the sub-adults ($\theta = 0.09$) and finally adults ($\theta = 0.08$). In contrast, the among sites component was greatest among the adults ($\theta = 0.05$), followed by the sub-adults ($\theta = 0.03$), and least among new recruits ($\theta < 0.01$, not significant). An overall hierarchical analysis of molecular variance found genetic partitioning was structured among years ($\theta = 0.05$), and size classes ($\theta = 0.08$), but not among sites ($\theta < 0.01$). Recruits at each site were most genetically similar to a different population of adults each year, and although some were apparently self-recruits, no consistent patterns of assignment or genetic similarity to natal populations were observed across years, indicating that there is no predictable source of recruitment. Temporal differentiation was stronger than spatial differentiation, and in the full hierarchical analysis, the among years and among size class components of variance explain the majority of observed population structure. Most studies of population genetic structuring are snap-shots based on single collections that lack explicit temporal components, and may provide an incomplete picture of population structure as a result. Overall, these data are consistent with the combined influence of all four hypothesized mechanisms acting synergistically to create fine-scale population genetic structure in marine species, because none of them alone appears sufficient to account for the observed population structure in *P. cinctipes*.

## 1 INTRODUCTION

Over microevolutionary time, rates and patterns of gene flow determine the potential for, and scale at which, local adaptation can evolve; over macroevolutionary time, rates and patterns of gene flow are related to evolutionary persistence and rates of speciation (Futuyma 1997; Hartl & Clark 1997). Because direct measures of gene flow are nearly impossible in many marine populations, indirect measures of gene flow inferred from the spatial distribution of alleles present an attractive alternative for marine systems (reviewed by Palumbi 1996; Neigel 1997; Grosberg & Cunningham 2001; Hellberg et al. 2002; Selkoe et al. 2009; Hellberg 2009). Even in cases where tracking of dispersal is possible (e.g., Carlon & Olson 1993; Jones et al. 1999; Planes et al. 2009), direct estimates of gene flow will typically underestimate the importance of rare long-distance dispersal of individuals (e.g., Lewis et al. 1997; Petit & Mayer 1999; reviewed by Hellberg et

al. 2002; Hellberg 2009). Although rare long-distance dispersal events are unlikely to be detected by direct methods, they may prove extremely important determinants of the biology and evolutionary history of natural populations (reviewed by Neigel 1997; Grosberg & Cunningham 2001; Hellberg et al. 2002; Selkoe et al. 2009; Hellberg 2009). Another potential issue with direct estimates of dispersal is that data and theory now indicate that marine larval dispersal events often happen in rare but intense pulses or "spikes" of recruitment. Across a suite of approaches ranging from Lagrangian simulation studies (e.g., Mitarai et al. 2008; Siegel et al. 2008; Pringle et al. 2009) to direct tracking, kinship and genetic assignment tests (e.g., Jones et al. 2005; Selkoe et al. 2006; Almany et al. 2007; Buston et al. 2009), evidence is accumulating that larval mixing is far less than had been generally assumed, and that dispersal paths within years are highly correlated. Although larvae in a given year may experience similar dispersal probabilities, the annual stochasticity of dispersal also indicates that there is little to no correlation between the non-Gaussian dispersal paths of annual cohorts of larvae (e.g., Mitarai et al. 2008; Siegel et al. 2008; Cowen & Sponaugle 2009; Pringle et al. 2009). Patterns of larval dispersal only become a smooth distribution when averaged over many events through time (Siegel et al. 2008; Selkoe et al. 2009; White et al. 2010). Thus, indirect methods of estimating gene flow have the potential advantage of providing estimates of both aspects of dispersal, ranging from spiky annual assignment to the ultimate consequences of dispersal averaged into the distribution of dispersal events themselves (Neigel 1997; Ouborg et al. 1999; Selkoe et al. 2009).

Such indirect approaches generally assume that patterns of genetic structure among geographic populations are stable over ecological time scales (reviewed by Lessios et al. 1994; Neigel 1997; Ouborg et al. 1999; Grosberg & Cunningham 2001). In the absence of natural selection, mutation, and migration, allelic frequencies in an infinitely large and randomly mating population remain constant through time (Hartl & Clark 1997; Futuyma 1997). In nature, however, populations are neither infinite or randomly mating, and spatial patterns of genetic structure must change through time as species respond to a suite of stochastic (e.g., reproductive variance and genetic drift) and deterministic (e.g., selection and gene flow) forces (reviewed by Hedgecock 1994; Palumbi 1996; Neigel 1997; Ouborg et al. 1999; Grosberg & Cunningham 2001; Hellberg et al. 2002; Selkoe et al. 2009; Hellberg 2009). Despite the operation of both stochastic and deterministic evolutionary forces, does genetic structure remain sufficiently constant that one can reliably infer patterns and levels of gene flow from a single time frame?

There are relatively few studies that examine whether the spatial distribution of alleles or allelic frequencies vary significantly through time (e.g., Kordos & Burton 1993; Hedgecock 1994; Viard et al. 1997; Hauser et al. 2002; Turner et al. 2002; Lee & Boulding 2009). For example, a quick search of the BIOSIS database revealed 5126 studies, published between 1985 and 2000, categorized using the keywords "population genetic structure." In contrast, only 360 (7%) of those studies included "temporal" as an additional keyword. The trend has not improved much through time: a similar search in ISI Web of Science returns 2200 studies published between 2000 and 2009, and a search for "temporal" within those returned only 117 (5%) hits. Most studies that assess genetic stability among populations through time focus on terrestrial species, and the handful of marine examples are divided between those documenting that the genetic constitution of populations tends to remain relatively stable (e.g., Berger 1973; Burton & Feldman 1981; Lessios 1985; McClenaghan et al. 1985; Waples 1990b; White and Svendsen 1990; Garant et al. 2001; Palstra et al. 2006; Shen & Tzeng 2007), and those that find significant changes in allele frequencies drawn from the same population over periods of time as short as a few generations (e.g., Powers & Place 1978; Johnson & Black 1984; Piertney & Carvalho 1995; Kusumo & Druehl 2000; Robainas et al. 2005; Florin & Höglund 2007; Lee & Boulding 2007).

Allele frequencies are expected to show the greatest rate of change in species with a short generation time, small and fluctuating population sizes, and with low levels of gene flow among populations because drift is stronger under such conditions (Lessios et al. 1994; Futuyma 1997; Hartl & Clark 1997). Thus, marine invertebrate taxa with relatively high fecundity, long-lived planktonic larvae, few obvious barriers to dispersal, broad species ranges, and very large and relatively stable population sizes ought to be among the most likely candidates to show temporal stability of allelic frequencies (e.g., Shen & Tzeng 2007). Existing literature runs contrary to that prediction, however, with studies of marine taxa demonstrating temporally stable genetic structure more often coming from species from the opposite end of the spectrum, such as the tidepool copepod *Tigriopus* (e.g., Burton 1997; Burton et al. 1999; Edmands & Harrison 2003), or the intertidal isopod *Excirolana* (e.g., Lessios et al. 1994). Studies of organisms that match the characteristics predicted to reduce temporal variability tend to commonly find that gene frequencies can change significantly through time, even in species that disperse broadly across large species ranges and maintain

large population sizes (e.g., Johnson & Black 1984; Li & Hedgecock 1998; Johnson & Wernham 1999; Robainas et al. 2005; Lee & Boulding 2007).

This contradiction between theoretical expectations of temporal stability and finding of substantial variability in the field requires an explanation of the underlying mechanisms. Unexpected patterns of genetic structure in marine species can be spatial as well as temporal. Another common pattern seen in studies of marine population genetic structure is an unexpected pattern of spatial variability with high genetic differentiation among adjacent sites compared to lower differentiation found between distant sites. A variety of such counterintuitive findings of population genetic structure have come to be known in the marine literature as "chaotic genetic patchiness" since the term was coined by Johnson & Black (1982; 1984). Chaotic genetic patchiness (defined as population differentiation at fine scales that lack geographic pattern and changes through time) is fairly often reported among marine population genetic studies, although sound theoretical mechanisms to explain these patterns are few. Alternative hypotheses to explain chaotic genetic structure generally encompass four alternatives: 1) variation among differentiated source populations from which larvae recruit, 2) self-recruitment and local differentiation of broadly-distributed species, 3) high variance in reproductive success (sweepstakes reproduction), and 4) pre- or post-settlement natural selection (reviewed by Hedgecock et al. 2007, Hellberg 2009).

This study presents a size-structured analysis of spatial and temporal genetic variation that seeks to examine the relative contribution of these hypothesized mechanisms for generating a non-geographic pattern of genetic patchiness in the common intertidal anomuran, *Petrolisthes cinctipes*. An initial study of geographic structure across the species range (Toonen 2001) revealed significant population structure (overall $\theta = 0.11$), and some pairwise comparisons between adjacent sites (e.g., Bodega Bay & Dillon Beach, only 10 km apart, pairwise $F_{ST} = 0.16$) were greater than comparisons across much of the species range (e.g., Bodega Bay, CA to Neah Bay, WA, nearly 1200km apart, $F_{ST} = 0.14$). *P. cinctipes* is a sedentary marine invertebrate that produces long-lived planktonic larvae with apparently great potential for mixing, has an enormous census population size (likely on the order of $10^8$ across the species range), a relatively high fecundity, an essentially continuous geographic range along the linear coastline of North America, and a maximum lifespan on the order of about eight years (Jensen 1990). Thus, by all expectations outlined above, *P. cinctipes* seems an obvious choice to expect temporal stability of gene frequencies over ecological time scales. We used polymorphic microsatellite loci with sufficient allelic variation and power (Kalinowski 2002; Ryman et al. 2006) to characterize the year-to-year variation in genetic structure among the recruits, subadults and adults of *P. cinctipes* and compare the patterns of temporal and spatial genetic structure to that seen in the initial geographic survey of adults.

Our study was designed to characterize the genetic structure among years, sites and among age classes through time in order to evaluate the relative contribution of stochastic (e.g., individual reproductive success, recruitment variation) and deterministic (e.g., selection and gene flow) forces in shaping the evolutionary trajectory of this species. Here we outline the data from this multi-year study of age and site structured population sampling to evaluate support for each of the common hypotheses advanced to explain chaotic genetic patchiness in species such as *Petrolisthes cinctipes*.

## 2 MATERIALS AND METHODS

### 2.1 Study organism

*Petrolisthes cinctipes* makes an excellent study organism to examine fine-scale population genetic structuring for a variety of reasons. First, the species is easily identified and distinguished from co-occurring congeners (Stillman & Reeb 2001). *P. cinctipes* occurs along a linear coastline ranging from the northern limit around the Dixon Entrance (British Columbia, Canada) to around to Point Conception (California, USA). Our field survey located individuals as far north as Prince Rupert, BC (roughly 54.3ºN, 130.4ºW) and as far south as Morro Bay, CA (roughly 35.3ºN, 120.8ºW), but we were unable to locate more than a few scattered individuals anywhere south of Morro Bay. Despite being restricted to a relatively narrow vertical range of the rocky intertidal (from roughly 0 to 1.8 m above MLLW, Jensen & Armstrong 1991), *P. cinctipes* is one of the most abundant decapod crustaceans in the Eastern North Pacific, typically occurring in densities of 100-600/$m^2$ (Donahue 2004), and occasionally as high as 3,933 individuals/$m^2$ (Jensen 1990). Males and females of all size classes, from newly settled postlarvae (~1.5 mm) to large adults (up to 20 mm carapace

width), live together in large aggregations among rocky cobble (Jensen & Armstrong 1991). Dense aggregations result largely from individual substrate preference (lower bound) and physiological tolerance (upper bound) of the adults, reinforced by gregarious settlement of the larvae with conspecific adults (Jensen 1989, 1991a, b; Jensen & Armstrong 1991; Stillman 2002; Akins 2003; Stillman 2004; Donahue 2004; Donahue 2006). For example, Akins (2003) found that adult *P. cinctipes* strongly preferred to live under boulders set on cobbles and pebbles, to those under which there was sand; there was a strong correlation ($R^2$=0.84) between the overall abundance of *P. cinctipes* at a site and the percent of cobble habitat per boulder. Adults compete for access to high quality feeding spots characterized by high flow rates (Jensen 1990). Individual growth rates decline with increasing conspecific density in both the lab and the field, with juveniles suffering most from competition because adults are able to monopolize the preferred feeding spots (Donahue 2004; 2006).

*Petrolisthes* spp. are gonochoric and females brood fertilized eggs until they hatch and begin pelagic larval development. Timing of reproduction in *P. cinctipes* varies with latitude, but in northern California, females typically produce broods from February through mid-April (Morris et al. 1980). However, some individuals may produce a second brood or be delayed, and brooding females can be found at low frequency as late as September (Toonen 2001; Toonen 2004). Newly extruded broods may contain up to about 1,300 embryos, each roughly 0.1 mg in dry weight and 800 µm in diameter (Donahue 2004). Although the exact mechanism of copulation in *P. cinctipes* remains unknown, mating occurs in the hard-shelled state, and large males defend territories in an attempt to monopolize access to females (Molenock 1974, 1976). Despite male guarding and attempts to monopolize fertilization, the majority do not appear successful because 71-100% of females produce broods of multiple paternity (Toonen 2004).

The exact duration of pelagic larval development is uncertain but feeding larvae likely spend months in the plankton before becoming competent to settle. Due to the extended pelagic larval duration they have the potential to drift passively >1,000 km, depending on current patterns and velocity. After larvae molt into megalopae, they settle quickly in response to a waterborne cue associated with live conspecific adults (Jensen 1989, 1991a, b). Megalopae of *P. cinctipes* do not molt at the time of settlement but rather lose the ability to swim due to degeneration of their pleopods; metamorphosis to the pigmented first instar juvenile occurs a week or more after settlement (Jensen 1991). This delay of metamorphosis after settlement makes identification of newly settled postlarvae straightforward. New recruits take advantage of the defended space beneath adults for about a year after settlement, until reaching roughly 5 mm carapace width (CW), when adults actively begin to expel them (Jensen 1991). Colonization of new habitats occurs most frequently by crabs of ~ 5mm CW, presumably because they are forced to seek new shelter when expelled by adults (Jensen 1991). Additionally, maximal growth and survival of individuals occurs at intermediate densities (~50 per rock), and there is both density- and size-dependent intraspecific competition for preferred feeding sites (Donahue 2004; 2006). Despite the potential motility and evidence of strong intraspecific competition of porcellanid crabs, adults are remarkably sedentary in the field. Mark-release-recapture data show that >50% of crabs were recovered within only a few meters of the point of release after two months (Jensen & Armstrong 1991).

Although the chelae of *P. cinctipes* may account for nearly 50% of the body mass, these crabs are suspension feeders that appear to gain most of their nutrition from capturing diatoms and tiny zooplankton from the water column. The impressive chelae are rarely used even in defense when being harassed, and appear to serve primarily for territorial disputes and mating displays. Chelae are important determinants of mate choice in other crabs (e.g., Sainte-Marie et al. 1999), and the same is likely true of *P. cinctipes* (Jensen, pers. comm.). *Petrolisthes* readily autotomize a cheliped as a defensive strategy when threatened, making non-lethal sampling quite simple.

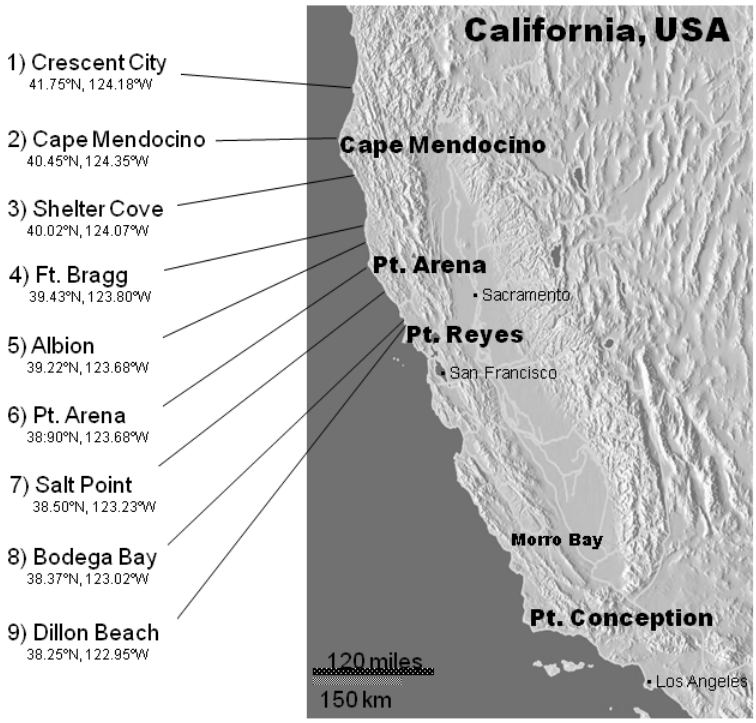


Figure 1. Northern California sampling sites for *Petrolisthes cinctipes*. Each site is numbered from north to south (1-9) and the approximate GPS location for each is listed below the site name.

## 2.2 Sample collection and DNA extraction

*Petrolisthes cinctipes* were collected from rocky cobble around large boulders at each site in Figure 1. The original study design sought to visit all sites from Dillon Beach, CA northward to Crescent City, CA on a single good low-tide series in each sampling year for collection. However, the distance proved too great to reach on a single tidal series and no collection could be made at Crescent City in the summer of 1997. Unfortunately, the crab population at Dillon Beach was wiped out during the winter storms that year, and did not recover in the following years. Thus, Dillon Beach was only sampled in 1997, and then replaced with Crescent City in the remaining (1998 & 1999) sampling seasons. Samples were collected during a good low tide series in August of 1997, September of 1998 and July of 1999. At each site, boulders were selected based on the presence of dense aggregations of crabs, and the same boulder was used for each annual collection at each site. We collected all individuals encountered at a site, regardless of size, and stored the live crabs in a mesh container filled with fresh kelp (to minimize stress), until at least 50 individuals of each size class were found or the incoming tide ended the collection. After a tidal cycle, the collections were counted, and subadults and adults were individually harassed to obtain a cheliped from each, which was preserved immediately in 95% ethanol (EtOH). Live crabs were then released at the site of collection. New recruits (unpigmented post-larvae) were preserved whole in 95% ethanol. Ethanol-preserved samples were kept on ice in the field for the roughly 4-5 days required to complete the collection, after which the EtOH was decanted and replaced. Vials containing individually preserved chelipeds, from subadult and adult crabs, or whole post-larvae were subsequently stored at -40ºC until DNA could be extracted.

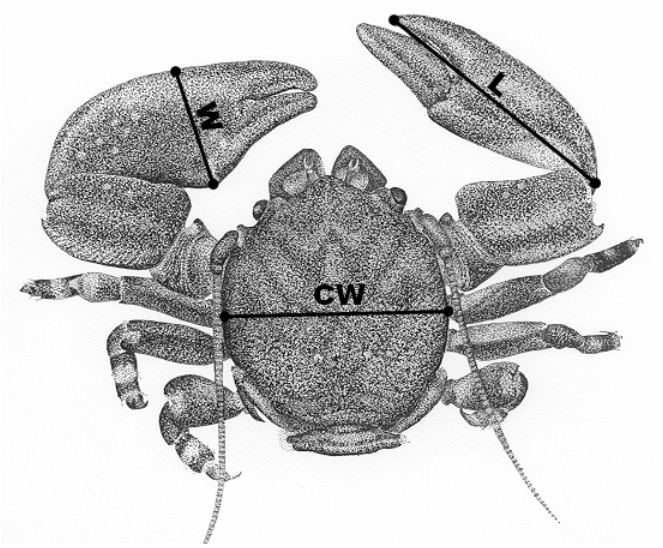


Figure 2. Measurement points for *Petrolisthes cinctipes*. Claw length (L) is measured from the tip of the non-articulating finger of the chela to the joint with the carpus. Claw width (W) is measured across the widest part of the chela, and carapace width (CW) is measured across the widest dimension of the carapace (drawing by Josh Ferrris).

In the laboratory, each cheliped was assigned a unique identification number and when possible 50 individuals per size class from each site were selected randomly for subsequent DNA extraction and genetic analysis. If fewer than 50 individuals of each size were collected at a site, all samples of that size class were included in the study. Each sample selected for inclusion in the study was removed from the ethanol and the length and width of the chela was measured to the nearest 0.01 mm with digital calipers (Figure 2). After dissection of 3-5 mg of muscle tissue from the claw, the remaining sample was placed back into storage as a backup, and the muscle tissue was labeled only with the six-digit unique ID number. DNA extraction, PCR amplification, electrophoresis and scoring were all done blindly using these unique crab ID numbers. Carapace width (size class) and site of collection for individual samples were reassigned to numbered samples only after all samples had been scored.

Because whole crabs were not available after being released at the site of collection, individuals were assigned to size classes (recruits, subadults or adults) according to their claw length and claw width, which correlate very well to carapace width (Figure 3). Carapace width (CW) was estimated from the best-fit regression model using each claw width and claw length

DNA was extracted from the dissected muscle tissue using a modification of the Gentra System PUREGENE marine invertebrate protocol. Extracted DNA pellets were rehydrated in 50 µl of low-TE (10mM Tris, 0.1mM EDTA) prior to being stored at -40ºC for subsequent PCR amplification.

2.3 PCR amplification and electrophoresis

The isolation of Simple Sequence Repeat (SSR) loci and development of primers used in for amplifying these loci in *Petrolisthes cinctipes* is described in detail in Toonen (2001) using the protocol of Toonen (1997). In brief, we developed primers for 17 putative microsatellite loci, and excluded loci that: 1) produced substantial stutter such that the scoring of amplicons was unreliable; 2) had significant null alleles; 3) failed Slatkin's (1994; 1996) exact test for a fit to the Ewen's sampling distribution for neutral alleles; 4) failed to amplify a product from the original individual from which the microsatellite library was developed; 5) amplified more than 2 alleles or did not amplify a product of the expected size; or 6) exhibited a non-Mendelian pattern of inheritance (Toonen 2001). After applying strict criteria for quality control (following Selkoe & Toonen 2006), 15 of the 17 loci were excluded from the analysis, and only 2 loci remained for this study. The two polymorphic microsatellite loci, which each have more than 30 alleles, have sufficient power for the analyses reported here (see Section 4.2 for further details). The primer sequences for

amplifying the two highly polymorphic microsatellite loci used in this study, Pc156s and Pc170s, are presented in Table 1.

Table 1. Microsatellite loci developed for *Petrolisthes cinctipes*. Forward and reverse primer sequences (5′ to 3′) with the fluorescent label used, originally cloned repeat motif, the number of crabs for which the locus was scored (N), number of alleles per locus, and the observed and expected heterozygosities for each locus pooled across residents of all populations studied (see Toonen 2001 for details of development, quality control and marker selection).

| Locus | Primer Sequence | Repeat motif | N | No. of alleles | Heterozygosity | |
|---|---|---|---|---|---|---|
| | | | | | Observed | Expected |
| Pc156s | **F:** HEX–TTG GCT TTG AAG ACC CTG TGG<br>**R:** CGG GGG ATC ATT GCT TTG TC | (TG) | 3602 | 34 | 0.60 | 0.68 |
| Pc170s | **F:** 6FAM-TGG CCG TTG CTG TTG TTG TC<br>**R:** GGC ACC AGT CAT TCC CAG TTG | (TGT)..(TGT)..(TG) | 3588 | 47 | 0.76 | 0.83 |

PCR was performed as outlined in Toonen (2001). Briefly, in a final volume of 10 µl, PCR reaction mixes contained Perkin Elmer 10X Buffer II at 1X concentration, 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 1X BSA, 0.5 mM of each the forward and reverse primer, 1 unit of Taq polymerase, and 1-50 ng of template DNA. PCR amplifications were performed using a touchdown protocol beginning with an initial 5 min denaturation at 94ºC, followed by 2 cycles with 30s denaturation at 94ºC, 30s of annealing at 68ºC, and 30s of extension at 72ºC, stepping down to 2 cycles of annealing at 65ºC, 2 cycles of annealing at 63ºC, and then 24 cycles of annealing at 60ºC, before a final extension at 72ºC for 30 min to ensure all amplicons were +A.

Amplified PCR products were sized by gel electrophoresis on an Applied Biosystems ABI 377 XL automated sequencer and scored using the STRand analysis software (Hughes 1998), as outlined in Toonen & Hughes (2001).

2.4 Analyses of population genetic structure

Standard statistical analyses (ANOVA, post-hoc comparisons among means, and linear regression) were all performed using JMP 4.0.2 (SAS Institute 2000). For genetic analyses, STRand stores allelic data generated by the ABI 377XL in the Microsoft ACCESS relational database, which allows direct export of fragment sizes into Microsoft EXCEL. Allelic data were then translated into ARLEQUIN input format using the MS TOOLKIT (Park 2001) macro for EXCEL. Summary statistics were all calculated using ARLEQUIN v2 (Schneider et al. 2000). Global tests for deviation from expectations of Hardy-Weinberg Equilibrium (HWE) were also performed using ARLEQUIN. These tests for HWE employ a Markov-chain method (Guo & Thompson 1992), and chain lengths for these tests were 500,000 steps with a 10,000 step dememorization.

Weir & Cockerham's (1984) unbiased estimator of Wright's FST ($\theta$) was calculated locus-by-locus using the Tools for Population Genetic Analyses (TFPGA) software (Miller 1997). TFPGA uses the method and terminology outlined in Weir & Cockerham (1984; Weir 1996). Because only two loci are included in this data set, however, bootstrapping and jackknifing are not meaningful, and therefore no mean, standard error or confidence intervals for $\theta$ are presented.

We constructed UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrograms (Michener & Sokal 1957) using the genetic distance $D_A$ (Nei et al. 1983), based on its superior performance in simulations (Takezaki & Nei 1996). $D_A$ was calculated in ARLEQUIN or DISPAN (Ota 1993), and population dendrograms were drawn using DISPAN or TFPGA. Population dendrograms give an indication of the degree of genetic similarity among populations; the longer the branches separating a pair of

populations, the more genetically dissimilar are the populations sampled from those geographic locations. If gene flow depends primarily on the number of dispersal steps between populations, then sites that are geographically proximate are expected to cluster more closely than with distant sites.

Finally, we used ARLEQUIN to perform an analysis of molecular variance (AMOVA), which partitions observed genetic variance into components analogous to traditional analysis of variance analyses (Excoffier et al. 1992; Excoffier 2000; Rousset 2000). Using the AMOVA framework within ARLEQUIN, we partitioned observed genetic variation components and calculated hierarchical *F*-statistic analogs (Φ) in order to test the relative contributions of year, site and size class in partitioning the observed genetic variation among sampled groups. The significance of each of these values was tested by 100,000 matrix permutations in ARLEQUIN (Excoffier et al. 1992).

2.5 Assignment tests and inference of recruitment sources

As outlined above, Nei's distance $D_A$ was calculated, and population dendrograms were also used to infer which breeding population was most closely related to the sample of new recruits collected at each site. This approach requires the assumption that recruits show the minimal genetic distance from the breeding population which produced them, which may not be true in cases of sweepstakes reproduction (Hedgecock 1994). Thus, source populations for recruits were also inferred using Bayesian likelihood methods to assign each sampled recruit back to a source population based on its multilocus microsatellite genotype as implemented in GENECLASS2 (Piry et al. 2004). Assignment tests are an independent method to infer patterns of recent gene flow, because they identify migrants, or recent descendents of migrants, through identifying disequilbrium within multilocus genotypes (Wilson & Rannala 2003). Multi-locus assignment is obviously a weak test with only two loci on which to assess disequilibrium, but this assignment testing provides an independent approach with which to compare the results of the genetic distance approach above.

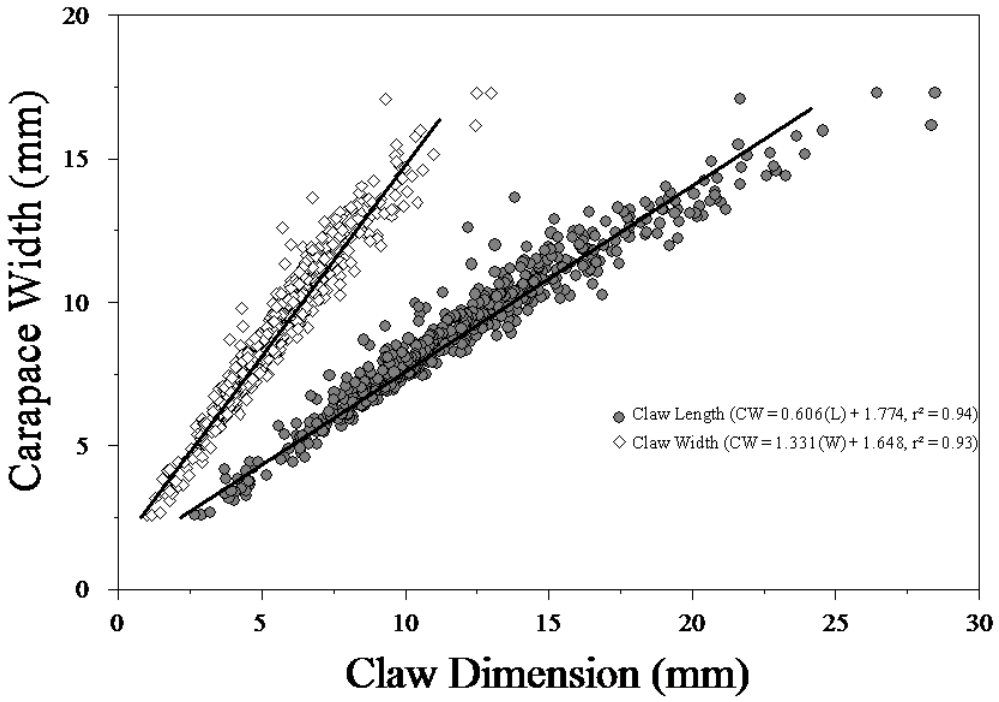


Figure 3. Carapace width as related to claw size in *Petrolisthes cinctipes.* Regression equations for carapace width (CW) based on each claw length (L) and claw width (W) were used to estimate the CW of the released animals and bin individuals into size classes for this study as described in the text.

## 3 RESULTS

Determining how genetic variation is partitioned among age classes, and among geographic locations through time can provide crucial insights into processes such as gene flow and local adaptation. Thus, in the sections that follow, the data are analyzed by site, year and size class to compare the relative contributions of each factor to the overall pattern of geographic population structure. Further details and the data not shown here can be found in Toonen (2001).

### 3.1 Carapace width measurements and size class assignment

The two preliminary collections made at Ft. Bragg and Salt Point, CA revealed that both claw size dimensions were a reliable predictor of crab carapace width (Figure 3), and that male and female crabs showed similar allometry ($r^2 = 0.99$, $n = 543$, $p < 0.001$), data not shown. Thus, there was no need to sex crabs in the field, and a single regression model for each claw length ($CW = 1.774 + 0.606 \cdot L$) and claw width ($CW = 1.648 + 1.331 \cdot W$) was sufficient to reliably estimate crab carapace width for binning into size classes from the preserved chela in the laboratory (Figure 3). If the CW estimated from the claw length regression model differed from that estimated from claw width by more than 1.0 mm, the sample was excluded from further analysis; if the CW estimates derived from both length and width regressions were within 1.0mm, the CW was calculated as the average of the two estimates. Recruits were defined as non-pigmented postlarvae that must have settled within at most a couple of weeks prior to collection (Jensen 1991). Subadults were defined as individuals between 5.0 and 7.0 mm CW, likely corresponding to individuals at least one year, but not more than two years of age. Adults were those individuals > 9.0 mm CW, corresponding to individuals likely 3+ years old. We caution that the relationship between carapace width and age is, at best, an approximation because there is considerable variation in conditions among sites and individual growth rates among locations and even within a site depending on habitat quality and individual density (Donahue 2004).

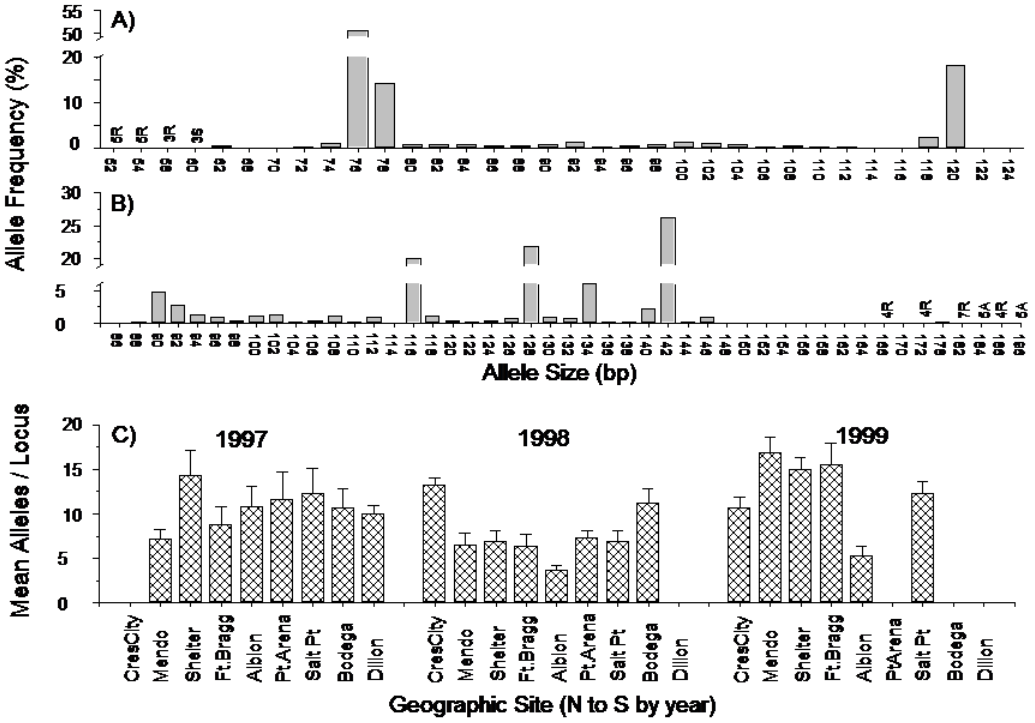


Figure 4. Allele frequency distribution for locus [A] Pc156s and [B] Pc170s from all *Petrolisthes cinctipes* individuals sampled across sites and years. Numbered bars (e.g., 5R) represent private alleles found only in site 5 (Albion) in the R (recruit) size class. The mean number of alleles per locus (error bars represent the maximum value) at each site [C] is also plotted.

3.2 Overall patterns of microsatellite variation - all samples pooled

As outlined above, Dillon Beach (site 9) was only sampled in 1997, whereas Crescent City (site 1) was only sampled in 1998 and 1999. The samples from Pt. Arena (site 6) and Bodega Bay (site 8) in 1999 were lost, and thus the overall collections included 978 crabs genotyped from the 8 sites sampled in 1997, 1,372 crabs genotyped from the 8 sites sampled in 1998, and 1,252 crabs genotyped from the 6 sites sampled in 1999. Expected heterozygosities ranged from 0.68 to 0.83 across all 3,602 crabs genotyped in this study (Table 1).

In most cases, the number of individuals of each size class scored at each site was > 50 and was sufficient to minimize bias in estimates of genetic structure and distance (Ruzzante 1998; Kalinowski 2005). Exclusion of those sites with fewer than 50 individuals available did not alter our conclusions, (data not shown), therefore all samples are included in the analyses presented here. The microsatellite loci used in this study are highly polymorphic, with 34 alleles for locus Pc156s, and 47 alleles at locus Pc170s, but most alleles were rare and only 3 or 4 alleles were found in the majority of individuals (~80%) scored (Figure 4). The overall allelic size ranges were 52 - 124 bp for Pc156s and 86 - 196 bp for Pc170s and the allele frequency distributions are presented in Figure 4. Private alleles occurred at very low frequencies at each locus, and seven of the ten private alleles detected in this study were found among recruits, but interestingly all occurred in the central portion of the northern California range, between Shelter Cove (#3) and Salt Point (#7).

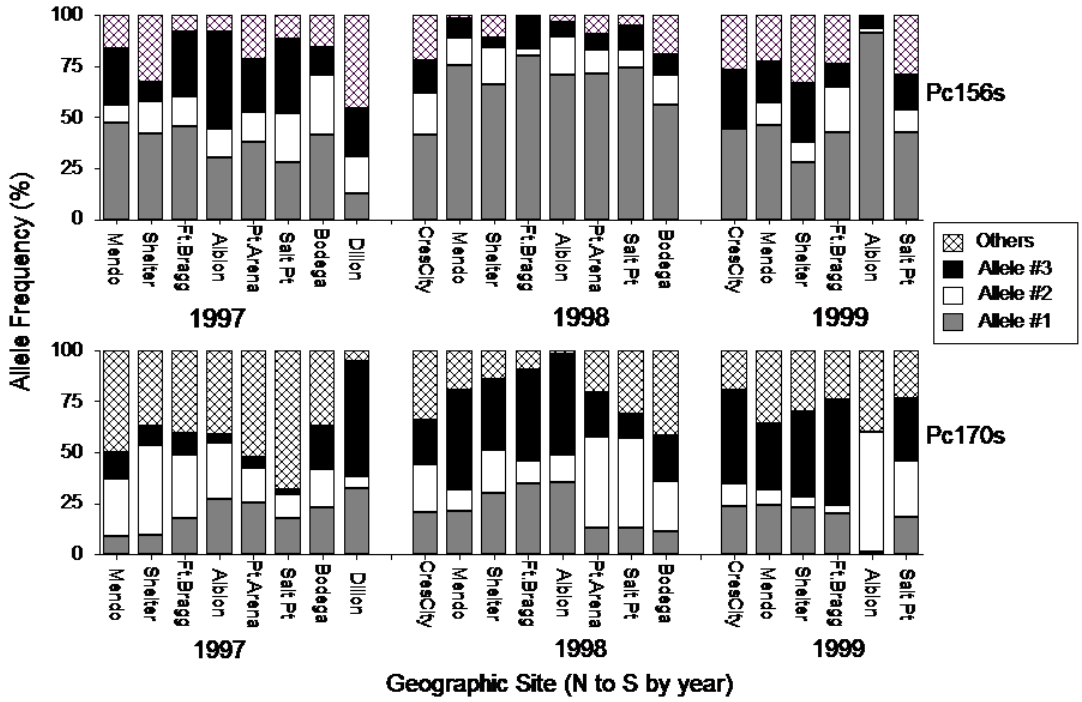


Figure 5. Allele frequency shifts in *Petrolisthes cinctipes* sampled across sites and years. The three most common alleles (76, 78 & 120 for Pc156s; 116, 128 & 142 for Pc170s) are each plotted as Alleles #1, 2 & 3, and all other alleles are binned into the 'Others' category for purposes of illustration here.

Geographic patterns of variation in the number of alleles per locus at each site were similar for both loci (ANOVA, $F = 1.12$, $df = 1$, $p > 0.05$). The mean number of alleles per locus varied from a low of three (1998, residents at Albion) to a high of 22 (1999, residents at Ft. Bragg), but differences among sites (ANOVA, $F = 1.86$, $df = 7$, $p > 0.05$) and size classes (ANOVA, $F = 0.08$, $df = 2$, $p > 0.05$) were not significant (Figure 4). Although there were no significant differences within a sampling year, the mean number of alleles per locus (13.90 ± 1.06 SE in 1997, 9.17 ± 0.95 SE in 1998, and 13.59 ± 1.12 SE in 1999)

did vary significantly across years (ANOVA, $F = 11.30$, $df = 2$, $p < 0.05$). Detailed locus-by-locus allele frequency distributions by site, size class, and year are available in Toonen (2001).

Table 2. Summary of microsatellite data collected for each size class of *Petrolisthes cinctipes* pooled across sites in Northern California in each of 1997-99. The number of individual crabs genotyped from each size class at each site is presented (N) along with the observed and expected heterozygosities for each locus, and the probability value for conformity to Hardy-Weinberg equilibrium (see text). *P*-values less than 0.5 are highlighted in bold, although after False Discovery Rate (FDR) correction for multiple tests none of the deviations from H-W expectation are significant.

| Year | Size class | N | Heterozygosity | | H-W *p*-value ± SE |
|---|---|---|---|---|---|
| | | | Observed | Expected | |
| **Locus Pc156s** | | | | | |
| **1997** | recruits | 186 | 0.65 | 0.76 | 0.25 ± 0.01 |
| | subadults | 402 | 0.70 | 0.74 | 0.18 ± 0.01 |
| | adults | 390 | 0.54 | 0.76 | **0.03 ± 0.01** |
| **1998** | recruits | 518 | 0.51 | 0.58 | 0.11 ± 0.01 |
| | subadults | 428 | 0.51 | 0.57 | 0.35 ± 0.02 |
| | adults | 426 | 0.47 | 0.50 | 0.24 ± 0.01 |
| **1999** | recruits | 320 | 0.66 | 0.71 | 0.27 ± 0.01 |
| | subadults | 492 | 0.67 | 0.77 | 0.24 ± 0.01 |
| | adults | 440 | 0.65 | 0.78 | 0.11 ± 0.01 |
| **Locus Pc170s** | | | | | |
| **1997** | recruits | 184 | 0.65 | 0.91 | 0.10 ± 0.01 |
| | subadults | 396 | 0.84 | 0.87 | 0.13 ± 0.01 |
| | adults | 390 | 0.81 | 0.88 | 0.06 ± 0.01 |
| **1998** | recruits | 516 | 0.74 | 0.78 | **0.01 ± 0.01** |
| | subadults | 428 | 0.72 | 0.80 | 0.06 ± 0.01 |
| | adults | 426 | 0.74 | 0.80 | 0.24 ± 0.01 |
| **1999** | recruits | 316 | 0.72 | 0.82 | 0.20 ± 0.01 |
| | subadults | 492 | 0.79 | 0.78 | 0.16 ± 0.01 |
| | adults | 440 | 0.80 | 0.80 | 0.24 ± 0.02 |

Because there are only two loci included in these analyses, jackknifing and bootstrapping across loci to calculate confidence intervals and standard errors for estimates of population structure is not informative. In almost all cases the general trend revealed by both loci was concordant, and differences in estimates of population subdivision and genetic distance generated by each locus were slight (Tables 2 & 3).

3.3 Conformity to HWE among size classes, sites and years – pooled sites

When each size class, site and year sample was examined individually, there were 25 significant deviations from Hardy-Weinberg expectations, even after correction for multiple tests (data not shown). When sites were pooled within years, however, observed heterozygosities were much closer to expectations, and data generally conformed to expectations of HWE for each size class and each year tested (Table 2). There was one significant deviation from HWE for each locus Pc156s and Pc170s, but neither was significant after FDR correction for multiple tests (Benjamini & Yekutieli 2001).

Table 3. Variation in genetic structure among years partitioned by size class for *Petrolisthes cinctipes* populations along the Northern California coastline. The overall hierarchical analysis evaluates how structure is partitioned among years ($\theta_Y$), among sites ($\theta_S$) and among size classes ($\theta_C$) and is presented below for comparison.

| **Locus** | **N** | ***F*** **(within total)** | $\boldsymbol{\theta_Y}$ **(among years)** | $\boldsymbol{\theta_S}$ **(among sites)** | ***f*** **(within years & sites)** |
|---|---|---|---|---|---|
| **Recruits** | | | | | |
| **Pc156s** | 1024 | 0.15 | 0.11 | 0.03 | 0.05 |
| **Pc170s** | 1016 | 0.12 | 0.12 | 0.0005 | - 0.001 |
| **Overall** | 1016 | 0.13 | 0.12 | 0.01 | 0.02 |
| **Subadults** | | | | | |
| **Pc156s** | 1322 | 0.22 | 0.11 | 0.04 | 0.02 |
| **Pc170s** | 1316 | 0.05 | 0.08 | 0.02 | - 0.01 |
| **Overall** | 1316 | 0.13 | 0.09 | 0.03 | 0.002 |
| **Adults** | | | | | |
| **Pc156s** | 1256 | 0.10 | 0.09 | 0.06 | 0.12 |
| **Pc170s** | 1256 | 0.06 | 0.07 | 0.05 | - 0.03 |
| **Overall** | 1256 | 0.08 | 0.08 | 0.05 | 0.04 |

| Overall hierarchical analysis all 3588 samples for which both loci were scored | | | | | |
|---|---|---|---|---|---|
| **Locus** | ***F*** | $\boldsymbol{\theta_Y}$ **(years)** | $\boldsymbol{\theta_S}$ **(sites)** | $\boldsymbol{\theta_C}$ **(size classes)** | ***f*** |
| **Pc156s** | 0.13 | 0.05 | - 0.01 | 0.08 | 0.06 |
| **Pc170s** | 0.08 | 0.04 | 0.01 | 0.08 | - 0.02 |
| **Overall** | 0.10 | 0.05 | - 0.0001 | 0.08 | 0.02 |

3.4 Allele frequencies among sites and years - size classes pooled

As highlighted in Figure 4, three common alleles account for roughly 80% of the data for each locus. Because rare alleles do not contribute much to the overall estimation of $F_{ST}$ and are difficult to visualize, we plot these three most common alleles and a binned category of 'other' alleles (Figure 5) to illustrate the

significant year effect across sites in annual allele frequency fluctuations (ANOVA, $F = 12.35$, $df = 7$, $p < 0.01$). In contrast to the year effect, there are no obvious patterns of variation among sites and a similar analysis of the site effect across years is not significant (ANOVA, $F = 0.96$, $df = 23$, $p > 0.05$).

3.5 Genetic structure among sites and years - adult crabs only

In order to compare these temporal results with an initial survey of population structure across the species range which included only adults (Toonen 2001), we also analyzed the adult crab size class collected in each year separately to examine how similar were patterns of geographic population structure in each of the four sampling collections. Estimates of population structure were highly consistent among adults collected in each year, with an estimated $\theta$ of 0.07 among the adults collected in 1997, 0.08 for 1998, and 0.08 for 1999. Each of these estimates is similar to that estimated in the original geographic survey across the species range, in which the estimate of $\theta$ across these same sites was 0.08, and across the species range was 0.11. Based on the relationship $N_em = (1 - F_{ST}) / 4F_{ST}$ (Wright 1978), the 95% confidence intervals for the effective number of migrants per generation was 1.75 - 3.43 for 1997, 4.39 - 11.34 for 1998, and 2.78 - 5.37 for 1999. When the overall data set is analyzed with samples from all three years pooled, the estimate of $N_em$ is 11.80 - 24.93 which is significantly higher than any of the single-year estimates.

Table 4. Hierarchical analysis of molecular variance (AMOVA) results for *Petrolisthes cinctipes* examining the relative contribution of size class, sampling year, and site. The relative proportion of genetic variation explained by years, sites, and size class are assessed. Probability of obtaining a more extreme variance component and $\Phi$ statistic by chance alone was determined by permutation test (500,000) in ARLEQUIN, and significant values ($\alpha = 0.01$) are denoted by an asterisk.

| Source of Variation | d.f. | Variance component | % of variation | $\Phi$ statistic |
|---|---|---|---|---|
| **Sites within Years** | | | | |
| Among years | 2 | 19547.953 | 5.29 | $\Phi_{CT} = 0.05$* |
| Among sites, within years | 18 | 2660.934 | 0.52 | $\Phi_{SC} = 0.005$* |
| Within sites | 7170 | 501213.447 | 94.19 | $\Phi_{ST} = 0.06$* |
| **Size Classes within Sites** | | | | |
| Among sites | 7 | 6837.629 | 0.83 | $\Phi_{CT} = 0.008$ |
| Among size classes, within sites | 16 | 6756.074 | 1.31 | $\Phi_{SC} = 0.01$* |
| Within size classes | 7164 | 509842.098 | 97.86 | $\Phi_{ST} = 0.02$* |
| **Size Classes within Years** | | | | |
| Among years | 2 | 19551.381 | 4.67 | $\Phi_{CT} = 0.05$* |
| Among size classes, within years | 6 | 25504.378 | 5.13 | $\Phi_{SC} = 0.05$* |
| Within size classes | 7194 | 478379.715 | 90.20 | $\Phi_{ST} = 0.10$* |

3.6 Genetic structure by year, site and size class

Estimates of subdivision among recruits, subadults, and adults are all of roughly the same magnitude in each sampling year, and averaging across size classes in each year returns the consistent estimate for θ of roughly 0.08 seen in previous analyses. There are, however, some interesting trends in the data. First, recruits have significantly more structure from year-to-year at a given site than do the residents (ANOVA, $F = 7.88$, $df = 1$, $p = 0.03$). Likewise, recruits show less geographic structure among sites within any given year than do the residents (ANOVA, $F = 7.23$, $df = 1$, $p = 0.03$).

Table 5. Variation in genetic structure among years partitioned by size class (pooled across loci and sites) for *Petrolisthes cinctipes* populations along the Northern California coastline as compared to the initial geographic survey of population structure (Toonen 2001) across the same range. Genetic differentiation standardized for marker heterozygosity ($\phi'_{ST}$) is calculated following Meirmans (2006).

| Size class | N | F (within total) | θ (among sites) | f (within sites) | $\phi'_{ST}$ (among sites) |
|---|---|---|---|---|---|
| **1997** | | | | | |
| **Recruits** | 224 | 0.20 | 0.08 | 0.14 | 0.48 |
| **Subadults** | 402 | 0.08 | 0.08 | 0.00 | 0.41 |
| **Adults** | 390 | 0.15 | 0.07 | 0.09 | 0.31 |
| **Overall** | 1016 | 0.15 | 0.07 | 0.09 | 0.42 |
| **1998** | | | | | |
| **Recruits** | 462 | 0.09 | 0.10 | - 0.01 | 0.39 |
| **Subadults** | 428 | 0.06 | 0.06 | 0.02 | 0.19 |
| **Adults** | 426 | 0.01 | 0.08 | - 0.08 | 0.22 |
| **Overall** | 1316 | 0.01 | 0.08 | - 0.08 | 0.25 |
| **1999** | | | | | |
| **Recruits** | 322 | 0.09 | 0.12 | - 0.03 | 0.51 |
| **Subadults** | 492 | 0.04 | 0.05 | - 0.01 | 0.22 |
| **Adults** | 442 | 0.11 | 0.08 | 0.03 | 0.38 |
| **Overall** | 1256 | 0.11 | 0.08 | 0.03 | 0.35 |
| **Initial Geographic Survey** | | | | | |
| **Mixed collection** | 776 | 0.08 | 0.08 | 0.04 | 0.33 |

Using θ in the hierarchical nesting scheme of Weir (1996), subdivision among sites, among size classes, and among years can be estimated simultaneously. For clarity, we denote the various hierarchical θ values as $\theta_Y$ for among years, $\theta_S$ for among sites, and $\theta_C$ for among size classes. The hierarchical analysis indicates that for recruits, there is far greater similarity among geographic sites within a year ($\theta_S < 0.01$) than at a single site among years ($\theta_Y = 0.05$). There is an order of magnitude more structure among years ($\theta_Y = 0.12$) than among sites ($\theta_S = 0.01$) for recruits, whereas for adults the among year and among site

differentiation are of approximately equal magnitude ($\theta_Y = 0.08$, $\theta_S = 0.05$; Table 3). Overall population subdivision among sites is lowest for recruits ($\theta_S = 0.01$), but the among sites differentiation increases sequentially for subadults ($\theta_S = 0.03$) and adults ($\theta_S = 0.05$). Estimates of $\theta_Y$ among years, on the other hand, are highest for recruits ($\theta_Y = 0.12$), but the among year differentiation decreases sequentially for subadults ($\theta_Y = 0.09$) and adults ($\theta_Y = 0.08$). It is noteworthy that in each year of sampling, there is more genetic structure among size classes than among sites (ANOVA, $F = 121$, $df = 1$, $p < 0.01$). Comparison of the relative effects of year, site and size class in a single hierarchical analysis (Table 3) indicates that the observed population subdivision results from significant variation among size classes ($\theta_C = 0.08$) and years ($\theta_Y = 0.05$) rather than among sites ($\theta_S < 0.01$, not significant).

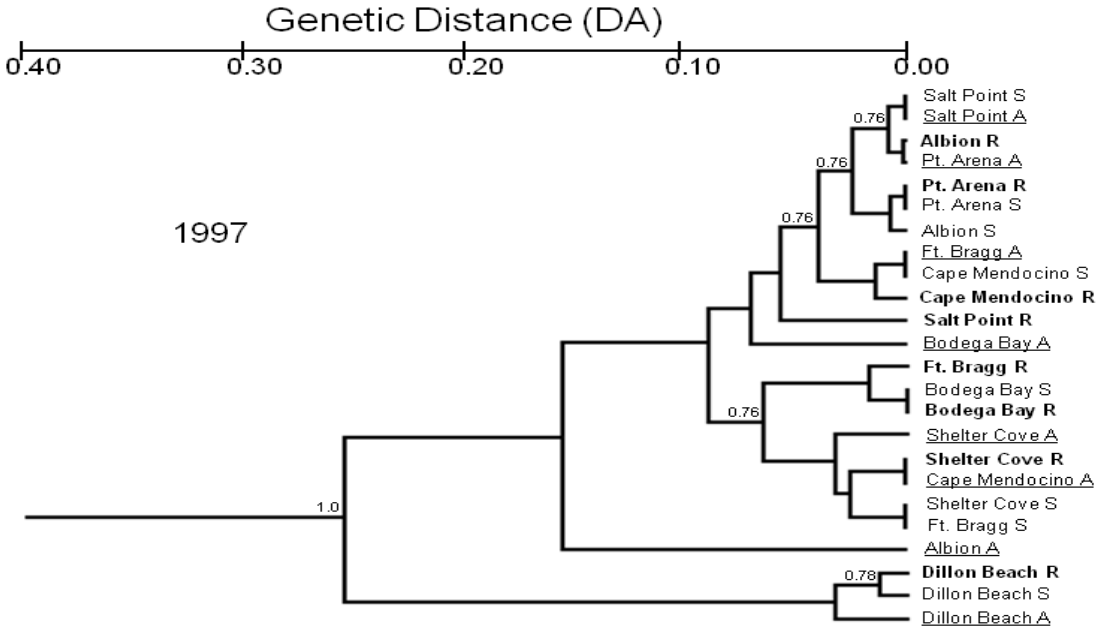


Figure 6. Genetic similarity dendrogram for *Petrolisthes cinctipes* size classes from each geographic site sampled in 1997, based on the DA genetic distance of Nei. Sites of collection are named, and recruits sampled from each site are denoted by R in bold (**Site R**), adults denoted by A are underlined (Site A) and subadults denoted by S are presented in normal text (Site S). Values from 10,000 bootstraps that are ≥.0.75 are presented next to the nodes.

The analysis of molecular variance (AMOVA) supports the same interpretation. Regardless of the grouping chosen, variation among sites explains less than 1% of the overall genetic variation (Table 4). Although only about 10% of the variation is explained by the best model (which partitions among size classes within years, and does not include the site), the result is consistent across all analyses that differences among size classes and years exceed those among geographic sites (Table 4). The pattern of significantly greater differentiation among recruits than among residents at each site is also consistent whether fixation indices are standardized for within population heterozygosity (Hedrick 2005; Meirmans 2006) or not (Table 5).

3.7 Genetic distance among populations among years

Although the exact clustering of sites and the branch length varied among different measures of genetic distance, results were qualitatively similar regardless of whether the UPGMA or NJ was used to construct the dendrogram, and whether the distance measure used was Cavalli-Sforza & Edwards chord distance (Edwards & Cavalli-Sforza 1965; Cavalli-Sforza & Edwards 1967), Nei's $D_A$ (Nei 1972, 1978), coancestry (Reynolds et al. 1983), or $(\delta\mu)^2$ (Goldstein et al. 1995), data not shown. Thus we present only the dendrograms based on Nei's $D_A$ genetic distance here. Overall we found little consistency among sampling years in the patterns of

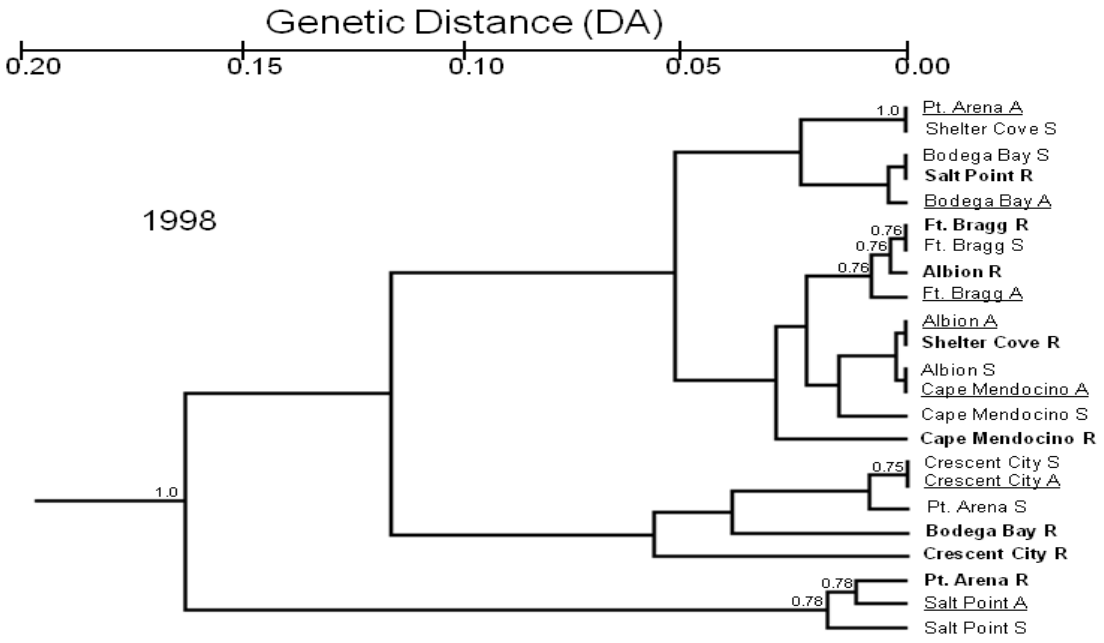


Figure 7. Genetic similarity dendrogram for *Petrolisthes cinctipes* size classes from each geographic site sampled in 1998, based on the DA genetic distance of Nei. Sites of collection are named, and recruits sampled from each site are denoted by R in bold (**Site R**), adults denoted by A are underlined (Site A) and subadults denoted by S are presented in normal text (Site S). Values from 10,000 bootstraps that are ≥.0.75 are presented next to the nodes.

genetic similarity within and among sites (Figures 6-8). For example, residents (adults and subadults) at Salt Point show little genetic differentiation ($D_A < 0.10$) from residents at most other northern California sites in 1997 and 1999 (Figures 6 & 8). In 1998, however, residents at Salt Point are separated from residents at all other sites sampled by the greatest genetic distance ($D_A > 0.15$) measured in that year (Figure 7). Likewise, residents at Albion in 1998 cluster in the middle of the Northern California sampling sites, whereas in 1999 Albion is highly differentiated from residents at any other site sampled (Figures 7 & 8). Neither the magnitude of differentiation among samples (ranging from $D_A$ ~0.17 in 1998 to > 0.40 in 1999), nor the pattern of clustering of sites or size classes are consistent from year-to-year (Figures 6-8). In addition to the used the genetic clustering methods Bayesian assignment tests confirmed annual dispersal variability, with the population of origin inferred from Figures 6-8 being one of the top five scores for assignment of most recruits (data not shown).

## 4 DISCUSSION

Here we show a striking similarity of $F_{ST}$ estimates from four replicate surveys of population structure in the porcelain crab *Petrolisthes cinctipes*, with an average estimate of $\theta = 0.08$ (Table 5). Despite this consistent and repeatable geographic structure, there are pairwise comparisons among nearby sites that exceed those across most of the species range (e.g., Bodega Bay & Dillon Beach, only 10 km apart, pairwise $F_{ST} = 0.16$) were greater than comparisons across much of the species range (e.g., Bodega Bay, CA to Neah Bay, WA, nearly 1200km apart, $F_{ST} = 0.14$). This chaotic genetic structure is a common finding among studies of marine populations, but an understanding of the drivers of such non-spatial genetic structure is lacking. Despite long-standing and growing evidence that temporal genetic analysis can contribute substantially to our understanding of population dynamics and structure in marine species (e.g., Koehn et al. 1976; Todd et al. 1988; Hedgecock 1994; Johnson & Wernham 1999; Moberg & Burton 2000; Hauser et al. 2002; Turner et al. 2002; Lee & Boulding 2009), studies that compare the genetic composition of populations through time, or of different age classes within a population remain the exception rather than the rule. Interpreting patterns of genetic structure in natural populations is problematic because a particular pattern may be generated by

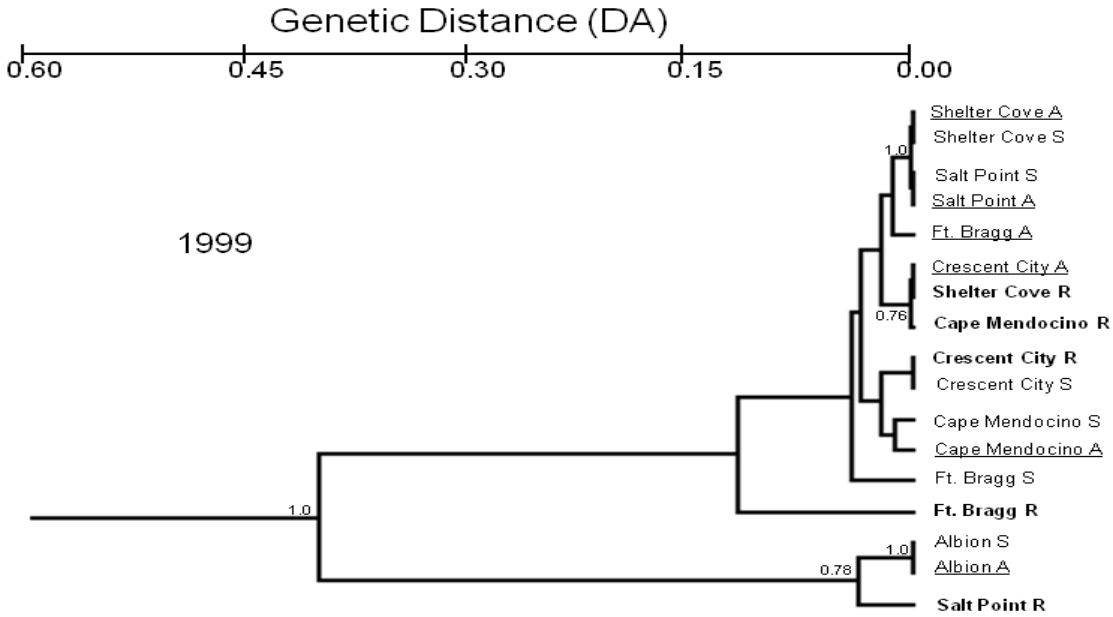


Figure 8. Genetic similarity dendrogram for *Petrolisthes cinctipes* size classes from each geographic site sampled in 1999, based on the DA genetic distance of Nei. Sites of collection are named, and recruits sampled from each site are denoted by R in bold (**Site R**), adults denoted by A are underlined (Site A) and subadults denoted by S are presented in normal text (Site S). Values from 10,000 bootstraps that are ≥.0.75 are presented next to the nodes.

both historical and contemporary processes, acting singly or in combination, at a variety of different spatial and temporal scales (reviewed by Grosberg & Cunningham 2001; Hellberg et al. 2002; Palumbi 2003; Marko 2004; Hellberg 2009). Furthermore, most methods for detecting patterns of genetic structure and inferring gene flow assume that any genetic signal from historical events has been erased by the combined effects of migration and drift (reviewed by Luikart & England 1999; Grosberg & Cunningham 2001). However, few studies of population structure among marine organisms test this assumption explicitly (e.g., Ayre 1990; Lessios et al. 1994; Hellberg 1995; Marko 1998; Wares & Cunningham 2001; Fauvelot & Planes 2002), and many authors have argued that few marine species satisfy fully the expectations of equilibrium between genetic drift and migration across the entire species range (reviewed by Palumbi 1994; Neigel 1997; Benzie 1999; Grosberg & Cunningham 2001; Hellberg et al. 2002; Marko 2004; Hedgecock et al. 2007; Hellberg 2009). The detailed spatial and temporal sampling herein provides insights into the processes structuring populations of *P. cinctipes* and allows us to infer the relative roles of each of the hypothesized mechanisms for creating chaotic genetic patchiness in marine populations, which we discuss below.

4.1 Larval dispersal as a function of PLD

A pelagic larval duration (PLD) of more than a month for *Petrolisthes cinctipes* is generally thought to imply great potential for larval dispersal and mixing. Looking at the estimates of $N_em$ derived from $F_{ST}$ here (1.75 - 11.34), we would infer that larvae fall far short of their potential for dispersal. This inference is misleading relative to the overall size structured data set however, which shows evidence for more broad dispersal of larvae within each year of study; looking at the population structure by size class and year, we would draw the opposite conclusion because $\theta_S$ is not significantly different from zero for the recruit size class (Table 6).

Intuitive predictions of dispersal potential based on PLD have a variety of caveats. Although many reviews report a significant relationship between larval dispersal potential and degree of genetic subdivision (reviewed by Waples 1987; Bohonak 1999; Shanks et al. 2003; Siegel et al. 2003; Kinlan et al. 2005), there are numerous cases in which dispersal potential only weakly predicted genetic structure (reviewed by Todd et al. 1998; Grosberg & Cunningham 2001). For example, Bird et al. (2007) showed that sister-species of endemic Hawaiian limpets with similar ecology, life-history and larval development times show dramatically

different patterns of population structure across their range. These exceptions highlight that our understanding of processes generating genetic structure among marine populations, especially the role of contemporary dispersal, is far from complete (e.g., Johnson & Black 1982; Johnson & Black 1984a, b; Watts et al. 1990; Kordos & Burton 1993; Edmands et al. 1996; Moberg & Burton 2000). In fact recent meta-analyses of the relationship between PLD and estimates of dispersal question whether the intuitive relationship is supported by the literature at all. Several recent meta-analyses find that contrary to earlier reviews the overall relationship between PLD and population genetic structure is weak at best, and that other factors override the diffusive predictions of dispersal based on the length of time larvae spend developing in the plankton (Bradbury & Bentzen 2007; Bradbury et al. 2008, Weersing & Toonen 2009; Shanks 2009; Ross et al. 2009).

4.2 Genetic markers and variability

We originally intended to include many more microsatellite loci in this study, but only two of the initial set of 17 loci passed our quality control testing (Toonen 2001). It has become standard practice in the literature to include 4 to 10 loci in any population genetic survey based on microsatellites, but the exact reasons for this rule of thumb are obscure, other than in general, the use of many loci is essentially an insurance of genomic concordance because each marker can be considered a single sample of the genome, and may reconstruct a slightly different genealogical history due to recombination, random drift or selective forces (Selkoe & Toonen 2006). However, there are also empirical and theoretical studies showing that power is not always increased by the inclusion of more loci; in some cases, increasing numbers of loci can even decrease power (Ryman & Jorde 2001). This counter-intuitive result can result from the large number of factors affecting power, including the number of samples, the size and equivalency of those samples, the magnitude of the true divergence, the number and type of loci assayed, the polymorphism of the markers, and their frequency distributions (Ryman et al. 2006). The common finding of these recent surveys is that, all else being equal, power is a function of the total number of alleles included in the analysis, regardless of how those alleles are distributed (Ryman & Jorde 2001; Kalinowski 2002; Ryman et al. 2006). The equivalent utility of alleles, whether distributed within or across loci, for estimating genetic distances described means that the same power is attained using few highly polymorphic loci or many loci of low polymorphism; the only requirement is that a sufficient number of alleles be examined (Kalinowski 2002). For the sample size (~50) and degree of divergence ($F_{ST}$ = 0.08) in this study, power approaches 1.0 with greater than 10 alleles (Ryman et al. 2006), and for $D_A$ the estimate of genetic distance has minimal variance by 32 alleles (Kalinowski 2002). Thus, the two polymorphic microsatellite loci here, which each have more than 30 alleles, have sufficient power for the analyses reported here. Additionally, initial analyses of mtDNA COI sequences from each of these sites revealed similar geographic structure to the microsatellite results reported here (Toonen, unpubl. data). Also, there is a striking similarity of $F_{ST}$ estimates from the initial geographic survey of adults, and each of the three annual surveys presented in this study (Toonen 2001), with average estimates of θ across all four surveys being 0.08 (Table 5). The patterns and interpretation are similar whether or not we apply an $F_{ST}$ correction for within-population heterozygosity (Hedrick 2005, Meirmans 2006). Although the magnitude of divergence among samples is quite different (Table 5), the overall inference from these data is similar whether looking at standardized or unstandardized fixation indices across all analyses (data not shown). Insofar as the loci used in this study are neutral and provide an accurate representation of the genetic makeup of *Petrolisthes cinctipes*, there is no reason to doubt that inferences regarding genetic structure of this species drawn from these two loci are reliable.

Although there are caveats (Whitlock & McCauley 1999), theory predicts that drift is a weak force in large populations, and the temporal shift in allele frequencies should be small; hence a single sampling of a population at drift/migration equilibrium is sufficient. Although the census size of many marine populations is enormous, the effective population size, $N_e$, which determines the strength of drift in those populations may be orders of magnitude smaller (e.g., Hedgecock 1994, Turner et al. 2002; reviewed by Hedgecock et al. 2007). In the case of *P. cinctipes*, the census size is immense and even if $N_e$ was three orders of magnitude smaller, the effective population size would still be sufficient to make drift a weak force. The presence of many alleles is expected in a species with a census population size on the order of $10^8$; and the probability of persistence for any new allele that arises in the population is a function of population size ($1-1/2N_e$). Thus the persistence of rare alleles is more likely in larger populations (Hartl & Clark 1997). Just

as the probability of losing a rare allele is lower, the probability that common alleles persist at the same frequency from generation-to-generation is higher the larger the population (Hartl & Clark 1997). The number of alleles per locus and the prevalence of rare alleles are both consistent with a large population size. The expected temporal consistency from such life history characters is not supported, however; there is substantial temporal variation seen among years within sites (Figure 5). Although counter to expectations, this study is not alone in showing temporal variation in allele frequencies among species in which stability would be expected; a growing number of studies are finding substantial temporal variation in allele frequencies even in species that do not show significant spatial structure at any given time point (e.g., Toonen 2001; Robainas et al. 2005; Florin & Höglund 2007; Lee & Boulding 2007, Lee & Boulding 2009). The typical hypotheses to explain such unexpected non-geographic patterns of population structure include: 1) variation in source of larval recruits, 2) self-recruitment and local subdivision, 3) variance in reproductive success (sweepstakes reproduction), and 4) pre- or post-settlement natural selection. Below, we discuss each of these hypotheses in turn.

4.3 Variation in the source of larval recruits

The source of recruits varies unpredictably from year-to-year (Figures 6-8). Although assignment tests do not place individuals with confidence into source populations, the results are concordant with the genetic distance approach (data not shown). Compiling a distribution of the top 5 sites for each individual recruit leads to a very similar inference to that drawn from the figures. Although neither analysis is entirely convincing, the concordance of the two different approaches with different assumptions lends some confidence to these conclusions.

Interestingly, the overall trend of genetic differentiation among sites generally matches the expectation of ENSO-driven currents. 1997-99 coincided with a major El Niño-Southern Oscillation (ENSO) event. The El Niño of 1997 resulted in the weakest upwelling recorded to date the 54-year database spanning 1946 to 1999 (NOAA Environmental Research Division – www.pfeg.noaa.gov). The 1998 El Niño transition showed an upwelling index that was not significantly different than the rest of the 54-year average (Diehl et al. 2007), although it is important to note that most of the preceding 21 years show anomalously low upwelling due to the warm phase of the Pacific Decadal Oscillation (NOAA PFEG). The 1999 La Niña, in contrast, had stronger and more consistent upwelling than either of the previous years. General predictions of the effect of wind-driven currents on crustacean recruitment throughout California are reviewed in Diehl et al. (2007), but essentially the 1997 and the spawning season of 1998 were El Niño years are characterized by weak upwelling and warmer sea water temperatures, whereas 1999 La Niña was dominated by unusually low temperatures and strong upwelling. Thus, there should be a major shift in the likelihood of larval transport alongshore between the 1997-1998 El Niño (in which frequent relaxation events should bring larvae up the coast from the south) and the consistent upwelling of the 1999 La Niña event (which should bring larvae down from the north). Despite the unpredictability of the annual source of recruits, our data support that prediction; the $D_A$ trees from 1997 and 1998 do not cluster recruits with a single population of adults to the north of the site at which recruits were collected. In striking contrast, none of the recruit samples collected in 1999 cluster with adult populations located to the south, and three of the five cluster with populations to north. Assignment tests support similar conclusions, with recruits generally assigning as self-recruits or to populations from the south during the 1997-1998 El Niño, and self-recruits or populations to the north during the 1999 La Niña. Significant ENSO-induced shifts in population genetic structure have been reported in a variety of other taxa, such as the work by Fauvelot et al. (2006) on tropical butterflies.

There are several different mechanisms proposed by which variation in the source of larval recruits may result in fine-scale chaotic genetic patchiness. The first possible mechanism is that substantial microgeographic population structure exists from a localized Wahlund effect. A Wahlund effect, the departure from Hardy Weinberg equilibrium due to mixing of individuals from groups with different allelic frequencies, could account for heterozygote deficiencies on small scales in some species (e.g., Johnson & Black 1984b; Kijima et al. 1987; Waples 1990a). In the case of the limpet *Siphonaria*, there is little geographic variation in allozyme frequencies at the scale of the species range (Johnson & Black 1982; Johnson & Black 1984b). Despite this large scale uniformity, however, there exists significant fine scale genetic patchiness, which is repeated, rather than accumulated, on the larger scale (Johnson & Black 1984b). Neither geographic or temporal variation in allelic frequencies can explain the pattern. Instead, the fine-scale structure appears to result from binomial sampling variance among small local breeding groups that results in

a localized Wahlund effect (Johnson & Black 1984a, b). This hypothesis predicts that there should be a significant deficiency of heterozygotes across sites that disappears as the scale of sampling is reduced to the point that samples are drawn from only the actual interbreeding group. We see no consistent evidence for a Wahlund effect at any scale in these data.

Table 6. Summary of population structure observed for *Petrolisthes cinctipes* by size class and year. Arrows highlight that the among sites component of variation increases from non-significant among recruits through sub-adults and is greatest in adults. The converse is true for the among years component of variation, which is greatest among the recruits and decreases sequentially through sub-adults and is least among adults. (ns - denotes values that are not-significantly different from zero)

| N | F (within total) | θ (among sites) | θ (among sites) | f (within sites) |
|---|---|---|---|---|
| **Recruits** | | | | |
| 1016 | 0.13 | 0.12 | $<0.01^{ns}$ | 0.02 |
| **Subadults** | | | | |
| 1316 | 0.13 | 0.09 | 0.03 | $<0.01^{ns}$ |
| **Adults** | | | | |
| 1256 | 0.08 | 0.08 | 0.05 | 0.04 |

A second possible mechanism is that genetically distinct source populations exist from which planktonic larvae recruit, and the source population varies through time. Differences in seasonal current patterns, spawning season or developmental times among geographic populations may result in temporal variation in the allelic frequencies of recruits arriving at a given site (e.g., Kordos & Burton 1993). Such genetic variation may accumulate within populations and lead to small-scale genetic structure if inter population gene flow is not sufficient to overcome population differentiation resulting from genetic drift and/or natural selection (e.g., Koehn & Williams 1978; Kordos & Burton 1993). The larval source hypothesis predicts that the genetic affinity of recruits to the geographic populations of spawners should vary with time and location, but further requires that adult populations exhibit sufficient differentiation to account for the variation detected among recruits. Temporal variance could also be generated if recruits were coming in from differentiated populations outside the scope of the study region (e.g., Kordos & Burton 1993). In this case the initial geographic survey of population structure across the species range found spatial differentiation was only slightly greater ($\theta = 0.11$) than within the geographic focus area ($\theta = 0.08$) presented here (Toonen 2001). Additionally the greatest pairwise values of θ occurred within the geographic area included here and all private alleles detected across both loci in *P. cinctipes* were found within this central portion of the range (Toonen 2001). Thus, as with the localized Wahlund effect above, the genetic distinctiveness of breeding populations and variation in larval source is insufficient to explain the observed pattern of differentiation among sites and size classes through time (Table 6).

4.4 Self recruitment and local subdivision

The data from both the genetic distance clustering and the assignment tests are consistent with self-recruitment and local retention of larvae in some cases, but the pattern varies among years such that no site shows a consistent source-sink relationship with any other. A mechanism of local retention for along the Pacific coast of North America is the upwelling-relaxation flow mechanism proposed by Botsford, Wing and colleagues (Botsford et al. 1994; Wing et al. 1995a,b; Wing et al. 1998; Morgan et al. 2000). This oceanographic dispersal hypothesis predicts differential delivery of larvae to sites with increasing distance from a headland because of the retention of larvae in transient gyres formed by promontories interrupting southward flow during upwelling (Wing et al. 1995a,b). The resulting differential delivery of larvae to sites

within embayment between promontories (labeled points on Figure 1) should result in frequent and consistent settlement directly to the north of a headland, while settlement of larvae at sites further north will be more sporadic and unpredictable, and transport to the coast south of the headland ought to be minimal. As outlined above, the change in recruit source does generally corresponds to the frequency and intensity of ENSO-driven upwelling, but the pattern of similarity among sites does not conform to predictions of the upwelling-relaxation hypothesis. For example, in 1997, although recruits appear self-recruiting or generally arrive from the south, they also appear to originate consistently from residents of a different embayment rather than the same embayment. In each year of sampling, the pattern of recruitment differs, but the tendency for recruits to cluster with adults from beyond the scope of entrainment predicted by the upwelling-relaxation hypothesis is consistent (Figures 6-8). These data argue that the effect of these oceanic retention zones is variable by year. Nevertheless, Moberg & Burton (2000) emphasize that data such as these do not constitute a strong test of the upwelling-relaxation flow hypothesis, because the temporal and spatial scale of sampling for recruits may not be fine enough. They point out that the effects of retention zones may only apply at the temporal scale of individual relaxation events, and not over the scale of seasons or years. Regardless, the role of self-recruitment and local differentiation across the range of sampling sites is again insufficient to explain the overall patterns of genetic structure reported here.

4.5 Variance in individual reproductive success

In addition to variation among larval sources made possible by long-lived pelagic larvae, variation in individual reproductive success may be great due to the considerable fecundity and high early mortality of many marine species. In highly fecund species, a small minority of individuals may effectively contribute all recruits for the entire population in each generation by a sweepstakes chance matching of reproductive activity with oceanographic conditions conducive to spawning, fertilization, larval survival and successful recruitment (Hedgecock 1986; 1994). The sweepstakes-reproductive success hypothesis can account for both the widespread, large discrepancies commonly found between effective and census population estimates and for local genetic differentiation despite high gene flow (reviewed by Hedgecock 1994; Hedgecock et al. 2007). The sweepstakes hypothesis predicts that allelic frequencies should exhibit significant temporal variation owing to genetic drift, and that genetic diversity should be significantly reduced and temporally variable among related recruits relative to a random sample of the potential spawning population (e.g., Edmands et al. 1996; Li & Hedgecock 1998; Moberg & Burton 2000; Flowers et al. 2002; Planes & Lenfant 2002; Turner et al. 2002; Waples 2002; Selkoe et al. 2006; Lee & Boulding 2009; Buston et al. 2009; Christie et al. 2010). The sweepstakes-reproduction hypothesis is made possible by the enormous fecundity ($10^6$ –$10^8$ eggs\female\reproductive season) and high larval mortality of most marine animals, which makes them fundamentally different from typical mammalian or fruit fly model species (10 to 1000 eggs per reproductive season) (reviewed by Hedgecock et al. 2007). From this perspective, *P. cinctipes* is a low fecundity organism, more similar to mammalian than to the marine species for which the sweepstakes hypothesis was originally proposed. The output of a few individuals clearly cannot contribute the majority of recruits, and thus the results are difficult to explain by sweepstakes reproduction.

The fact that there are significant temporal fluctuations in allelic frequencies and numbers of alleles in the batches of recruits (Toonen 2001), and that the greatest differentiation occurs among annual batches of recruits (Table 6) argues that successful reproduction represents only a small portion of the potential breeding population each time. In all comparisons, recruits differed most between years, whereas adults differed least. Furthermore, if we compare each batch of recruits to the most closely-related resident population (Figures 6-8), the heterozygosity of recruits is significantly reduced relative to that of the residents (paired t-tests, $p < 0.05$). In contrast if recruits are pooled across years and sites, the heterozygosity of recruits is slightly higher than that of pooled resident populations, although this is not significant (paired t-test, $p < 0.05$). The simulations of Turner et al. (2002) demonstrate that variance in reproductive success among different local populations can be a more potent force in reducing effective population size relative to the census population. Although the sweepstakes-reproduction hypothesis is insufficient to account for the pattern of genetic structure reported in *P. cinctipes*, our results are consistent with both individual and population variation in adult reproductive success leading to reduced effective population size and contributing to temporal genetic structure in this species.

4.6 Pre- or post-settlement natural selection

Finally, variable small-scale patterns of population genetic structure may result from pre- or post-settlement natural selection among populations (e.g., Koehn et al. 1976; Powers & Place 1978; Johnson & Black 1984a; Hilbish & Koehn 1985; Gardner & Palmer 1998). Despite geographic proximity of sites, abiotic (such as wave exposure, salinity, nutrient supply, etc.) and biotic (such as competitors and predators) differences among sites have the potential to exert differential selective pressure over very small spatial scales. Although we routinely assume selective neutrality of the genetic markers used in surveys of population genetic structure such as this, markers may be linked or directly subject to natural selection that can either alter or maintain the allelic frequencies among samples obtained from a given site (e.g., Koehn et al. 1976; Powers & Place 1978; Koehn et al. 1980; Hilbish & Koehn 1985; Karl & Avise 1992; McGoldrick et al. 2000), or even among size-classes or tidal heights within a site (e.g., Gardner & Palmer 1998). In general, researchers have inferred the action of natural selection when population surveys using putatively neutral genetic markers revealed clinal or habitat-specific patterns of allelic frequency change (e.g., Koehn et al. 1976; Powers & Place 1978; Hilbish & Koehn 1985; Schmidt & Rand 2001; reviewed by Sotka & Palumbi 2006), or marked differences among loci or classes of genetic markers (e.g., Reeb & Avise 1990; Karl & Avise 1992; Schmidt & Rand 2001). The ecological genetics required to test this hypothesis are not trivial, and with more complex spatial patterns, isolating the specific causes and effects of natural selection can be even more difficult (e.g., Gardner & Palmer 1998).

Using Slatkin's exact test (1994, 1996) to compare the allele frequency distributions to neutral expectations, we see no evidence of selection causing a departure of allele frequency distributions from the Ewens sampling distribution at either of these two loci. Despite the lack of direct evidence for selection, it is difficult to envision a neutral mechanism by which recruits show no significant spatial structure in each year of sampling, but spatial genetic differentiation accumulates sequentially in older age classes to a consistent value in each of 4 replicate studies of the adults (Tables 5 & 6). The probability of randomly selecting two loci under similar selection pressure is small, and populations show significant among year variation of both number of alleles and the frequencies of alleles within sites from year-to-year (Figures 4 & 5). Further, the 1997-99 ENSO event was one of the most extreme that has been recorded to date, and the coastal environmental and nearshore oceanography of 1997 and 1999 ought to be maximally different during this study, but the genetic data do not match that expectation. Thus, selection alone cannot explain the overall results of this study, despite the fact that the pattern of sequentially increasing population structure with age class is suggestive of post-settlement selection, and further study along these lines may be fruitful.

### 4.7 Concordant changes and final conclusions

The $F_{ST}$ estimates drawn from each of the four independent surveys of *P. cinctipes* reported here are highly consistent (Table 5), but could be misleading relative to the picture drawn from the overall analysis of temporal and spatial sampling of age-structured samples presented in this study. In sum, these data provide a rare look into the detailed patterns of population genetic structure across space and time in a replicated study structured explicitly by size-class and location through time. The detailed inference here provides a much richer interpretation of the relative role of different mechanisms in structuring these populations relative to the standard single-time snapshot survey of population genetic structure. Further, these findings emphasize that even replicate studies finding the same overall magnitude of population genetic structure may overlook the underlying variability revealed by this explicit temporally- and spatially-structured analysis.

We find evidence for each of the four primary hypothesized mechanisms driving fine-scale population genetic structure in marine species with larval development, but none of the mechanisms alone appears sufficient to explain the overall pattern of population genetic structure among sites, years and age classes reported herein. Thus, these data are consistent with the action of each of the potential mechanisms, and indicate that it is most likely a synergistic effect of multiple mechanisms that interact to produce the observed population structure in *P. cinctipes*. More similarly detailed empirical studies are necessary to confirm the pattern that these hypotheses attempt to explain. Are there general patterns consistent among groups of marine organisms, or does each species present a unique combination of life history mode, natural history and evolutionary background that effectively prevent useful generalizations? In order to refine theoretical expectations for marine populations that may often be far from equilibrium, which are linked by complex patterns of dispersal, and in which a variety of contemporary and historical processes are likely to acting synergistically, much more detailed empirical studies are needed to characterize the important generalities and differences among species.

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