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Chaotic genetic patchiness without sweepstakes reproduction in the shore crab *Hemigrapsus oregonensis*

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ABSTRACT: Fine-scale spatial and temporal variation in the genetic composition of benthic recruits, known as chaotic genetic patchiness, is often observed in marine and estuarine species with planktonic larvae. Several explanations have been proposed for chaotic genetic patchiness, including sweepstakes reproductive success, variability in larval source, and natural selection. In a survey of the green shore crab *Hemigrapsus oregonensis* in Bodega Bay, California, USA, allele frequencies at a mitochondrial single nucleotide polymorphism were found to differ significantly among samples of first-stage zoeae and between zoeae and adults. Sweepstakes reproductive success is unlikely to be responsible because the fecundity of this species is too low and there was no reduction in genetic diversity among zoeae. In principle, influxes of larvae from genetically distinct populations over 500 km to the north could have caused these differences; however, coalescent estimates indicated that gene flow from these distant populations has been very low and it is unlikely that first-stage zoeae would have been transported such great distances. The possibility remains that natural selection, directly or indirectly, is responsible for the observed patchiness in mitochondrial allele frequencies.

KEY WORDS: Mitochondrial DNA · Single nucleotide polymorphism · Natural selection · Phylogeography

INTRODUCTION

In the 1990s, the hypothesis of sweepstakes reproductive success (SRS) was promoted to explain temporal changes in allozyme frequencies in populations of oysters and other marine species with high fecundity and planktonic larvae (Hedgecock 1994). SRS requires individual fecundity to be so high that it is possible for just a few individuals, the lucky 'sweepstakes winners,' to spawn the majority of a generation's progeny (Hedrick 2005). Such extreme variance in reproductive success would result in genetic drift strong enough to create substantial temporal changes in allele frequencies (reviewed by Hedgecock & Pudovkin 2011).

Another phenomenon cited as evidence of SRS is 'chaotic genetic patchiness' (CGP), whereby small differences in allele frequencies occur among cohorts of settling larvae. The concept of CGP was introduced by Johnson & Black (1982) to describe the fine-scale spatial heterogeneity in allozyme frequencies they observed in an intertidal limpet. They found that patches of recruits that were settling at different times differed in allozyme frequencies because the genetic composition of the larval pool changed over time (Johnson & Black 1984). They also concluded that the genetic changes in the larval pool were caused by natural selection because those changes were larger than could be explained by geographic variation in larval sources. Since then, SRS, rather

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than selection, has often been favored as an explanation for both temporal variance in allele frequencies and CGP (Hedgecock & Pudovkin 2011). Although these 2 explanations might appear compatible, i.e. natural selection could cause variance in reproductive success (e.g. the 'Elm-Oyster Model' of Williams 1975), the hypothesis of SRS specifically excludes selection as the mechanism responsible for the enormous reproductive success of just a few individuals. Instead, SRS assumes that non-selective mechanisms arise from stochasticity in the effects of oceanographic processes on spawning success and the fates of planktonic larvae (Hedgecock & Pudovkin 2011).

It is now appreciated that CGP occurs in many species with planktonic larvae and that selection, SRS, and other mechanisms are considered as the underlying cause (Harrison 1990, Hedgecock 1994, Johannesson et al. 1995, Larson & Julian 1999, Flowers et al. 2002, Turakulov & Easteal 2003, Selkoe et al. 2006, 2008, 2010, Virgilio & Abbiati 2006, Virgilio et al. 2006, Hedgecock et al. 2007, Petersen 2007, Arnaud-Haond et al. 2008, Sella et al. 2009, Hey 2010, Hogan et al. 2010, Hedgecock & Pudovkin 2011, Broquet et al. 2013, Iacchei et al. 2013, Jue et al. 2014, Kesaniemi et al. 2014, Moody et al. 2015). In what may be the most extreme case of CGP, Kordos & Burton (1993) reported large and rapid temporal shifts in allozyme allele frequencies among cohorts of blue crab megalopae settling along the coast of Texas, USA. They proposed that either natural selection or changes in the source of larvae carried by shifting currents were responsible. Additionally, Selkoe et al. (2006) suggested that incomplete mixing of a genetically heterogeneous larval pool could cause CGP in fishes. This appears to be the case for the bicolor damselfish *Stegastes partitus*, where recruits from separate settlement pulses differed in microsatellite allele frequencies and had lower heterozygosity than adults (Christie et al. 2010). These patterns are both consistent with SRS, although there was also direct evidence that the larvae tended to settle near where they were spawned. Thus, genetic patchiness in recruits of the bicolor damselfish appears to be due, at least in part, to incomplete mixing of the larval pool. Although Christie et al. (2010, p. 1051) referred to this as a 'sweepstakes effect,' they were careful to point out that they 'do not believe that the magnitude of this effect equals that of other published studies [of SRS],' in part because 'estimates of the effective number of breeders included infinity as both the lower and upper 95% confidence limits.' We suggest that a distinction should be made between the strict sense of SRS, whereby an entire generation or cohort is dominated by a very small number of breeders (i.e. 10 or fewer), and situations wherein the entire adult population is not uniformly represented in a single localized settlement pulse.

The interpretation of CGP in benthic recruits is often ambiguous because it could be caused by very dissimilar mechanisms that might occur during spawning, larval development, dispersal, or settlement, or during the post-settlement transition to benthic recruitment. The range of possibilities can be narrowed by investigating earlier life stages. For example, if the genetic composition of early-stage larvae is spatially and temporally uniform, genetic heterogeneity that developed later, e.g. among recruits, could not have originated by SRS but instead by some later-acting mechanism, such as variability in the source of later-stage larvae or natural selection. Conversely, genetic heterogeneity at early life stages cannot be easily explained by mechanisms that are expected to act during later stages.

To focus on a subset of the potential mechanisms that could cause CGP, we surveyed mitochondrial DNA (mtDNA) variation in both adults and first-stage larvae (zoeae) of the green shore crab *Hemigrapsus oregonensis* in northern California, USA. SRS requires that the combined fecundity of a small number of individuals is sufficient to produce most of the progeny in a cohort (Hedrick 2005). For example, the hypothesis that SRS causes temporal variance in the American oyster *Crassostrea virginica* is plausible because the maximum brood size for this species is on the order of 10^7 eggs (Davis & Chanley 1956, Choi et al. 1993). In contrast, the green shore crab has a maximum brood size of approximately 10^4 eggs (Garth & Abbott 1980), which is orders of magnitude less than the abundance of first-stage larvae in Bodega Harbor during the spawning season (see 'Discussion'). It is therefore not possible for the early-stage larvae within the harbor to be the product of SRS. Furthermore, because first-stage larvae are recently spawned, it is unlikely that they arrived from distant and consequently genetically divergent populations.

Adult green shore crabs are restricted to protected shorelines; the Bodega Harbor population is isolated from others by the sparseness of suitable habitat along a predominately open coastline. Although the larvae of *Hemigrapsus* spp. are planktonic for several weeks, all stages can be found in plankton samples within 1 km of shore in Bodega Bay, while late larval stages are common as far as the middle of the continental shelf (Morgan et al. 2009, Morgan & Fisher 2010).

Petersen (2007) surveyed mtDNA sequence variation in *H. oregonensis* from 8 locations along the coasts of California and Oregon (Fig. 1). She found a phylogeographic break between the 4 locations sampled in Oregon, which we refer to as the northern locations, and the 4 locations sampled in California, which we refer to as the southern locations. These 2 groups of populations are separated by Cape Blanco, a prominent headland and potential barrier to planktonic dispersal. Haplotype and nucleotide diversities were high in samples from all locations, but were highest in samples from southern locations. One group of related haplotypes was common in northern locations but not in the southern locations. In pairwise comparisons by analysis of molecular variance (AMOVA) (Excoffier et al. 1992), every southern location was significantly different from every northern location, but there were no significant differences among southern locations, and only 2 of 6 comparisons among northern locations were significant (Petersen 2007). Petersen (2007) concluded that the 2 regional populations represented by the northern and southern locations have long been separated by a barrier to dispersal, while dispersal within each region has been 'common.' She also suggested that the barrier to dispersal could be caused either by a physical oceanographic mechanism, such as offshore jets at Cape Blanco, or by natural selection acting on mitochondrial variation.

The goal of our study was to determine whether CGP occurred at the first zoeal stage of *H. oregonensis*, which would indicate a mechanism other than either SRS or variation in the source of larvae. To this end, we focused on a mitochondrial single nucleotide polymorphism (SNP) that differs in frequency between northern and southern locations. We chose this SNP because the frequencies of its alleles would differentiate larvae that were spawned in the northern region from those originating in the southern region. We also considered this SNP as a potentially useful marker to detect the effects of selection on mtDNA, which may have maintained the uneven distribution of haplotypes between northern and southern locations (Petersen 2007).

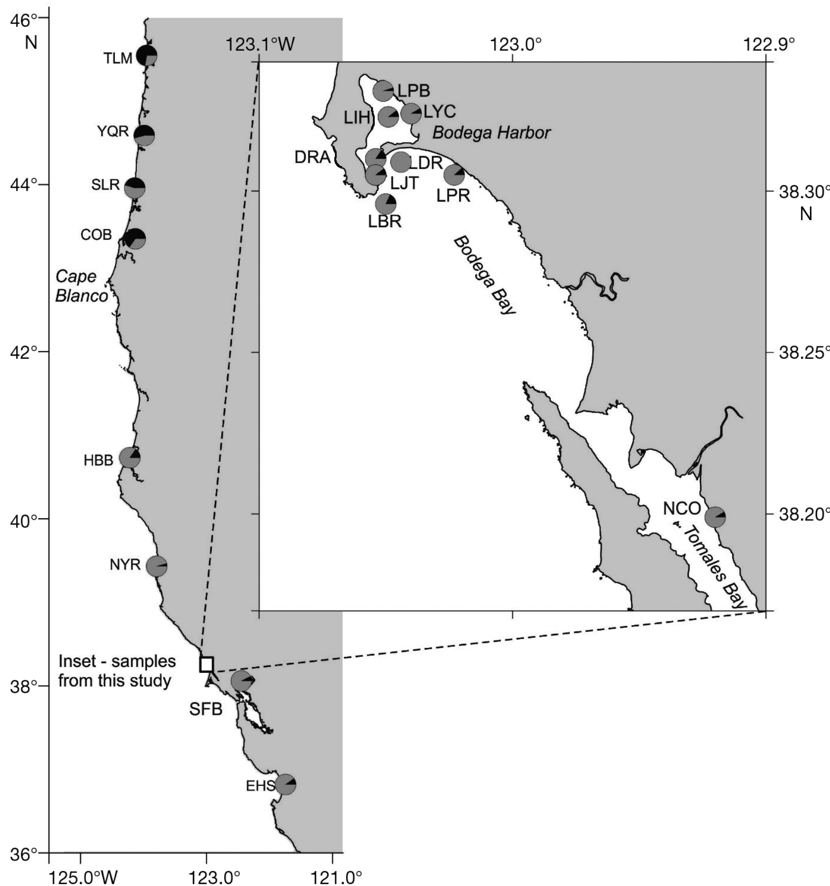

Fig. 1. Sampling locations for planktonic larvae and intertidal adults of green shore crabs *Hemigrapsus oregonensis*. Locations sampled by Petersen (2007) are shown on the large-scale map to the left, locations for this study are shown on the inset map to the right. The black fraction of each circle represents the proportion of individuals for which the northern allele of SNP-461 was found. Abbreviations for locations from which larvae were collected in plankton tows begin with L (full place names and coordinates are provided in Table 1)

MATERIALS AND METHODS

Specimen collections

Larvae of *Hemigrapsus oregonensis* were collected in plankton tows during the summer of 2006 along 3 transects inside Bodega Harbor, California, 3 outside the harbor but within Bodega Bay, and 1 from offshore waters outside Bodega Bay (Table 1, Fig. 1). The plankton net consisted of a 0.5 m ring fitted with a 335 µm mesh net, and transects were approximately 100 m long. Immediately after collection, plankton samples were coarsely sieved to remove large pieces of plant material (typically sea grasses), gelatinous organisms, and debris. Samples were drained of seawater, rinsed twice with 70% ethanol that had been pre-chilled to 10°C, and then transferred to polypropylene bottles with 70% ethanol. Samples were

Table 1. Sample locations and sample sizes from this study and from Petersen (2007). Nseq: number of individual green shore crabs *Hemigrapsus oregonensis* for which a partial COI sequence was available; Nsnp: number of individuals for which a SNP-461 genotype was determined

Location	Site	Nseq	Nsnp	Latitude (°N)	Longitude (°W)
Larvae inside Bodega Harbor					
Porto Bodega	LPB	61	250	38.331	123.051
Yacht Club	LYC	0	166	38.324	123.049
Inner Harbor	LIH	22	123	38.323	123.049
Larvae in Bodega Bay					
Jetties	LJT	40	333	38.305	123.054
Doran	LDR	0	19	38.309	123.044
Pinnacle Rock	LPR	58	236	38.305	123.023
Larvae offshore					
Bodega Rock	LBR	0	66	38.296	123.050
Adults					
Tillamook Bay, OR	TLM	47	47	45.521	123.918
Yaquina River, OR	YQR	35	35	44.619	123.940
Siuslaw River, OR	SLR	51	51	44.005	124.132
Coos Bay, OR	COB	46	46	43.364	124.132
Humboldt Bay, CA	HBB	45	45	40.720	124.215
Noyo River, CA	NYR	46	46	39.432	123.797
Doran Park, CA	DRA	161	185	38.310	123.054
Nick's Cove, CA	NCO	0	71	38.199	122.920
San Francisco Bay, CA	SFB	40	40	38.028	122.489
Elkhorn Slough, CA	EHS	41	41	36.807	121.742

stored temporarily in the field in coolers at approximately 5 to 10°C until being transferred to the Bodega Marine Laboratory for long-term storage at 5°C.

Adults of *H. oregonensis* were collected between 9 and 11 July 2010 during low spring tides from inside Bodega Harbor (DRA; see Table 1 and Fig. 1 for site names and locations) and from Nick's Cove (NCO) near the mouth of Tomales Bay, California (Fig. 1). Within the harbor, adult crabs occurred at 2 upper intertidal locations: a mud flat at the southeastern edge of the harbor near Doran City Park and at a nearby rocky section of shoreline near the Doran Beach boat launch. Immediately outside of the harbor, the exposed coastline is devoid of suitable habitat for *H. oregonensis*, and the nearest population we located was 17 km to the south at Nick's Cove. Live specimens were brought to the Bodega Marine Laboratory where they were photographed and euthanized. From each specimen, a walking leg or chela was preserved in 80% ethanol for later DNA extraction.

DNA extraction, sequencing, and SNP genotyping

Under a dissecting microscope, individual larvae tentatively identified as zoeae of *H. oregonensis* were removed from chilled plankton samples and transferred to 96-well plates. DNA was extracted and purified from individual larvae with either a modified Puregene 'DNA purification from 5–10 mg marine invertebrate tissue' protocol (Gentra Systems) or a modified Nucleospin DNA extraction protocol (Machery-Nagel). The Puregene protocol was modified to increase the recovery of DNA by reducing all volumes to one-sixth of those specified in the protocol and eliminating the RNaseA incubation step. The Machery-Nagel protocol was modified for use with the Eppendorf 5075 TMX Automated Pipetting System, and the final elution of each extraction was dried down and resuspended in 10 µl dH_2O to provide a sufficiently concentrated DNA solution.

We used PCR to amplify a portion of the mitochondrial *cytochrome c oxidase subunit 1* (COI) gene for our analysis. The sequences, locations, and orientations of PCR primers and TaqMan probes are listed in Table 2. Because the complete sequence of the COI gene for *H. oregonensis* has not been determined, we based our numbering of sequence positions on homologous positions in the complete COI sequence of the confamilial species *Eriocheir hepuensis* (GenBank Accession FJ455506). We amplified a 560 bp amplicon with a single pair

Table 2. PCR and sequencing primers and TaqMan primers and probes

Name	Orientation	Sequence (5'-3')	Position
COI-1Fb	Forward	TCT TGC TGG AGT CTC GTC AA	446–465
COI-1R	Reverse	ATY TCY CAT ATT GTT AGY CAA GAA TC	753–778
COI-2F	Forward	GGA GGA TTT GGA AAT TGA TTA GTW CC	219–244
COI-2R	Reverse	CTC TTT TTG TKT GAG CYG TA	538–557
SNP-461-F	Forward	GGC CTC TGT TGA TTT GGG TAT T	413–434
SNP-461-R	Reverse	GAT ACC TCT TTT TGT TTG AGC CG	533–555
SNP-461-A	Forward	TGC TGG AGT CTC ATC	449–463
SNP-461-G	Forward	TGG AGT CTC GTC AAT	452–466
Horeg-F	Forward	CCT TCC TTA TCT GCT GCT ATC G	382–403
Horeg-R	Reverse	TCA CTA CAT CTT GCT GGA GTC TCG	438–461
Horeg-Probe	Forward	CCT CTG TTG ATT TGG GTA TTM GB	416–438

of primers (COI-2F with COI-1R) for DNA extracted from adults, or with 2 pairs of overlapping primers (COI-2F with COI-2R, and COI-1Fb with COI-1R) for extractions from ethanol-preserved zoeae, which tended to yield slightly degraded DNA. Our sequence analysis was limited to a 375 bp portion of the amplicon that overlaps with the sequences reported by Petersen (2007) and excludes primers and regions of lower sequence quality. PCR amplifications were conducted in 15 µl reactions with Applied Biosystems Sequencing Buffer, 2.5 mM $MgCl_2$, 1.2 mM of each primer, 0.2 mM dNTPs, 0.6 U AmpliTaq Gold (Applied Biosystems), and between 10 and 40 ng of genomic DNA. The PCR thermal profile consisted of an initial step at 95°C for 10 min to denature the template and activate AmpliTaq Gold, followed by 45 cycles of 95°C for 20 s, 45°C for 20 s, and 72°C for 30 s, and a final extension step at 72°C for 2 min. Residual primers were degraded and residual dNTPs dephosphorylated by incubation with exonuclease I (2 U) and Antarctic Phosphatase (1 U) at 37°C for 2 h in a volume of 8.2 µl (New England Biolabs), followed by incubation at 72°C for 15 min to inactivate the enzymes.

Cycle sequencing reactions were performed with BigDye Terminator v1.1 chemistry (Applied Biosystems). Both strands were sequenced with the following thermal profile: 96°C for 1 min, followed by 45 cycles of 96°C for 15 s, 55°C for 10 s, and 60°C for 4 min and 20 s. Reaction products were ethanol precipitated, resuspended in 20 µl Hi-Di Formamide (Applied Biosystems), and electrophoresed on 310 Genetic Analyzers (Applied Biosystems). Sequencing Analysis Software v 5.2 from Applied Biosystems was used for base calling. Contig assembly and sequencing editing was done with the SeqMan Pro module of the Lasergene software package (DNASTAR).

TaqMan assays were designed with TaqMan assay design software from Applied Biosystems. Two assays were designed: one for a SNP at which alleles (nucleotides) are strongly correlated with regional (northern vs. southern) population differentiation and the other that distinguishes COI haplotypes found in *H. oregonensis* from those of other species with morphologically similar larvae that were found in our plankton samples. The SNP detected by the first assay was selected as follows. Individuals from the most characteristically northern (TLM, YQR, and SLR; see Table 1 and Fig. 1 for sites) and southern (HBB and NYR) of Petersen's locations were grouped. For each position in the sequence, a χ^2 value was calculated for counts of observed nucleotides in the 2 groups versus expected counts based on the groups combined. A TaqMan assay was designed to distinguish the 2 most common nucleotides at the SNP with the highest χ^2 value, i.e. SNP-461. The primers for this assay were SNP-461-F and SNP-461-R, and the probes were SNP-461-A and SNP-461-G.

The second TaqMan assay was designed to verify that zoeae isolated from plankton samples were correctly identified as larvae of *H. oregonensis*. This was a particular concern for larvae from which only SNP genotypes (not full sequences) were determined. This species verification assay was designed to detect a region of the COI gene that was diagnostic for *H. oregonensis* in an alignment of sequences for *Cancer antennarius*, *C. branneri*, *C. gracilis*, *C. magister*, *C. oregonensis*, *Aegla platensis*, *Pugettia* sp., *Carcinus maenas*, and *H. nudus*. The primers used for this assay were designated Horeg-F and Horeg-R, and the probe was Horeg-Probe.

The SNP-461 and species verification assays were multiplexed on a StepOnePlus Real-time PCR System (Applied Biosystems). When neither of the 2 major alleles (A or G) was unambiguously detected or the signal from the species verification probe was weak, the amplicon was sequenced to resolve the anomaly. Amplicons were also sequenced for haphazardly selected samples from each 96-well plate to verify the accuracy of the TaqMan assays.

Sequence analysis

MrAIC (Nylander 2004) with PhyML 3.0 (Guindon & Gascuel 2003) was used to determine the most appropriate model of sequence evolution based on Akaike's information criterion corrected for sample sizes (Akaike 1974) and to estimate the transition/transversion ratio. Arlequin 3.5 (Schneider et al. 2000) was used to estimate haplotype diversity and AMOVA-based F_{ST} estimates. GenePop 4.1 (Rousset 2008) was used to perform exact tests of population differentiation, with the setting for the number of batches increased from 100 to 1000 to estimate p-values with greater accuracy. Randomization tests of differences in haplotype diversity among samples and a variety of data management tasks were performed with Perl scripts written by J. E. Neigel.

Coalescent analysis of historical gene flow

The COI sequences from this study were aligned with sequences provided to us by C. Petersen (from Petersen 2007), and the alignment was trimmed to an

overlapping 375 bp region that spans homologous positions 291 to 665 in the COI sequence of *E. hepuensis* (GenBank Accession FJ455506). The program IMa2 (Hey & Nielsen 2007, Hey et al. 2010) was used to estimate population sizes, divergence time, and rates of gene flow for the population we sampled as adults in Bodega Harbor (DRA) and the closest northern population sampled by Petersen (2007) in Coos Bay, Oregon (COB). Details of program settings, runs, and output are provided in the Supplement at www.int-res.com/articles/suppl/m548p139_supp.pdf.

RESULTS

Analysis of mitochondrial sequences

We analyzed sequences of a 375 bp portion of the COI gene for a subset (165 of 189) of adult green shore crabs sampled from Bodega Harbor (BH), and a subset (193 of 1195) of zoeae collected in plankton tows. The larvae were from 2 locations within Bodega Harbor and 2 outside the harbor in Bodega Bay (Table 1, Fig. 1). Bodega Bay, including Bodega Harbor, is within the southern part of the region encompassed by Petersen's (2007) survey of COI sequence variation in *Hemigrapsus oregonensis* (Table 1, Fig. 1). Among the combined 358 sequences were 136 polymorphic sites and 177 unique haplotypes. Among the 351 sequences from Petersen's (2007) study, which included samples from the less variable northern populations, there were 87 polymorphic sites and 149 distinct haplotypes for the same 375 bp region. The combined set of 709 sequences from Petersen's (2007) and our survey included 153 polymorphic sites and 291 haplotypes.

F_{ST} was used as a measure of differences in haplotype frequencies, and an AMOVA (α = 0.05) was used to test for significant differences (Table 3). Haplotype frequencies in adults (DRA) and larvae (LPB, LIH) from Bodega Bay were not significantly different from those in any of Petersen's (2007) 4 southern samples (HBB, NYR, SFB, EHS; $F_{ST} < 0.01$), but were different from each of Petersen's northern samples (TLM, YQR, SLR, COB; F_{ST} between 0.08 and 0.29, $p < 0.00001$). There appeared to be some differentiation between larvae from inside Bodega Harbor (LPB) and adults from within the harbor (DRA), but this difference (F_{ST} = 0.0091) was not significant after a sequential Bonferroni correction for multiple comparisons (p = 0.0166). These results are consistent with Petersen's (2007) scenario of a long period of isolation between southern and northern populations and high gene flow among southern populations. Although we have no comparisons for samples taken from the same locations at different times, Petersen's samples of adults were collected in 2001 and 2002, our plankton tows were collected in 2006, and we collected adults from Bodega Harbor in 2010. The lack of significant differences in haplotype frequencies among these samples suggests that haplotype frequencies did not change appreciably over that 8 to 9 yr period.

We found no evidence of a reduction in haplotype diversity in larvae relative to adults (Fig. 2). Haplotype diversity in samples of larvae was slightly

Table 3. Population differentiation of COI sequences in green shore crabs *Hemigrapsus oregonensis*. Data are from Petersen (2007) and this study. Values above the diagonal are estimates of F_{ST}, values below are p values from Fisher's exact test. Site abbreviations as in Table 1

Site	N	Northern region				Southern region				Bodega Bay and vicinity				
		Adults									Larvae			
											Inside harbor		Outside harbor	
		TLM	YQR	SLR	COB	HBB	NYR	SFB	EHS	DRA	LPB	LIH	LJT	LPR
TLM	47		0.0181	0.0444	−0.0060	0.2471	0.2997	0.2996	0.2899	0.2580	0.2487	0.2864	0.2357	0.2852
YQR	35	0.0977		0.0039	0.0077	0.1274	0.1772	0.1735	0.1640	0.1438	0.1434	0.1624	0.1231	0.1708
SLR	51	0.0078	0.2822		0.0361	0.0768	0.1100	0.1099	0.1041	0.1088	0.1046	0.1047	0.0803	0.1154
COB	46	0.5186	0.2178	0.0176		0.2294	0.2882	0.2870	0.2729	0.2344	0.2315	0.2755	0.2172	0.2735
HBB	45	<0.0001	<0.0001	<0.0001	<0.0001		0.0044	0.0013	−0.0035	−0.0018	0.0086	0.0090	−0.0005	0.0088
NYR	46	<0.0001	<0.0001	<0.0001	<0.0001	0.2002		−0.0065	0.0002	0.0022	−0.0006	0.0083	0.0048	0.0020
SFB	40	<0.0001	<0.0001	<0.0001	<0.0001	0.3457	0.8731		−0.0070	−0.0028	−0.0022	−0.0010	−0.0007	−0.0044
EHS	41	<0.0001	<0.0001	<0.0001	<0.0001	0.6123	0.4063	0.8545		0.0012	−0.0003	−0.0001	0.0036	0.0097
DRA	161	<0.0001	<0.0001	<0.0001	<0.0001	0.5869	0.2754	0.6377	0.3506		0.0091	0.0161	0.0036	−0.0006
LPB	61	<0.0001	<0.0001	<0.0001	<0.0001	0.0440	0.4932	0.6719	0.4600	0.0166		0.0007	0.0070	0.0040
LIH	22	<0.0001	0.0010	<0.0001	<0.0001	0.1387	0.1348	0.5215	0.4404	0.0606	0.4268		0.0074	0.0152
LJT	40	<0.0001	<0.0001	<0.0001	<0.0001	0.4688	0.1582	0.4785	0.2315	0.2022	0.0576	0.1602		0.0077
LPR	58	<0.0001	<0.0001	<0.0001	<0.0001	0.1035	0.2705	0.7188	0.0889	0.4658	0.1455	0.0547	0.1094	

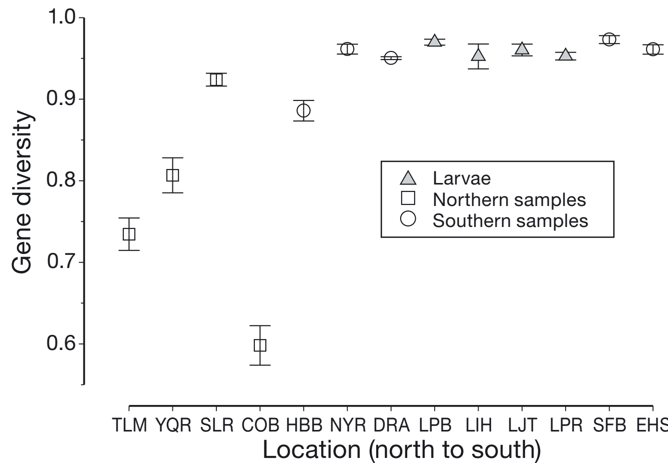

Fig. 2. Estimates of COI haplotype diversity with approximate 95% confidence limits for samples of green shore crabs *Hemigrapsus oregonensis*. Full site names are given in Table 1

higher than in samples of the adults from inside the harbor (DRA) and slightly lower than in samples of 3 of the 4 other populations south of Cape Blanco (NYR, SFB, EHS). We used 2 criteria to judge whether these small differences in haplotype diversity were statistically significant. First, pairs of random samples of the same sizes as the original samples were drawn with replacement from pooled samples to create a distribution of haplotype diversity differences under the null hypothesis that the 2 samples have identical haplotype frequencies. The observed difference in haplotype diversity between the paired samples was then compared with this distribution to estimate a p value. Even without adjustment of the Type I error rate for multiple comparisons, none of the comparisons between samples (adults or larvae) from the southern region approached significance ($p > 0.33$ for all comparisons). Second, variances for estimates of haplotype diversity were calculated and used to construct approximate 95% confidence limits under the assumption that estimates of haplotype diversity follow Gaussian distributions. Although these approximate confidence limits, which are shown as error bars in Fig. 2, are very small, they overlap for larvae and adults from Bodega Harbor.

The number of alleles (or haplotypes) in a sample is a more sensitive indicator of population bottlenecks than heterozygosity or haplotype diversity (Spencer et al. 2000), which should also be true for the bottleneck effects of sweepstake reproduction. The number of COI haplotypes in samples of *H. oregonensis* increased close to linearly with sample size ($R^2 = 0.92$), averaging 1 additional haplotype for every 1.74 additional individuals sampled (Fig. 3). When scaled by this relationship, the numbers of haplotypes in samples of larvae were no lower than either the numbers of haplotypes in Petersen's (2007) southern samples (HBB, NYR, SFB, EHS) or our sample of adults from Bodega Harbor (DRA). As with haplotype diversity, there is no evidence of reduced numbers of haplotypes for larvae. The number of haplotypes in a sample of larvae also indicates the minimum number of females that must have contributed progeny to that sample. For the 3 largest samples of larvae (40, 58, and 61 individuals) the numbers of distinct haplotypes were 30, 38, and 46 respectively.

Coalescent analysis of isolation and migration

Under a scenario in which mtDNA variation is selectively neutral and the patchiness in haplotype frequencies in Bodega Bay was caused by differences in the proportions of larvae from the genetically different northern populations identified by Petersen (2007), the level of gene flow between populations should be high enough to have detectable effects on larval haplotype frequencies but also low enough to allow the persistence of genetic differences between populations. We used the coalescent method implemented in IMa2 (Hey et al. 2010), which assumes that the genetic markers are selectively neutral, to estimate gene flow between the adult population in Bodega Harbor and the nearest of Petersen's northern populations at Coos Bay, Oregon (Petersen 2007).

Joint peak locations and posterior probabilities used for likelihood ratio tests of nested demographic models are shown in Table S1 in the Supplement, and estimates of demographic parameters from marginal peak locations are shown in Table S2 in the Supplement, along with plots (histograms) of the marginal distributions of parameters (Figs. S1 & S2 in the Supplement). Estimates of gene flow rates for either the full model or the model with a single migration rate were essentially 0, with upper confidence limits on the order of 5×10^{-7}, effectively ≤ 1 female per generation. Unless these estimates are several orders of magnitude below actual contemporary rates of larval dispersal from northern populations to Bodega Harbor, it is unlikely that any of the 1195 larvae that we genotyped (either by sequencing or detection of SNP-461) originated from the northern population represented by the Coos Bay sample. Thus, this analysis indicates that influxes of larvae from northern populations would not likely be a cause of CGP in Bodega Bay.

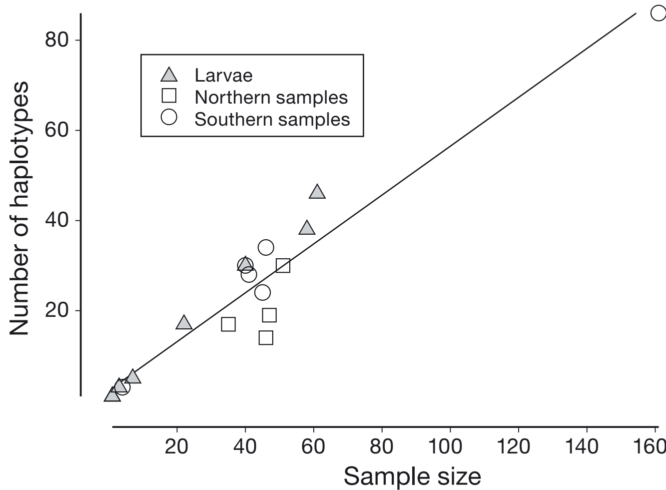

Fig. 3. Regression of the number of individual green shore crabs *Hemigrapsus oregonensis* in a sample against the number of unique haplotypes in the sample

SNP analysis

Although no SNPs in our alignment of COI sequences were diagnostic for either the northern or the southern samples of *H. oregonensis*, the differences in nucleotide frequencies for a few SNPs corresponded to χ^2 values over 1000 (Table 4). Those SNPs have considerable power to distinguish samples that differ in proportions of northern and southern haplotypes. We designed TaqMan assays to detect the 2 most common nucleotides (A and G) at the SNP with the highest χ^2 value, SNP-461. In northern samples, adenine (SNP-461-A) was the most common nucleotide (allele), while guanine was the most common in southern samples. When neither A nor G was detected, the amplicon was sequenced to determine which of the other 2 possible nucleotides was present. In this way, all 4 nucleotides were detected. For the subset of 186 individuals to which both the TaqMan and sequencing methods of genotyping were applied, identical results were obtained, indicating the accuracy of the methods. In tests of homogeneity of SNP-461 allele frequencies across samples or groups of samples, genotypes determined by sequencing were combined with those determined by TaqMan assays to provide greater statistical power. From all sources of data combined, SNP-461 genotypes were assessed for a total of 1806 individuals including 1195 larvae (Table 1). We began by testing the most general hypothesis of homogeneity across samples, followed by more specific hypotheses concerning groups and subsets of those samples. Where individual sample sizes were relatively small, we pooled samples that were similar in life stage, time of collection, and location to maintain statistical power. This hierarchical sequence of hypotheses tests was intended to reduce the severity of Type I error rate corrections required for multiple comparisons. For tests of homogeneity among southern samples, we excluded the 4 northern samples as well as the sample from Humboldt Bay (HBB), which could be considered a transitional population with a higher frequency of SNP-461-A than other southern samples (Fig. 4).

Table 4. Single nucleotide polymorphisms for which the χ^2 statistic for north (N) region vs. south (S) region sequences exceeded 1000

Position	A		C		G		T		χ^2
	N	S	N	S	N	S	N	S	
461	0.59	0.07	0.01	0.00	0.39	0.93	0.01	0.00	6197
404	0.00	0.00	0.40	0.92	0.00	0.00	0.60	0.08	5947
554	0.01	0.00	0.41	0.91	0.56	0.07	0.02	0.02	5450
167	0.55	0.06	0.00	0.01	0.45	0.94	0.00	0.00	5389

Heterogeneity in SNP nucleotide frequencies among southern samples of adults and larvae

Although SNP-461 strongly differentiates northern from southern samples, its frequency appears to be similar among samples from southern locations, including the 2 southern locations that we sampled (DRA and NCO) 8 yr after Petersen's (2007) survey (Fig. 4). For a test of homogeneity among southern samples, we included our sample of harbor adults (DRA), Petersen's closest sample to the north of Bodega Bay (NYR), and groupings of adults from south of Bodega Harbor (NCO, SFB, and EHS), larvae from inside the harbor (LPB, LYC, and LIH pooled), larvae from outside the harbor but near the coast (LJT, LDR, and LPR pooled), and a sample of offshore larvae (LBR). The sample from NYR was thus the most northern population in this set. However, NYR does not appear to be a transitional population between northern and southern groups because it had the lowest frequency of the characteristically northern SNP-461-A allele. Homogeneity among these groups was rejected ($p = 0.0060$, $F_{ST} = 0.012$), providing the first evidence of temporal or spatial heterogeneity among southern locations. We then conducted a similar test after eliminating the sample from NYR because of its unusually low frequency of SNP-461-A, and pooling all larvae

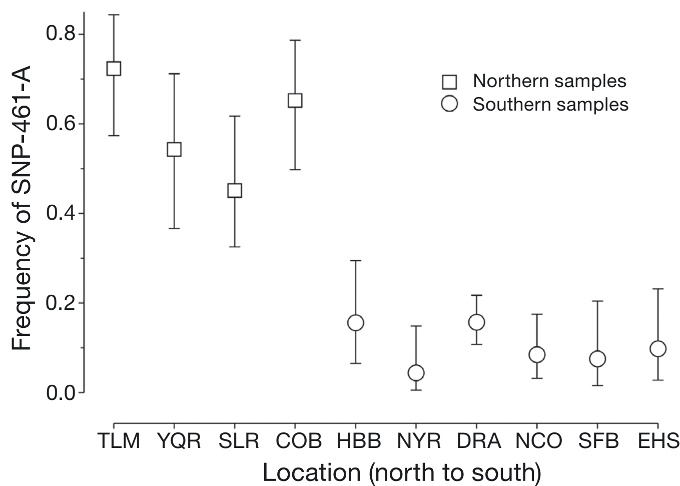

Fig. 4. Frequency of the SNP-461-A allele at the locations sampled by Petersen (2007) and in this study (DRA and NCO; full site names are given in Table 1). Error bars represent approximate 95% confidence limits

from inside the harbor into a group and all larvae from outside of the harbor into another. This test rejected homogeneity among harbor adults, harbor larvae, bay larvae, and adults from south of the harbor ($p = 0.013$, $F_{ST} = 0.0087$). We then performed all 6 pairwise tests among groups with a Bonferroni adjustment of α to 0.0083. Homogeneity between harbor adults and harbor larvae was rejected ($p = 0.0058$, $F_{ST} = 0.0343$), but no other comparisons were significant. The significant difference in the frequency of SNP-461 alleles between adults and larvae from the same population (harbor) could be a consequence of a change in the population's SNP allele frequencies between the time that larvae were sampled (summer 2006) and the time that adults were sampled (summer 2010). Such a rapid change in allele frequencies over a few generations would also be consistent with Johnson and Black's original concept of chaotic patchiness (Johnson & Black 1982, 1984)

Heterogeneity in SNP frequencies among samples of larvae grouped by location

The homogeneity of SNP-461 nucleotide frequencies among larvae pooled from inside Bodega Harbor (LPB, LYC, and LIH), nearshore but outside the harbor in Bodega Bay (LJT, LDR, and LPR), and offshore in Bodega Bay (LBR) was rejected ($p = 0.028$, $F_{ST} = 0.010$). Homogeneity of larvae from inside the harbor compared with outside the harbor was also rejected ($p = 0.016$, $F_{ST} = 0.0067$). These samples were all collected in the summer of 2006, so if the differences represent temporal shifts, they would have occurred over the course of a single spawning season. The frequency of the characteristically northern SNP-461-A was highest for larvae collected offshore, intermediate for larvae collected inside Bodega Bay but outside the harbor, and lowest for larvae from inside the harbor, with exclusion of the LDR sample because of its small size (Fig. 5). However, the frequency of SNP-461-A in adults collected from Bodega Harbor was as high as it was in the sample of offshore larvae, and significantly higher than in larvae from within the harbor.

DISCUSSION

Fecundity and the plausibility of sweepstakes reproduction

The frequency of SNP-461 alleles in the mitochondrial genome of *Hemigrapsus oregonensis* differed among samples of larvae in Bodega Bay and between adults and larvae within Bodega Harbor. Population-wide SRS is unlikely because the fecundity of *H. oregonensis* is too low for a small number of females to have produced the majority of the zoeae in Bodega Harbor. In a survey, the density of zoeae of *Hemigrapsus* spp. at the northern end of Bodega Bay averaged between 1 and 2 m^{-3}, about an order of magnitude greater than late-stage larvae (Morgan & Fisher

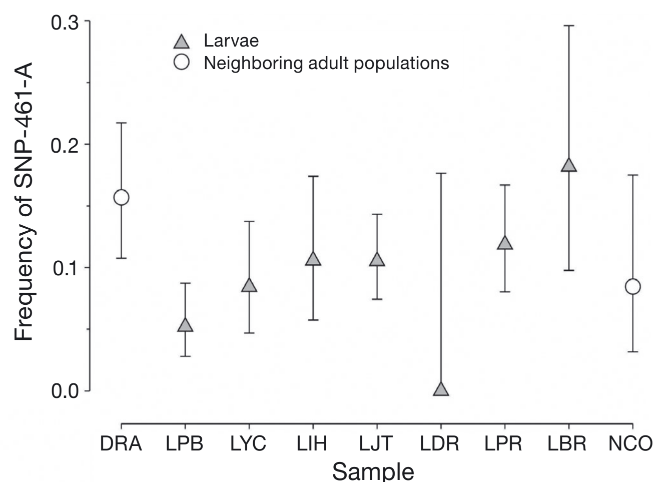

Fig. 5. Frequency of the SNP-461-A at the locations where planktonic larvae of green shore crabs *Hemigrapsus oregonensis* were sampled (see Fig. 1) and the 2 locations where adults were sampled in this study (DRA and NCO; full site names are given in Table 1). Error bars are 95% CI

2010). In plankton samples collected at the time of this study, densities of first-stage larvae of *H. oregonensis* averaged 5.9 m^{-3} for 46 tows conducted over a 2 d period of intensive sampling within the harbor during March of 2006 (Morgan et al. 2014). Larvae are distributed throughout the 15 to 20 m water column, so the number of larvae within a square kilometer (roughly the area of Bodega Harbor) is on the order of 15 to 40 million. This number is equivalent to the full reproductive output of several thousand females that each spawn 10^4 larvae. Variance in reproductive success among males would not have affected maternally inherited mtDNA polymorphisms.

We can also address the possibility of a small-scale 'sweepstakes-like effect' (sensu Christie et al. 2010), in which genetically distinct patches of larvae, each produced by a small number of females, were sampled by our plankton tows (transects ~100 m long). For the individuals from which we obtained COI sequences, we did not detect the reductions in either haplotype diversity or numbers of haplotypes in samples of larvae relative to adults in Bodega Harbor which would be expected from a sweepstakes-like effect. The diversity of COI sequences is very high in *H. oregonensis*, providing considerable statistical power to detect reductions in haplotype diversity or number, had individual patches of larvae been spawned by small numbers of females.

Gene flow among divergent populations

It is unlikely that the differences in SNP-461 allele frequencies in samples of larvae from Bodega Bay reflect varying proportions of larvae from genetically distinct populations. This would require differences in haplotype frequencies between populations to be at least as large as the deviations we observed for samples of larvae. Differences in frequencies of COI haplotypes or SNP-461 alleles among samples of adults from southern locations, including our samples from Bodega Harbor (DRA) and Nick's Cove (NCO), were small and not statistically significant, which suggests that an influx of larvae from other southern populations cannot easily explain the deviations we observed in samples of larvae. The northern and southern populations differ substantially in COI haplotype frequencies, especially for those distinguished by SNP-461. However, as indicated by our coalescent analysis of gene flow between Coos Bay and Bodega Harbor populations, the extent of differentiation between northern and southern populations implies levels of gene flow so low that for selectively neutral markers we would not expect any northern larvae in our samples. This situation exemplifies the dilemma of attempting to explain chaotic patchiness by the arrival of larvae from other populations: without invoking natural selection to maintain genetic differences among populations, levels of gene flow high enough to cause detectable fluctuations in allele frequencies would rapidly eliminate those genetic differences. There are plausible scenarios that avoid this dilemma: (1) arrival of exogenous larvae is a rare event that we just happened to observe during the sampling period; (2) exogenous larvae do not affect gene flow because of selection against non-resident genotypes, phenotype–environment mismatches (Marshall et al. 2010), or an unbearable cost to long-distance transport itself (Ballard & Rand 2005, Peteiro et al. 2011); or (3) the Bodega Harbor population receives larvae from multiple, genetically different sources, but these sources do not receive larvae from Bodega Harbor or exchange larvae with each other. In the third scenario, variability in the contributions of larvae from different sources creates CGP, while the lack of gene flow among source populations preserves their distinctiveness. However, in the present study, a problem with any explanation based on the influx of exogenous larvae, and especially one based on larvae arriving from northern populations, is that the larvae that were sampled in Bodega Harbor were unlikely to have traveled far from their spawning sites. Only larvae in the first zoeal stage were sampled, and this stage has a duration of just 7 to 10 d (Lehto et al. 1998). Although planktonic duration is not an accurate predictor of dispersal distance (Shanks 2009), first-stage larvae are unlikely to travel far in the 7 to 10 d after being released in the harbor. Furthermore, although *H. oregonensis* larvae are transported out of the harbor by the third stage (Morgan et al. 2014), they complete their development nearshore (Morgan & Fisher 2010). This would restrict probable source populations to those from which we did sample adults (DRA and NCO). The high abundance of first zoeal stage larvae within Bodega Harbor is also consistent with a local origin.

Variable selection across space and time

CGP in mtDNA haplotype or SNP-461 allele frequencies in the Bodega Bay population of *H. oregonensis* could be caused, either directly or indirectly, by natural selection. Speculation about the role of selection in shaping genetic variation in marine species is often viewed with skepticism because selec-

tion is compatible with so broad a range of observations that it is difficult to falsify as a hypothesis (Hellberg et al. 2002); instead, the case for selection is often built on the elimination of competing explanations. However, despite the potential for high levels of gene flow from larval dispersal, local adaptation appears to be common in marine invertebrates (Sanford & Kelly 2011). Selection may be particularly effective on the mitochondrial genome because of hitchhiking (Smith & Haigh 1974): selection on any maternally transmitted factor, including any site in the mitochondrial genome or symbionts, can alter the frequencies of other maternally transmitted factors. Direct or indirect selection on the mitochondrial genome has been convincingly demonstrated in other species including marine crustaceans (Burton et al. 2006, Ellison & Burton 2008).

Selection was proposed as the mechanism that maintains the phylogeographic break between northern and southern populations of *H. oregonensis* (Petersen 2007). If this were the case, the existence of the break and the inferred absence of gene flow across it would be consistent with a scenario in which larvae from north of the break arrive in Bodega Harbor to cause patchiness in mtDNA haplotype frequencies but are eliminated by selection before they contribute to gene flow. Although, as discussed above, first-stage zoeae in Bodega Harbor are unlikely to have originated from distant populations, northern populations could still be a source of genetic variants that transiently appear in southern populations before they are eliminated by selection. It is well established that selection acts on genetic variation in clines or patches in or near hybrid zones (Harrison 1990), including hybrid zones between marine invertebrate species (Bert & Harrison 1988, Gardner 1994). In hybrid zones, spatial heterogeneity in allele frequencies can result from the interplay between gene flow and selection (Barton & Hewitt 1985), as well as endogenous incompatibilities among loci (Bierne et al. 2011). It would be useful to investigate the phylogeographic break between northern and southern populations of *H. oregonensis*, ideally with nuclear as well as mitochondrial markers, to determine whether it is actively maintained by selection against gene flow.

Patchiness in the frequency of SNP-461 alleles within Bodega Bay could be the result of selection varying with location, time, or life-history stage. Selection could affect fecundity of spawning females, hatching success of eggs, or survival of larvae. Larvae of *H. oregonensis* occupy habitats distinct from those of adults and experience environmental heterogeneity across the small temporal and spatial scales over which genetic patchiness was detected. During the summer in Bodega Bay, surface water temperatures vary from <10 to >14°C as prevailing upwelling conditions alternate with periods of relaxation or downwelling (Roughan et al. 2005, Morgan et al. 2012). Within Bodega Harbor, temperature and salinity variation over days and weeks is driven by tides, rainfall, and other factors (Morgan et al. 2014). Ballard & Rand (2005) argued that selective forces play a larger role in the population biology of mitochondrial DNA than is generally realized and that thermal adaptation is a 'strong candidate' for selection on mtDNA. Selection by temperature could explain both the small-scale patchiness observed in our study and the large-scale north–south phylogenetic break discovered by Petersen (2007). Currents and upwelling create latitudinal variation in temperature within the range of *H. oregonensis* (Huyer 1983), with an upwelling jet that corresponds to the phylogenetic break at Cape Blanco (Barth et al. 2000, Petersen 2007). Further investigation to determine whether a relationship exists between haplotype frequencies and temperature would be useful in assessing this possibility.

If variable selection is responsible for patchiness in the frequency of SNP-461 alleles within Bodega Bay, it is notable that it has not led to the fixation of a single allele. In simple models with selection varying between generations, the allele with the highest geometric mean fitness is eventually fixed (Dempster 1955, Gillespie 1973). However, a polymorphism can be maintained if selection varies within generations (Borash et al. 1998) or if the environment is divided into multiple niches that favor different genotypes (Levene 1953). The literature on phenotypic selection offers many examples of temporal changes in the strength and direction of phenotypic selection (Siepielski et al. 2009, 2011) as well as selection acting in opposite directions at different life stages (Schluter et al. 1991). Another possibility is that gene flow from other populations dampens the effects of locally variable selection and prevents fixation. Although we have argued that it is unlikely for the genetic composition of early-stage larvae to be influenced by immigration, it remains plausible for late-stage larvae.

CGP as a phenomenon and SRS as a mechanism

CGP and reduced polymorphism have been considered signatures of SRS (e.g. Avise 2000). However, whether or not CGP is good evidence of SRS depends

on what is considered a 'sweepstakes winner' and what is considered a 'cohort.' In common usage, a sweepstakes is a contest or lottery in which one winner 'takes or appropriates everything' (Oxford English Dictionary). In this sense, SRS would mean that for an entire population, the progeny of just a few individuals are represented in the 'future pool of reproductively mature adults' (sensu Hedgecock & Pudovkin 2011) and that a cohort consists of all the individuals that recruit into a population over a generation or a spawning season. In contrast, CGP would only require that the progeny of some individuals are overrepresented within a small group of recruits arriving at a specific location over a short span of time. If there are many such groups of recruits, then there would be no winner-take-all reproductive success at the population level and there may be little actual reduction in effective population size (Selkoe et al. 2006). However, regardless of how broadly SRS is defined, there is an important distinction between CGP, which is an observable phenomenon, and SRS, which is a possible mechanism underlying CGP. As shown here, CGP in the larval pool can arise without SRS.

Early vs. late-acting mechanisms

A variety of mechanisms could hypothetically lead to CGP. Progress in understanding the causes and significance of CGP will require approaches that isolate or eliminate particular mechanisms. In this study, we sought to eliminate 2 of the most commonly invoked mechanisms by focusing on early-stage larvae. In general, different mechanisms are expected to act at different times throughout a life history. For example, the genetic effects of SRS (reduced diversity and spatial or temporal associations among siblings) should appear in the earliest life stages. The longer after spawning cohorts are sampled, the greater the opportunity for mechanisms other than SRS to cause CGP. For example, older larvae are more likely to have been transported from distant, genetically divergent populations. The opportunity for selection also increases over time, whether selection acts cumulatively throughout larval life or at particular stages of development. At the end of the larval phase, near the time of settlement, genetic differences in habitat preference, recruitment success, or behaviors that influence dispersal would also contribute to genetic heterogeneity among settlers. Overall, from the time of spawning to recruitment, the number of potential confounding influences on the genetic composition of cohorts will increase.

Another characteristic of early-stage larvae that is relevant to testing hypotheses about the cause of CGP is their abundance. In our study, the high abundance of zoeae in our study area combined with the relatively low fecundity of our study species allowed us to conclude that a large number of females were represented in the pool of individuals from which we sampled; this would not have been the case if we had sampled less abundant settlers or recruits. Higher abundance also makes larger sample sizes feasible, allowing greater statistical power to detect differences among samples.

The broader relevance of CGP

A central task of population genetics is to estimate the magnitudes of the fundamental microevolutionary forces of genetic drift, gene flow, and selection (Lewontin 1985). This has been especially difficult for marine and estuarine species: population sizes could be numerically large while genetically small (Hedgecock et al. 1992), planktonic dispersal could result in high gene flow but not necessarily so (Shanks 2009), and the effects of natural selection on large-scale spatial patterns are easily confounded with those of history. This is of more than theoretical concern. Marine ecologists and conservation biologists make frequent use of population genetic methods for inferences about dispersal, demography, stock size, and stock structure (Neigel 1997, Hellberg et al. 2002), methods that are often based on the assumption that the effects of selection can be ignored (Rand 1996, Yednock & Neigel 2011). The phenomenon of CGP challenges our understanding of how population genetic forces operate in marine populations while offering opportunities to investigate these forces at scales that are amenable to study. Investigation of the mechanisms that cause CGP, in a variety of taxa and at different life stages, should lead to a better overall understanding of the population genetics of marine species.

Data accessibility. DNA sequences generated and analyzed for this study have been deposited in the GenBank database, under accession numbers KU925912-KU926269. Perl scripts, sample locations, IMa2 input files, and SNP data have been deposited in the DRYAD database doi:10.5061/dryad.812mm.

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