POPULATION STRUCTURE OF DUNGENESS CRAB (*CANCER MAGISTER*) IN BRITISH COLUMBIA

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ABSTRACT Population structure of Dungeness crab (*Cancer magister*) from British Columbia was evaluated from a survey of variation of eight microsatellite loci in eight populations. Genetic differentiation among the populations surveyed was observed, with the mean F_{st} for all loci 0.031 (SD = 0.007). The Alison Sound population in the British Columbia central coast displayed less genetic variation and was distinct from all other populations in British Columbia, with pairwise F_{st} values >0.12, over 20 times the differentiation in other comparisons. The results were consistent with a high level of retention of larval crabs within Alison Sound, owing to reduced water exchange between the sound and adjacent waters. There was no evidence for an isolation by distance model of population structure of Dungeness crab in British Columbia, but there was some indication of differentiation between a west coast Vancouver Island population and a population adjacent to the southern Strait of Georgia.

KEY WORDS: British Columbia, Dungeness crab, microsatellites, population structure

INTRODUCTION

The Dungeness crab *Cancer magister* is widely distributed along the Pacific coast in North America from the Aleutian Islands in Alaska to southern California (Hart 1982). In British Columbia, Dungeness crab are harvested in recreational and commercial fisheries and comprise the second most valuable invertebrate fishery in the region (Phillips & Zhang 2004). Dungeness crab also continue to be an important part of the food, social, and ceremonial resource of coastal Aboriginal peoples. The primary fishery management tools are minimum size limits (Phillips & Zhang 2004), male-only fisheries in some areas (Zhang et al., 2002), gear limits, limited entry to the commercial fishery, and fishery closures.

Determination of population structure of exploited species is an essential component in successful management of fisheries, particularly with respect to development of appropriate management or conservation units. Tagging studies of adult Dungeness crab have indicated relatively local movements (Butler 1957, Snow & Wagner 1965, Gotshall 1978), providing the potential for the development of individual populations. However, after egg fertilization and hatching, young larval crab are planktonic for about four months (Reilly 1983), where the larvae are transported primarily by currents. Larval crab thus have the potential to move substantial distances from the local area of hatching (Reilly 1983, Jamieson & Phillips 1988, Jamieson et al. 1989). In the final larval stage (megalopa), the larval crab settles to the bottom and moults to a juvenile crab. Regional differences in megalopal size and diurnal distribution have been reported, which indicated a nonrandom distribution of megalopae. This nonrandom distribution may indicate larval retention in specific regions, like the southern Strait of Georgia in British Columbia (Jamieson & Phillips 1993), which may in turn provide the basis for the development of genetically distinct stocks.

For Dungeness crab, widespread dispersal of larval crabs without retention in local spawning areas suggests that development of local genetically distinct stocks would be unlikely, and thus delineation of management or conservation units based upon genetically distinct stocks may not be practical, unlike other marine invertebrates in British Columbia (Miller et al. 2006). Surveys of allozyme variation provided no evidence of genetically distinct local populations in Dungeness crabs (Soule & Tasto 1983), in accordance with expectations based upon widespread juvenile dispersal. However, allozymes display little variation in Dungeness crabs, and it may be that genetic markers selected for extensive variation may be more effective in detecting finer-scale population differentiation if it were present. DNA level markers have substantially increased the number of polymorphic loci that are available to be included in surveys of genetic variation. Microsatellites have been reported to display extensive variation in some decapod species (Tam & Kornfield 1996, Baker et al. 2000), and indeed variable microsatellites have been isolated in Dungeness crabs (Jensen & Bentzen 2004, Kaukinen et al. 2004). If there are areas where there are barriers to larval transport, then the potential exists for local population differentiation to occur.

The objective of the present study was to analyze variation at eight microsatellite loci to evaluate population structure of Dungeness crab populations in British Columbia. We were particularly interested in evaluating whether there was any evidence of genetic differentiation among Dungeness crabs from different locations in British Columbia, and if so, whether there was any evidence of local population differentiation. Local population differentiation may imply that differential rates of exploitation may be applicable in some local fisheries.

METHODS AND MATERIALS

Laboratory Analysis

Tissue samples were collected by removing a walking leg from individual Dungeness crab and preserving the sample in 95% ethanol. Dungeness crabs were sampled at eight geographically diverse coastal locations in British Columbia (Fig. 1). DNA was extracted from the tissue samples using a chelex resin protocol outlined by Small et al. (1998). Once extracted DNA was available, surveys of variation at eight microsatellite loci were conducted: *Cma102, Cma103, Cma107,*

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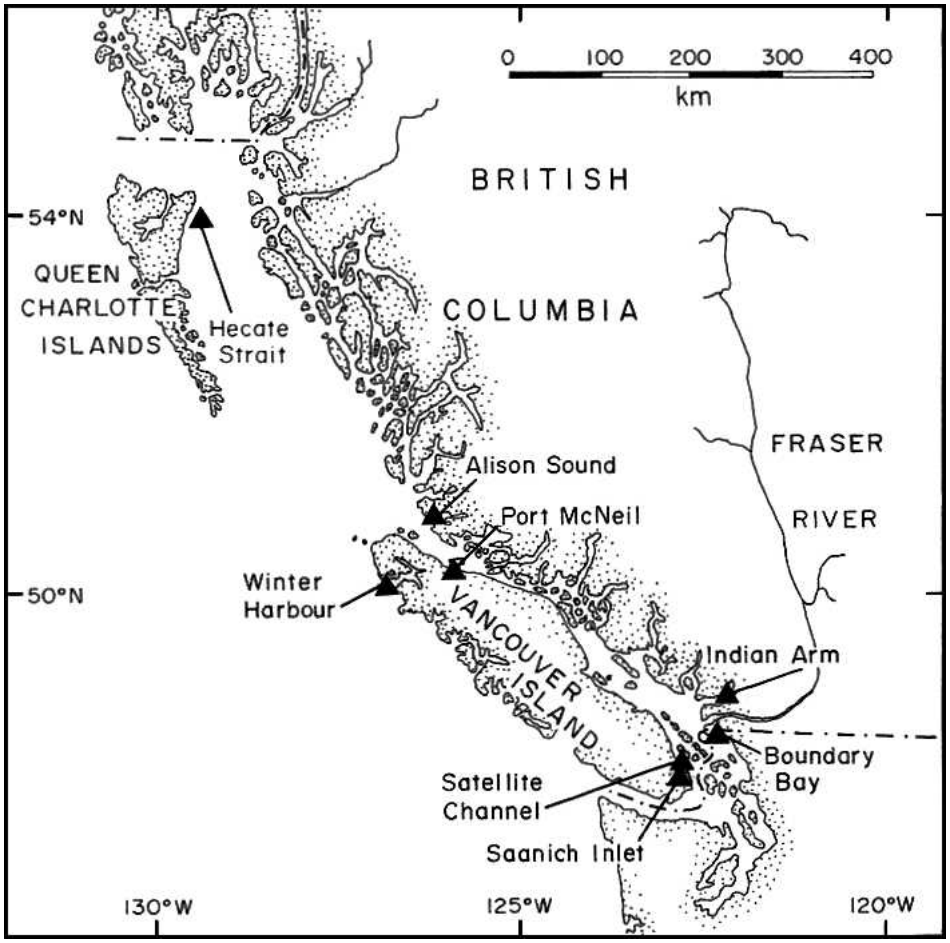

Figure 1. Locations of collection sites of Dungeness crab in British Columbia surveyed for microsatellite variation.

Cma108a, Cma108b, Cma114, *Cma117*, and *Cma118* (Kaukinen et al. 2004). Polymerization chain reaction (PCR) amplifications were performed using 35 cycles of 94°C for 30s, 48–54°C for 30s, and 70°C for 45s. Each 8.0 μL reaction contained 0.50 μL of a 1:2 dilution of Chelex extracted DNA (approximately 0.01–0.03 μg), 0.48 μM of each primer, 80 μM dNTPs, 0.15 units of HotStarTaq DNA polymerase (Qiagen, Valencia, CA) and 1× HotStarTaq PCR Buffer containing Tris-HCl, KCl $(NH_4)2SO4$, 1.5 mM $MgCl_2$, pH 8.7.

PCR fragments were initially size fractionated in denaturing polyacrylamide gels using an ABI 377 automated DNA sequencer, and genotypes were scored by Genotyper 2.5 software (Applied Biosystems, Foster City, CA) using an internal lane sizing standard.

Data Analysis

Each population at each locus was tested for departure from Hardy-Weinberg equilibrium (HWE) using GDA (Lewis & Zaykin 2001). Critical significance levels for simultaneous tests (8 populations, Table 1) were evaluated using sequential Bonferroni adjustment (Rice 1989). F_{st} estimates for each locus were calculated with FSTAT (Goudet 1995). Computation of the number of alleles observed per locus, as well as allelic diversity standardized to a common sample size, was carried out with FSTAT. Cavalli-Sforza and Edwards (CSE) (1967) chord distance was used to estimate distances among populations. An unrooted neighbor-joining tree based on CSE was generated using PHYLIP (Felsenstein 1993). An isolation by distance model of population structure was tested by regression of pairwise F_{st} values with geographic distance (shortest water distance between location in kilometres) calculated in FSTAT following Mantel (1967). The distribution of genetic variance was tested between northern British Columbia (populations 1–3) and southern British Columbia (populations 4–8) and among populations within regions with GDA (Lewis & Zaykin 2001). Allele frequencies for all location samples surveyed in this study are available at <http://www-sci.pac.dfo-mpo.gc.ca/mgl/Default_e.htm>.

RESULTS

Variation Within Populations

There was substantial variation in the number of alleles observed for the eight microsatellite loci surveyed in the study. The fewest number of alleles was observed at *Cma117* (six alleles), with the greatest number of alleles observed at *Cma107* (45 alleles) (Table 2). Lower heterozygosity was usually observed at loci with fewer than 10 alleles. The genotypic

TABLE 1.

Sampling location, sample collection years, and total number of individuals sampled for eight populations of Dungeness crab in British Columbia. Allele frequencies for all location samples surveyed in this study are available at http://www-sci.pac.dfo-mpo.gc.ca/mgl/default_e.htm.

Population	Year	N
(1) Hecate Strait	1999	119
(2) Port McNeil	2002	102
(3) Alison Sound	2002	75
(4) Winter Harbour	2002	99
(5) Indian Arm	2002	105
(6) Boundary Bay	2002	113
(7) Saanich Inlet	2003	83
(8) Satellite Channel	2003	23

frequencies at each locus generally conformed to those expected under Hardy-Weinberg equilibrium (HWE), with the possible exception of *Cma107*. At this locus, the genotypic frequencies in the Hecate Strait, Port McNeil, Winter Harbour, and Boundary Bay populations were not in HWE, possibly as a result of the wide range in allele size and reduced amplification of larger-sized alleles.

Genetic diversity in terms of number of alleles observed was very consistent among seven of the eight populations surveyed (Table 3). Dungeness crab from Alison Sound displayed the least number of alleles compared with all other populations surveyed (Table 3). Individuals from this population displayed on average only approximately 65% of the alleles observed in other populations. Not unexpectedly, the greatest differentiation in terms of allelic diversity among populations was observed at the locus (*Cma107*) with larger numbers of total observed alleles.

Population Structure

Genetic differentiation among the Dungeness crab populations sampled in our survey was clearly evident. The F_{st} value over all populations and loci was 0.031, with individual locus values ranging from 0.006 (*Cma103*) to 0.050 (*Cma102*) (Table 2). The most distinctive population was that from Alison Sound, with pairwise population F_{st} values all >0.10 for comparisons involving the Alison Sound population (Table 4). This population also displayed substantially reduced genetic diversity compared with other populations surveyed. The Winter Harbour population was also genetically distinct from the Saanich Inlet population, as were the Indian Arm and Satellite Channel populations ($P < 0.05$). The level of differentiation in the southern British Columbia comparisons was <20 times than the differences observed in the comparisons involving the Alison Sound population.

The distinctive genetic differentiation of the Alison Sound population was observed in the cluster analysis, with the Winter Harbour population displaying some distinctiveness (Fig. 2). Regional structuring of populations was generally not observed, with the populations in northern British Columbia (1–3) not discrete from those in southern British Columbia (4–8) (Fig. 2). An isolation by distance model of population structure was not supported for the eight populations surveyed ($r^2 < 0.01$, $P > 0.05$) (Fig. 3). The analysis was heavily influenced by the distinctive Alison Sound population (F_{st} values > 0.10 in Fig. 3), but removal of this population from the analysis did not provide any evidence of an isolation by distance model of population structure ($P > 0.05$).

Gene diversity analysis of the eight loci surveyed was used to evaluate the distribution of genetic variation between northern and southern British Columbia, and among populations within regions. With eight populations included in the analysis, no variation was observed among regions, and variation among populations within regions accounted for an average 3.2% of total observed variation. With the Allison Sound population removed from the analysis, no regional variation was observed, and variation among populations within regions accounted for 0.2% of total observed variation. Both the isolation by distance analysis and gene diversity analysis indicated no regional structure of Dungeness crab populations in British Columbia.

DISCUSSION

Some structure of Dungeness crab populations has been suggested based on life history variation, oceanographic currents, or models of larval drift (Tasto 1983, Stevens & Armstrong 1984, Dinnel et al. 1993). However, demonstration of genetically distinct populations has been difficult, and lack of detectable genetic divergence has usually been ascribed to the

TABLE 2.

Number of alleles per locus, F_{st}, expected heterozygosity (He), observed heterozygosity (Ho), and number of significant Hardy-Weinberg equilibrium tests (HWE) for 8 microsatellite loci surveyed in 8 populations of Dungeness crab listed in Table 1. Standard deviation of F_{st} is in parenthesis.

Locus	Number of Alleles	F_{st}	He	Ho	HWE
(1) Cma102	10	0.050 (0.050)	0.74	0.73	0
(2) Cma103	10	0.006 (0.008)	0.71	0.68	0
(3) Cma107	45	0.046 (0.046)	0.89	0.75	4
(4) Cma108a	19	0.048 (0.052)	0.71	0.70	1
(5) Cma108b	8	0.005 (0.008)	0.71	0.70	0
(6) Cma114	8	0.015 (0.018)	0.57	0.56	0
(7) Cma117	6	0.027 (0.018)	0.57	0.59	0
(8) Cma118	19	0.025 (0.026)	0.84	0.88	0
Total		0.031 (0.007)			

TABLE 3.

Mean number of alleles observed per locus at eight microsatellite loci for eight Dungeness crab populations. Populations were as numbered in Table 1. Allele numbers have been standardized to a sample size of 14 individuals per population.

	1	2	3	4	5	6	7	8
Cma102	6.4	6.5	3.4	6.4	6.5	5.8	6.5	6.7
Cma103	5.0	5.4	4.8	5.7	4.6	5.0	4.6	6.0
Cma107	14.6	14.1	4.9	13.8	16.0	14.5	15.2	16.2
Cma108a	7.7	6.8	4.5	6.9	7.2	7.0	7.4	6.9
Cma108b	4.9	5.4	4.9	5.1	4.6	4.8	4.8	5.5
Cma114	4.2	3.7	3.6	4.2	3.9	4.0	4.0	4.5
Cma117	4.1	3.9	3.3	3.9	4.4	3.9	3.8	4.1
Cma118	8.5	8.8	5.6	9.2	9.1	8.8	8.8	7.4
Total	55.4	54.6	35.0	55.2	56.3	53.8	55.1	57.3

TABLE 4.

Pairwise population F_{st} values observed over eight microsatellite loci for eight Dungeness crab populations, with populations numbered as in Table 1. Boldface font indicates significant difference ($P < 0.05$).

	1	2	3	4	5	6	7
1	0.000						
2	0.002						
3	**0.134**	**0.137**					
4	0.000	0.004	**0.119**				
5	0.001	0.001	**0.130**	0.001			
6	0.002	0.000	**0.145**	0.006	0.003		
7	0.003	0.000	**0.137**	**0.005**	0.000	0.000	
8	0.000	0.005	**0.147**	0.004	**0.005**	0.001	0.005

widespread dispersal of larval crabs, where mixing of larval crabs from different geographical areas and subsequent settling would tend to homogenize allele frequencies at genetic markers in adult crabs.

In British Columbia, the bifurcation of the Subarctic Current off the west coast of Vancouver Island into the southerly flowing California Current and the northerly flowing Alaska Current potentially allows differentiation into northern and southern population components of Dungeness crabs. Some seasonal variation in local current patterns adjacent to Vancouver Island are also observed, dependent upon winds, river discharges, and other factors (Thomson et al. 1989), but the expectation would be that populations in northern British Columbia should be genetically more similar to each other compared with populations in southern British Columbia. Thus, Dungeness crab population structure should be expected to fit an isolation by distance model.

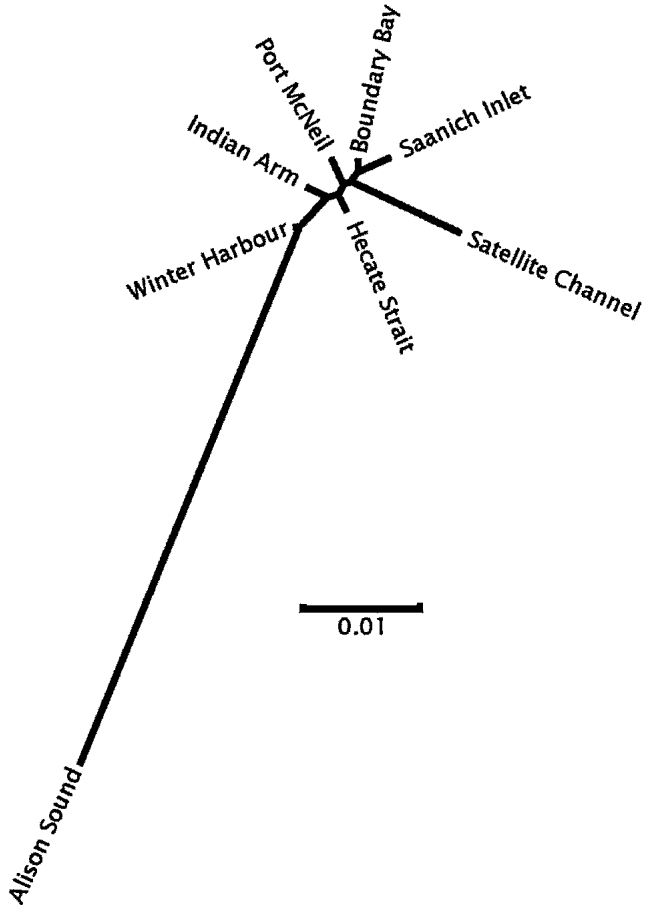

Figure 2. Neighbor-joining tree based on Cavalli-Sforza & Edwards (1967) chord distance among Dungeness crab collection sites. Geographic locations are outlined in Figure 1.

In British Columbia, there was no evidence for an isolation by distance model of population structure of Dungeness crab in British Columbia, but there was some evidence of local population differentiation. The Alison Sound population was clearly genetically distinct from all other populations sampled in British Columbia, and displayed reduced genetic variation than populations in other areas. Reduced genetic variation can be symptomatic of a recent bottleneck in population size or inbreeding. Based on the narrowness and shallow depth of the channel leading into Alison Sound, water volume exchange between Alison Sound and outside waters is limited (R. Thomson, personal communication, Institute of Ocean Science, Sidney, British Columbia). Therefore, there would likely be high level of retention of larval crabs within Alison Sound, and the reduced water exchange would constitute a barrier to immigration of larval crabs from other areas. It would be prudent to confirm the distinctiveness of the Alison Sound population through repeat sampling to document relative stability of allele frequencies over time.

Jamieson and Phillips (1993) reported regional differences in size and behavior between megalopae in the southern Strait of Georgia compared with those on the west coast of Vancouver Island and suggested that they may be indicative of stock differences between crabs in the two regions. The sole west coast of Vancouver Island population examined (Winter Harbour) was genetically distinct from the Saanich Inlet population, which is adjacent to the southern Strait of Georgia, supporting some level of differentiation between crab populations from these two regions. However, the apparent differentiation between outer coastal Vancouver Island populations and the inner coastal southern Strait of Georgia populations was substantially less than the differentiation between the Alison Sound population and other populations surveyed in British Columbia.

Genetic population structure in British Columbia has also been evaluated for geoduck clams *Panopea abrupta* and red sea urchins *Strongylocentrotus franciscanus*, species with a planktonic larval stage as in Dungeness crabs. An isolation by distance model of population structure was observed in geoduck clams, but not in red sea urchins (Miller et al. 2006). However, the isolation by distance population structure observed in geoduck clams was dependent on inclusion of Queen Charlotte Islands populations in the analysis, a region known for its distinctive biota (Kyle & Boulding 2000, Withler et al. 2003). An isolation by distance population structure was also reported for northern abalone *Haliotis kamtschatkana* in British Columbia (Withler et al. 2003), but this result was also dependent on inclusion of Queen Charlotte Islands populations in the analysis, similar to the results outlined for geoduck clams by Miller et al. (2006). Otherwise, no relationship between geographic distance and genetic differentiation was observed

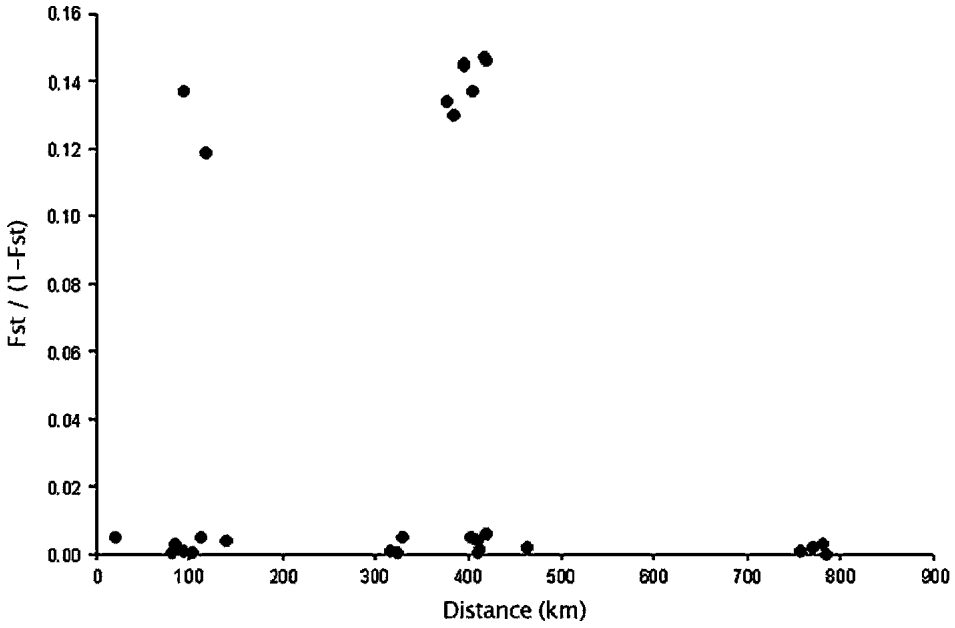

Figure 3. Isolation by distance for eight Dungeness crab populations in British Columbia. A single population (Alison Sound) accounted for all comparisons in which F_{st} values were >0.10.

in northern abalone populations. In British Columbia, distinct populations may be observed, either in specific regions like the Queen Charlotte Islands, or in specific areas like Alison Sound, but there is no general pattern of an isolation by distance population structure for species with planktonic larvae.

As microsatellites are relatively inexpensive to locate and develop (Kaukinen et al. 2004), they can provide an effective method of assessing population structure in a species with limited genetic information available. The nonlethal requirements for sampling, and ease of laboratory analysis and sample processing combine to provide a powerful tool in the assessment of population structure in exploited species.

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