Title

Post-invasion Genetic Structure of European Green Crab Populations on the US West Coast and Its Implications for Their Control

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Project Completion Report
Post-invasion Genetic Structure of European Green Crab Populations on the US West Coast and Its Implications for Their Control (Project R/CZ-161)

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Introduction

One of the most ecologically significant invaders in the coastal waters of the U.S. is the European green crab, *Carcinus maenas*. It is a voracious, generalist predator in its native range in Europe as well as invaded regions which include including South Africa, Australia, , Japan, and both coasts of North America (reviewed in Grosholz and Ruiz 1995, Cohen et al. 1995, Geller et al. 1997). Previous work in its native range and in the eastern U.S. has provided much evidence for its strong control over the abundance of benthic organisms in soft sediment communities (e.g. Ropes 1968, Reise 1977, 1985, Morgan et al. 1980, Jensen and Jensen 1985, Tettlebach 1986). Furthermore, work in Bodega Bay Harbor, CA has documented significant changes in the local food web as the direct result of green crab predation: Grosholz et al (2000) found 5- to 10-fold declines in the abundances of previously abundant invertebrate taxa. Lafferty & Kuris (1996) estimated that the annual net value of crab, mussel, oyster, and bait harvests threatened by green crabs in Western North America (WNA) is $43.7 million.

Understanding the factors that contribute to the spread of the green crab is key to developing control strategies. If the populations in one region are linked by larval dispersal to other regions, then control in one area will be negated by recruitment from areas outside of the managed region. For this reason, genetic markers can provide information about the nature of post-establishment spread. Metapopulation models and empirical evidence indicate that transient spatial clustering of genotypes can occur during an invasion, particularly if post-invasion spread follows a stepping-stone or leptokurtic pattern (Stone & Sunnucks 1993, Demelo & Hebert 1994, Ibrahim et al. 1996, Le Corre & Kremer 1998). Furthermore, if post-invasion spread follows a stepping stone pattern then a gradual loss of heterozygosity over time at the edges of the spreading population is expected (Le Corre and Kremer 1998) which, if functionally important, may limit the rate of spread. Observed patterns of spatial spread in WNA and Tasmania are saltatory (Grosholz 1996, G. Ruiz, pers. com), suggesting similarities to the stepping stone model.

The actual genetic consequences of range expansion depend primarily on the number of founders arriving in newly colonized habitat and the rate of migration to the edge areas following colonization.

History of Carcinus in WNA

Carcinus maenas was first identified in California in San Francisco Bay around 1989-1990. Identification was confirmed by the California Academy of Sciences from specimens collected by bait fishers in Redwood City. Green crabs were first identified outside of San Francisco Bay in Bodega Harbor in 1993. That same year, a single year class of new recruits (no adults) were found in Bolinas Lagoon, Tomales Bay, and Drake's Estero as well as Bodega Harbor. The first green crabs were found south of San Francisco Bay in Elkhorn Slough in 1994. The population expanded northward in 1995 with populations identified in Humboldt Bay. Subsequent surveys in 1996 showed green crabs also present in some small estuaries in Mendocino County (Little River). Northward expansion included records in Coos Bay in 1997 and in Washington in 1998. Green crabs were also found in Morro Bay in 1998 (EG, pers obs). Despite significant trapping efforts, green crabs have not been found in Morro Bay since 1998 and are not believed to be established there currently. Additional west coast records include crabs found as far north as Tofino on the western coast of Vancouver Island and in the Straits of Juan de Fuca in the port of Victoria and the port of Vancouver. Green crabs have not been recorded in the Puget Sound proper. Thus, the established green crab range extends from Gray's Harbor, WA to Elkhorn Slough, CA and extensive trapping has confirmed that this range includes all major embayments between these endpoints.

Objectives

We proposed to determine the extent of mixing between populations to assess the feasibility of regional removal efforts. Specifically, we aimed to test the following hypothesis:

H_{A1}: Microsatellite allele frequencies for the WNA populations are temporally and spatially variable.
H_{01}: No genetic differences exist among age classes or among different locales within the WNA populations.

H_{A2}: Heterozygosity is reduced in populations peripheral to San Francisco Bay, the presumed site of initial invasion.
H_{02}: Heterozygosity does not differ in populations near and distant to San Francisco Bay.

Methods

We deployed crab traps at sites in (from south to north) Morro Bay, CA, Elkhorn Slough, CA, San Francisco Bay, CA, Bolinas Lagoon, CA, Drakes Estero, CA, Tomales Bay, CA, Bodega Harbor, CA, Humboldt Bay, CA, Coos Bay, OR, Newport Bay, OR, Willapa Bay, WA, Gray's Harbor, WA.

At each site, modified minnow traps were baited and deployed by Grosholz and/or colleagues in mid to late summer. Multiple traps were deployed at several stations within each site and fished for a 24 hour period. Crabs were collected and size, gender and related parameters were measured for each crab. Because of the discrete nature of the reproductive season, crabs below a certain size were separated and unambiguously identified as new recruits of that year class. These small crabs were frozen at -20º C and shipped to MLML.

The years 1999 and 2000 were marked by near total recruitment failure for *Carcinus maenas*, rendering our goal of single-cohort genetic analysis over the present range of the crab in WNA impossible. Sufficient new samples could only be obtained at Bodega Bay, CA. Our analysis was therefore structured geographically, using previously collected specimens. However, crabs of a given year class were collected in Bolinas Lagoon, Bodega Harbor, and Tomales Bay, allowing a geographically limited analysis of single year recruitment classes.

Genomic library preparation.

Genomic DNA was prepared from fresh hepatopancreas and gill tissues frozen in liquid nitrogen. We used a Qiagen Genome-Tip DNA extraction kit to isolate and purify DNA, which was then shipped to Genetic Identification Service, Inc. (Chatsworth, CA) for construction of a genomic library in pUC 19. Libraries were enriched for plasmids containing dinucleotide, trinucleotide, and tetranucleotide repeats. Enriched libraries were plated onto LB/Amp/X-gal agar and colonies containing recombinant plasmids were selected and grown in liquid culture. Purified plasmids were sequenced on a Licor 4200-L2 automated sequencer. Sequences containing microsatellites and sufficient flanking information were identified and primers designed using Primer Premier 5.0 (Premier Biosoft International). Additional microsatellites were isolated from a separate $(CA)_n$ enriched library prepared in our laboratory using methods described in Bagley and Geller (1998).

DNA was extracted from gill tissue of crabs which were stored at –20^{o}C. Without thawing, gills were dissected from crabs, homogenized in a CTAB solution [50 mM Tris-HCl (pH 8.0), 0.7M NaCl, 10 mM EDTA, 1% CTAB (hexadecyltrimethylammonium bromide), 0.1% b-mercaptoethanol] and incubated at 55° C for 16 to 23 hours. These homogenates were extracted twice in chloroform:isoamyl-alcohol (24:1), then nucleic acids were precipitated with 0.1 volume of 3 M sodium acetate and two volumes of 100% ethanol at -20° C. Nucleic acids were pelleted in a microcentrifuge (10,000 X g for 10 minutes), dried under a vacuum, and resuspended in 30-300 ml of TE [10 mM Tris, 1 mM EDTA (pH 8.0)]. DNA quality varied considerable, probably due to the differences in age, history, and storage condition of different frozen samples. Prior to microsatellite amplification, all DNA samples were further purified using Qiagen DNeasy kits. Nucleic acids were quantified using Hoechst stain and a Hoeffer fluorometer, calibrated against a known standard of calf thymus DNA. Concentrations were adjusted to 5 ng/ μl.

Microsatellite Amplification and Analysis.

Nineteen primer sets were designed from sequences derived from Genetic Identification Service enriched libraries. These were synthesized and tested on high quality *Carcinus* DNA. We achieved replicable and scorable results from none of these primer sets. We have not yet diagnosed the problem with these primers. Primers did amplify the expected product from the plasmid from which they were designed, thus PCR conditions are not solely responsible. DNA quality from -20^{o} C frozen or ethanol preserved tissues was generally low, and we believe that this contributed to a high failure rate of DNA amplifications. Microsatellites derived from our own CA enriched library were amplifiable, but success rate was low (~50%). This too likely indicates problems with template quality. For the present, our data are restricted to two loci developed from our own CA enriched library, and these loci are termed CM6 and CM14.

PCR analysis was performed using 15 ng of genomic DNA in a 10 µl PCR mix that included 2mM $MgCl_2$, 200 µM each dNTP, 3 pmol of each of the primers CM6F, CM6R, CM14F, CM14R, and 0.6 U Taq polymerase (Gibco BRL) in 1x Taq buffer (Gibco BRL). Primers CM6F and CM14F were 5'-labeled with 6-FAM and HEX dyes, respectively. The cycling profile included an initial denaturation step at 95° C for 2 min, followed by 26 cycles of denaturation (95° C for 45 s) and annealing/extension (65° C for 3 min). The cycling profile terminated with a 72° C extension for 30 min. Following PCR, products were diluted 25-fold with water and two-fold with deionized formamide. A 1 µl aliquot of the diluted PCR was mixed with 0.08 µl of a 500 bp size standard (ROX-500, Applied Biosystems) and electrophoresed on a BaseStation DNA sequencer (MJ Research) with KilobasePack acrylamide and buffer (MJ Research). Allele sizes (bp) at each microsatellite locus were interpolated relative to the ROX-500 in-lane standard with the aid of the software Cartographer (MJ Research). We used Genepop 3.1 to look for differentiation between populations.. This provides an 'approximate' Fisher exact test that uses Markov chain procedures to estimate probabilities that samples were pulled from the same population.

Results

Table 1 lists sites sampled, year class assignments, and samples sizes. Sites in California were Arcata (ACM), Bodega Harbor (BCN, BBCM, BML), Tomales Bay (TCM), Bolinas Lagoon (BLCM), Drake's Estero (DCM), Hayward (HCM), Redwood City, San Francisco Bay (RCM), Triangle Marsh, San Francisco Bay (TrCM), and San Francisco Bay (SFCM). Sites in Washington State were Willapa Bay (WCM) and Gray's Harbor (GHCM). Where possible, samples were grouped into year classes (YC93, YC94, YC95) based on size distributions. Year class information was not available for some samples, so these were assumed to be mixtures of different year classes.

Table 2. presents allele frequencies at loci CM6 and CM14 for all samples, as well as expected heterozygosity assuming Hardy-Weinberg equilibrium.

Table 1. Sites sampled and sample sizes

Population	Sample Size (n)
ACM95mix	15
BBCM95mix	37
BCM YC93	62
BCM96mix	11
BLCM YC93	36
BLCM YC94	55
BLCM YC95	36
BLCM95mix	6
BML YC00	23
BML YC99	8
DCM YC93	28
GHCM99mix	78
H93CMmix	8
RCM 94mix	28
SFCM 95mix	2
TCM YC93	49
TCM YC94	46
TCM YC95	168
TCM YC96	42
TrCM95mix	5
WBCM99mix	91
Grand Total	834

Table 2. Allele frequencies and expected heterozygosity for loci CM6 (top) and CM14 (bottom).

Locus CM6

Population	Allele Size (bp)										Allele count	He
	259	267	271	273	277	279	283	285	289	293		
WBCM99mix	0.187	0.011	0.126	0	0.137	0.143	0.192	0	0.203	0	182	0.832
TrCM95mix	0.1	0	0.1	0.1	0.2	0.2	0.1	0	0.2	0	10	0.84
TCM YC95	0.149	0.021	0.052	0	0.174	0.113	0.198	0.01	0.277	0.003	328	0.816
TCM YC96	0.19	0.024	0.083	0	0.119	0.107	0.238	0.04	0.202	0	84	0.832
TCM YC93	0.125	0.063	0.094	0	0.208	0.156	0.208	0	0.146	0	96	0.839
TCM YC94	0.065	0.054	0.033	0.01	0.087	0.283	0.174	0.08	0.217	0	92	0.821
SFCM 95mix	0	0.25	0	0	0.25	0	0.25	0	0.25	0	4	0.75
RCM 94mix	0.25	0.018	0.018	0.07	0	0.125	0.089	0.14	0.286	0	56	0.806
H93CMmix	0.063	0.188	0	0	0.313	0.125	0.125	0.06	0.125	0	16	0.812
GHCM99mix	0.195	0.052	0.058	0	0.156	0.097	0.234	0.01	0.201	0	154	0.827
DCM YC93	0.26	0.12	0.06	0	0.14	0.22	0.08	0	0.12	0	50	0.826
BML YC99	0	0	0.188	0	0.188	0	0.25	0	0.375	0	16	0.726
BML YC00	0.152	0.022	0.065	0	0.13	0.152	0.217	0.02	0.239	0	46	0.827
BLCM YC93	0.222	0.056	0.042	0.04	0	0.097	0.111	0.15	0.264	0.014	72	0.829
BLCM95mix	0.5	0	0	0	0	0.333	0.083	0	0.083	0	12	0.625
BLCM YC94	0.091	0.009	0.055	0	0.073	0.255	0.127	0.06	0.327	0	110	0.791
BLCM YC95	0.111	0.042	0.083	0.01	0.056	0.292	0.125	0.04	0.236	0	72	0.817
BCM96mix	0.182	0	0.045	0	0.091	0.182	0.136	0.14	0.227	0	22	0.835
BCM YC93	0.177	0.048	0.04	0.02	0.032	0.194	0.137	0.17	0.169	0.016	124	0.85
BBCM95mix	0.162	0.014	0.054	0.11	0	0.297	0.149	0.07	0.149	0	74	0.822
ACM95mix	0.133	0	0.033	0	0.333	0.133	0.1	0.03	0.233	0	30	0.787

Locus: CM14

	Allele Size (bp)		Allele count	
	266	269		He
WBCM99mix	0.736	0.264	182	0.39
TrCM95mix	0.7	0.3	10	0.42
TCM YC95	0.726	0.274	336	0.4
TCM YC96	0.702	0.298	84	0.42
TCM YC93	0.694	0.306	98	0.42
TCM YC94	0.728	0.272	92	0.4
SFCM 95mix	1	0	4	0
RCM 94mix	0.661	0.339	56	0.45
H93CMmix	0.75	0.25	16	0.38
GHCM99mix	0.714	0.286	154	0.41
DCM YC93	0.714	0.286	56	0.41
BML YC99	0.438	0.563	16	0.49
BML YC00	0.717	0.283	46	0.41
BLCM YC93	0.736	0.264	72	0.39
BLCM95mix	0.25	0.75	12	0.38
BLCM YC94	0.704	0.296	108	0.42
BLCM YC95	0.778	0.222	72	0.35
BCM96mix	0.85	0.15	20	0.26
BCM YC93	0.75	0.25	124	0.38
BBCM95mix	0.757	0.243	74	0.37
ACM95mix	0.7	0.3	30	0.42

Hypothesis 1. Population differentiation. Table 3 summarizes all comparisons across space and time. In the next paragraphs, comparisons of year classes within and between site are presented.

Comparisons within year classes across sites. Data for 1993, 1994, and 1995 year classes from Bodega Harbor, Bolinas Lagoon, Drake's Estero, and Tomales Bay allow for within-year class comparisons, though all sites were not sampled in all years. Genetic differentiation here refers to significant differences in allele frequency distributions.

For locus CM6 in 1993, recruits to Bodega Harbor were genetically differentiated from those in nearby Tomales Bay, Drake's Estero, but not Bolinas Lagoon. Recruits in Bolinas Lagoon, however, were significantly different from those in Drake's Estero or Tomales Bay. Drake's Estero and Tomales Bay crabs were significantly differentiated. Locus CM14 was not significantly differentiated among any of these populations.

For loci CM6 and CM14 in 1994, there was no significant differentiation between Bolinas Lagoon and Tomales Bay. However, in 1995, allele frequencies were significantly different in these two sites for locus CM6.

Comparisons within sites across year classes. Data for Bodega Harbor, Tomales Bay, and Bolinas Lagoon are available for comparisons over time.

For Bodega Harbor, allele frequency distributions differed in all comparisons between year classes 1993, 1999, and 2000 for locus CM6. For locus CM14, year classes 1993 and 1999 were significantly different.

For Tomales Bay, allele frequencies for locus CM6 changed from year class 1993 to year class 1994, from 1994 to 1995, but not from 1995 to 1996. Allele frequencies at locus CM14 did not significantly change over year classes.

For Bolinas Lagoon, allele frequencies for locus CM6 changed from year class 1993 to 1994, but not 1994 to 1995. Allele frequencies at locus CM14 did not significantly change over year classes.

Comparisons of mixed year class samples. Samples taken at Arcata, Bodega Harbor, Bolinas, San Francisco, and Triangle Marsh (SF Bay) in 1995 were not sorted into age classes. The relative contribution of various ages classes to these samples is thus unknown, but comparisons of these samples may provide a general picture of differentiation of resident populations among sites. Unfortunately, sample sizes are mostly low, and results of these comparisons should be viewed cautiously. The Arcata sample was significantly different from Bodega and Bolinas populations, but not from the two SF Bay samples. The Bodega sample was also different from the San Francisco sample, but not that from Triangle Marsh nor Bolinas. The San Francisco sample and Bolinas sample differed, and the but neither were different from Triangle Marsh.

Mixed samples for Willapa Bay and Gray's Harbor in Washington State in 1999 were obtained. These samples did not differ in allele frequencies for either locus.

Hypothesis 2. Diminution of genetic variation.

There is no evidence for diminution of genetic diversity in peripheral populations (Willapa Bay, and Gray's Harbor) relative to California populations. Instead, northern populations had a slightly higher expected heterozygosity (H_e), (Table 4) A one-way ANOVA indicates that differences in H_e among populations and year classes are not significantly different (Table 4). These data provide no support for hypothesis H_{A2}

Table 4. One-way ANOVA comparing expected heterozygosity (H_e) in Californian (central) and Washington State (peripheral) populations in three year classes. Data do not include TrCM, SFCM or BLCM95mix since sample sizes were very small.

CM6

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
central93-94	8	6.574	0.82175	0.000351
central95-96	6	4.909	0.818167	0.000293
central99-00	2	1.553	0.7765	0.0051
peripheral99-00	2	1.659	0.8295	1.25E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.003775	3	0.001258	1.949724	0.168032	3.343885
Within Groups	0.009035	14	0.000645			
Total	0.01281	17				

CM14

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
central93-94	8	3.24	0.405	0.000657
central95-96	6	2.22	0.37	0.00368
central99-00	2	0.9	0.45	0.0032
peripheral99-00	2	0.8	0.4	0.0002

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.010511	3	0.003504	1.858025	0.183102	3.343885
Within Groups	0.0264	14	0.001886			
Total	0.036911	17				

Discussion

Comparisons of sites within year classes showed that recruits even in geographically proximate locations may differ significantly in allele frequencies. Populations from sites in central California (San Francisco Bay, Drake's Estero, Bolinas

Lagoon, Tomales Bay, and Bodega Harbor), all within 75 km, were differentiated in 1993 and 1995. Thus distance is not strongly correlated to genetic differentiation in any given cohort because nearby sites are as likely to be different as distant sites. Similarly, in comparisons between year classes at a given site, a given year's cohort is likely to be genetically different from the preceding or following year's cohort.

In general, *Carcinus* populations appear very prone to genetic drift. Presumably, genetic drift in these populations arises due to variation in reproductive success among breeders and multiple larval releases over a breeding season, resulting in heterogeneity in the pool of larvae in space and time. This heterogeneity is then compounded by variability in the oceanographic conditions that deliver larvae to each site

Thus, while samples from peripheral sites such as Arcata, Gray's Harbor, and Willapa Bay are frequently different in allele frequencies from central California sites in the matrix of all pairwise comparisons (Table 3), this is not clearly related to distance as opposed to the general pattern of stochasticity seen in these data. Because microgeographic and year-by-year variability is great, these data do not support our hypothesis of a stepping stone pattern of dispersal, nor do they strongly refute it. Sources of larvae for any particular site may well be from adjacent populations, but genetic drift obscures any such demographic relationship.

Heterozygosity observed in these populations was generally high, as expected for microsatellite loci. We predicted that, if populations were demographically connected in a stepping stone pattern and if sequential founding events were accompanied by sampling effects, genetic variation should be lower in populations distant from the presumed point of invasion. This hypothesis was disproved: heterozygosity was slightly higher in peripheral populations, and statistically there was no significant difference.

Conclusions

Allele frequencies for the two loci surveyed varied greatly among geographically close populations and from year to year. This pattern is similar to the "sweepstakes recruitment" pattern discussed by Hedgecock (1994) and points to the importance of behavioral traits and oceanographic features for genetic structure. Unfortunately, this pattern also makes it difficult to make probabilistic statements about potential sources of recruits in any population. We are unable to definitively conclude that peripheral populations do not have sources among central populations. From the perspective of control of *Carcinus* populations, we cannot conclude, therefore, that removal of crabs from peripheral populations will be an effective strategy for their elimination.

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