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Biogeographical genetic variation of the Dungeness crab (Cancer magister)

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BIOGEOGRAPHICAL GENETIC VARIATION OF THE DUNGENESS CRAB (*CANCER MAGISTER*)

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ABSTRACT

BIOGEOGRAPHICAL GENETIC VARIATION OF THE DUNGENESS CRAB (*CANCER MAGISTER*)

By Corinne H. Lardy

The genetic structure and phylogeography of Dungeness crab (*Cancer magister*) populations from Alaska, Washington, Oregon, and California were assessed by sequencing the mitochondrial Cytochrome *c* Oxidase subunit I (COI) and nuclear Elongation Factor α subunit (EF1α) genes. High COI haplotype diversity (350 haplotypes among 846 crabs, $h = 0.940$), and high nucleotide diversity (0.01435) was observed. The incidence of universal COI and EF1α haplotypes and low differentiation between populations (F_{ST}=0.00655-0.17956, G_{ST}=0.00237-0.01722) revealed an absence of population structure and suggest a high level of gene flow over the range of *C. magister*. This finding is supported by MANTEL and AIS tests (comparing geographic distance to genetic distance), which also show little geographic structure. Coalescence analysis indicates a genetic bottleneck occurred approximately 78,000-146,000 YBP. High dispersal capability and high genetic diversity suggest that a few, strategically placed marine reserves along the crabs' range might maintain the evolutionary potential of this species.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

History of the Dungeness crab fishery in California

The Dungeness crab (*Cancer magister*) (Fig. 1) is a large edible crustacean that is found along the west coast of North America, from the Aleutian Islands in Alaska (Hoopes 1973) to Baja California (Pauley *et al.* 1989). *C. magister* resides in shallow coastal waters of bays, inlets, and estuaries, off shore to ~100 m depth. The species is almost completely carnivorous throughout its life, feeding primarily on bivalves, crustaceans, and fish, although juveniles have been known to occasionally feed on diatoms and/or algae when food is scarce or competition is high (Jensen and Asplen 1998).

Figure 1. The Dungeness crab (*Cancer magister*).

C. magister is of high conservation priority for many people, as it supports important sport and commercial fisheries all along its range (Hankin *et al.* 1997). This is especially true in California, where the species has a long history of both cultural and economic importance.

Dungeness crabs have long been a part of the cultural history in coastal California. In fact, archeologists have found evidence that several early Native American tribes consumed these animals. However, a commercial crabbing industry did not begin in California until the late 1840's, when it was introduced by Italian immigrants in San Francisco. Dungeness crabs subsequently gained popularity as a food source and the industry spread, as immigrants continued to pour into the San Francisco area during the Gold Rush (Dahlstrom and Wild 1983).

From the San Francisco area (Bodega Bay, Sausalito, San Francisco, and Half Moon Bay), the California commercial Dungeness crabbing industry spread northward to Crescent City, Eureka, and Fort Bragg, where the majority of crabs have been landed in the state since 1945 (Dahlstrom and Wild 1983; Pauley *et al.* 1989). The industry also spread southward to Monterey Bay, and eventually, after World War II, to Morro Bay and Avila Bay (Port San Luis). Since their beginning, the industries in Monterey Bay and southward have always produced significantly less crab landings than the northern ports in California. This is mainly due to a low abundance of crabs in the southern limits of the species' range, believed to be caused by environmental constraints such as a higher water temperature (Dahlstrom and Wild 1983).

Dungeness crab fishery management in California

Almost since the very beginning of the Dungeness crabbing industry, Californians have showed concern for protecting their valuable resource. The industry grew quickly in the 1890's with the advent of gasoline-powered boats. Fishermen began to fear that the crab supply was decreasing, as they had to travel greater and greater distances away from the mouth of the San Francisco Bay for their catch (Dahlstrom and Wild 1983).

In response to the fishermen's concerns, in 1897 the California government enacted the first law to protect Dungeness crabs, prohibiting possession and sale of female crabs (Dahlstrom and Wild 1983; Didier 2002; Tasto 1979). As the number of crabs continued to decrease, even with this law, the State Legislature added more restrictions on crabbing, including a closed season between September 2 and October 31 in 1903 (Rogers-Bennett 2002; Tasto 1979), and a minimum size limit of 6 inches across the carapace in 1905. In 1911, the minimum size limit was increased to 7 inches in order to help eliminate the illegal sale of females, which rarely reach this size (Dahlstrom and Wild 1983; Didier 2002).

Since 1909, the fishing season for Dungeness crabs in California has changed many times. In general, the fishing season is closed during the period of time when males are molting (late summer and fall in California), allowing crabs time for their shells to harden and the meat to fill out before harvesting (Hankin *et al.* 1997; Rogers-Bennett 2002). Until 1929, the legal fishing season was the same for all of California. However, since then the timing of fishing season has been changed to reflect Dungeness

crabs' developmental differences in Northern and Central California: The opening date of the season in Northern California is usually 2-4 weeks later than Central California, since crabs reach market condition more slowly in colder water (Dahlstrom and Wild 1983). Since 1994, the timing of the crabbing seasons in California, Oregon, and Washington has been dictated by soft shell testing conducted by the Pacific States Marine Fishery Commission to determine percent meat yield per crab. Crabs are tested around November 1st each year. If meat recovery is less than 25%, the opening of the season is delayed until 25% is reached, or until January 15th, when the fishery must open (Didier 2002; Rogers-Bennett 2002).

Another way the Dungeness crab resource in California has been managed is through the fishing gear used to trap crabs. The first widely used nets in California were hoopnets. These traps had course netting surrounding two iron hoops of different sizes, allowing them to lie flat on the ocean bottom, but become bucket-shaped to contain the crabs as they were pulled up. Wide spaces in the netting allowed undersized crabs to escape, also making the load lighter for fishermen (Dahlstrom and Wild 1983).

In 1938, hoopnets began to be replaced in California by more efficient round crab traps, or pots, now used exclusively in commercial fisheries (Fig. 2). These Dungeness crab pots are usually made of a circular iron frame 36-48 inches in diameter, covered with a coarse steel mesh. Two entrance tunnels are opposite each other and are fitted with trigger bars to prevent escape. In 1956, the California State Legislature began requiring at least one 4-inch rigid circular escape port on each pot, in order to allow sub-legal size males and most females out. In 1969, the number of escape ports was

increased to two, and in 1974 the required size of the ports was increased to 4 ¼ inches, where it remains today (Dahlstrom and Wild 1983). In addition, all traps are now required to contain a "trap destruction device," allowing animals to escape, should a trap be lost (California Fish and Game Commission 2003; Hankin *et al.* 1997).

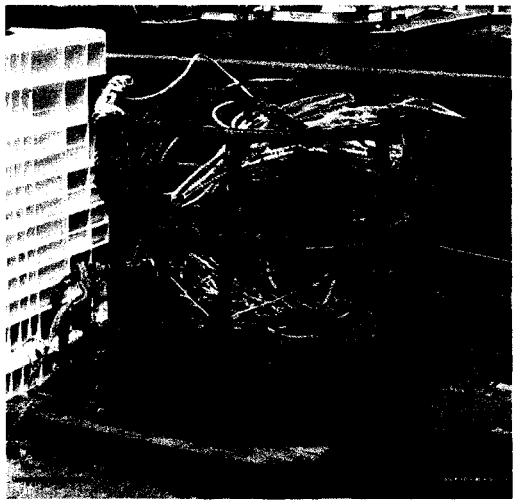

Figure 2. Dungeness crab traps (pots).

Sustaining Dungeness crab fisheries

Despite extensive regulations on the California fishing industry designed to protect the local Dungeness crab population, concerns remain about the sustainability of the fishery. A drastic decline in crab landings was seen in Central California around San Francisco from 1957 to 1982, amounting to an average loss of one to two million pounds per season (Farley 1983; Tasto 1979). The Dungeness crab fisheries in California continue to be of concern into the 21st Century. Although crab landings in Central

California increased by up to 78% during the 2000-2001 crabbing season, overall California commercial crab fisheries landed only 2,559 tons, a 36% decrease from the previous season, and the lowest landing on record for the state in 26 years. This decrease in yield led Dungeness crab prices to increase to an average of $2.23/lb, the highest in 10 years (Rogers-Bennett 2002).

Similar trends have been observed in the Dungeness crab fisheries in Southern Oregon, British Columbia (Jamieson *et al.* 1998; Zhang *et al.* 2004), and Alaska (Didier 2002; Rogers-Bennett 2002; Taggart *et al.* 2004), where crabs have equal commercial importance. A drastic decrease in Dungeness crab numbers even prompted the close of four Alaskan fisheries between 1980 and 1997 (Orensanz *et al.* 1998).

While the exact reason for the decline in *C. magister* numbers in many fisheries is unclear, there are many possible contributing factors. Normal interannual fluctuations in crab numbers have been attributed to a combination of naturally occurring density dependent selection and random environmental perturbations (Higgins *et al.* 1997). However, an increasing pressure on the marine system by humans may be causing a downward trend to these fluctuations. The human population along the Pacific Coast, especially in California, has increased substantially over the last several decades. With this increase in population comes increased potential for negative anthropogenic effects on the ocean, bays, and estuaries, all of which are essential for Dungeness crab reproduction, development, and survival (Wild 1983a). Factors that have been associated with a negative impact on Dungeness crab populations include: competition and predation associated with non-native introduced species, such as the green crab (*Carcinus*

maenas) (Banks and Dinnel 2000; McDonald *et al.* 2000), environmental contaminants (Horne *et al.* 1983; Tasto 1979), incidental mortalities of non-legal crabs in trawling fisheries (Reilly 1983b), and habitat destruction (Holsman *et al.* 2003; Visser *et al.* 2004; Wainwright *et al.* 1992).

An environmental factor that is often associated with a negative impact on Dungeness crabs is the increase of ocean water temperatures. High water temperatures have been found to lower fecundity of female Dungeness crabs (Wild 1983b), as well as increase the mortality of fertilized eggs, larval, and juvenile Dungeness crabs (Sulkin *et al.* 1996; Tasto 1979; Wild 1983c). Increasing water temperatures and global climate changes are of special concern in central California, where crabs are already at the southernmost end of their range, the extreme end of their natural tolerable conditions (Wild 1983a). With long-term climatic trends and the continuous anthropogenic strain on the Pacific Coast, it is uncertain at what level the Dungeness crab fisheries can be sustained in the future (Botsford *et al.* 1994).

Despite efforts to mitigate the decline of Dungeness crab fisheries, such as with essential habitat restoration (Visser *et al.* 2004; Warner and Visser 2000), some researchers assert that a change in management practices is needed. While most states have limited the number of crabbing licenses issued since 1995 (Didier 2002), there are no direct limitations on total commercial catches of male Dungeness crabs in any of the Pacific Coast fisheries; catches are only limited by the number of legal size males available each year. As a result, a "derby" situation has been created in many Dungeness crab fisheries, in which the majority of Dungeness crab catches occur in the first six

weeks of the fishing season (Didier 2002; Krachey and Hackett 2003). In addition, due to lack of catch limits, exploitation rates of legal size male Dungeness crabs are over 90% some years, thus leaving few large males to take part in mating in heavily fished areas. Although the effects of this selective harvest are uncertain, and female reproductive success seems to be unaffected (Oh and Hankin 2004), it could potentially have negative consequences on the future of *C. magister*. For example, selective harvest may cause selection for less desirable traits, such as earlier age and/or size of maturity, as well as shorter molt increments (Hankin *et al.* 1997; Jamieson *et al.* 1998).

A management option that has the potential to help sustain Dungeness crab fisheries is the implementation of marine reserves. Orensanz *et al.* (1998) argue that if the Dungeness crab fishing practices remain as they are now, with virtually all areas of the species range saturated with fisheries, overfishing with ultimately result. By establishing protected areas of *C. magister* habitat, these areas could serve as source populations to help "seed" areas outside the reserves, thus sustaining the population. However, in order for any reserve designed to protect Dungeness crabs to be effective, a clear understanding of larval dispersal mechanisms is needed (Shanks *et al.* 2003).

The importance of larval dispersal to marine reserve design

One of the most prominent strategies for protecting marine organisms is the implementation of marine reserves, protected ocean areas with restricted fishing within. When implemented correctly, reserves have to potential to conserve many exploited

marine species (Halpern 2003; Halpern and Warner 2003; Roberts 2005). However, many of the organisms that these reserves are designed to protect, including the Dungeness crab, have a mobile stage somewhere in their life histories; even organisms that are primarily sessile as adults, such as many invertebrates, have the potential of wide dispersal as eggs or larvae. Therefore, there is no guarantee that a species will remain within the boundaries of a marine reserve, or network of marine reserves, that was designed to protect that species. For this reason, the accurate understanding of patterns of larval dispersal is critical for marine reserve success (Botsford *et al.* 1994; Botsford *et al.* 2001; Shanks *et al.* 2003).

The dispersal capability of larvae can affect the survival of a species in two important ways. The first way is that it maintains gene flow between populations (Shanks *et al.* 2003), thus effectively opening up the gene pool, and, in turn, decreasing the threat of inbreeding depression and retaining maximum evolutionary potential for the species. The second way larval dispersal affects a species is by sustaining individual populations with new recruits, thus keeping the populations at an adequate size to survive environmental changes or stochastic events (Cowen *et al.* 2006; Palumbi 2003; Shanks *et al.* 2003).

In order for a marine reserve to successfully sustain the populations of a species of conservation concern, it must be understood as to how the individual populations of a species sustain themselves under natural conditions (Shanks *et al.* 2003). That is, do the young of a population remain in the location they were produced to help support that local population, or are they dispersed to a population elsewhere?

The level of movement into and out of a particular area depends on dispersal distance; if a certain organism has the capability to disperse very long distances, the sustainability of a particular population may greatly depend on processes occurring far from that location. For this reason, a reserve that may be set up to protect a locally valuable species, such as one that is economically important, may be ineffective in sustaining that local population (Roberts 1997). In a case such as this, it is necessary to increase the scale of management being implemented, perhaps leading to large-scale international cooperation.

While few outside recruits may be needed to maintain gene flow between separate populations, many are needed to maintain a sustainable population size (Cowen *et al.* 2006). This is because it is estimated that less than 10% of the individuals that recruit to an area will survive to reproduce (Shanks *et al.* 2003). Marine reserves, if they are to effectively sustain population size, need to be designed in size and spacing to provide the maximum number of recruits to all populations of concern. Shanks *et al.* (2003) suggest that this can be accomplished in two ways. A population within a reserve can be sustained by making the reserve large enough to keep most dispersing offspring within the protective boundaries. However, there are several problems with this strategy. The first problem is that, while it may be practical for species with very short dispersal distances (Botsford *et al.* 2001), if the dispersal distance of a species is very large, it may be impractical or impossible to make a reserve that large (Botsford *et al.* 2003; Palumbi 2003). A second problem is that many reserves are designed to replenish species of economic importance to fishermen. If reserves are to be effective for this purpose, some

individuals must recruit outside of the protected area where fishing is legal (Palumbi 2003).

The second strategy for marine reserve design proposed by Shanks *et al.* (2003), is to space reserve areas so that larvae have the ability to disperse to neighboring protected populations. Other biologists support this strategy (Botsford 2001; Botsford *et al.* 2003; Palumbi 2003; Roberts 1997). Because many populations depend on larvae from distant locations, networks of small interdependent reserves would help encourage gene flow as well as sustain population size. For coastal areas, Palumbi (2003) suggests that the best strategy for maintaining movement of individuals between populations may be to design marine reserves according to the stepping stone model (Fig. 3). In this model, individuals are not only able to move to nearby populations, but have the potential to be exchanged between distant populations as well.

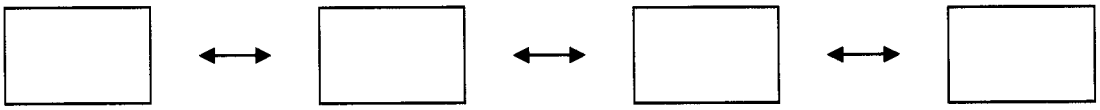

Figure 3. Stepping stone model for reserve design. Boxes represent reserve locations/populations. Populations close to one another may readily mix, while distant populations may mix less frequently by using neighboring reserves as "stepping stones."

One of the advantages to creating a reserve made of a network of smaller patches, is that it puts less economic strain on the conservation programs that must buy the land for reserves, allowing them to stretch out limited resources as effectively as possible. In addition, this strategy puts less strain on local economies, particularly for fishermen who

may be pushed out of a job by a larger reserve that retains all individuals of an economically important species. A potential drawback of this strategy, however, is that a large-scale management network, in most cases, requires the crossing of geographic, political, and social boundaries in international cooperation, a feat that may be very difficult in some cases (Roberts 1997).

Knowledge of larval dispersal distances can help focus limited conservation resources in other ways as well. Populations that provide larvae for other populations are referred to as "sources," while populations that rely on recruits from other populations are termed "sinks." Roberts (1997) suggested that these sources and sinks could be analyzed to determine which areas are more vulnerable to over-fishing, and thus are of greater conservation concern. According to Roberts (1997), areas that are completely dependent upon their own larvae for sustainability (essentially their own only source), or areas that serve as important sources for many other populations, should be of highest conservation priority.

Indirect estimates of larval dispersal

In order for larval dispersal to be effectively used as a tool in marine reserve design, we need accurate data for dispersal capabilities of all species involved in the reserve. This can be a difficult goal to obtain, as direct observations of larval dispersal and settlement are almost impossible to accomplish in the field (Todd 1998) and tagging larvae has proved to be difficult in many cases (Anastasia *et al.* 1998; Levin *et al.* 1993;

Thorrold *et al.* 2002). Almost all studies that have been able to make direct observations of larval movements have focused on large-sized, short-dispersing larvae that may be easily followed by eye (Shanks *et al.* 2003). Even in these cases, most studies took place in the lab where observed larval behavior may not reflect what actually takes place in the wild (Palumbi 2003; Todd 1998). In addition, life histories and local habitat differ widely between organisms, so even if it is possible to obtain accurate data for one species in the wild, it can probably not be assumed accurate for another (Grantham *et al.* 2003).

Because it is so difficult to quantify larval dispersal directly, dispersal most often must be indirectly estimated from other factors believed to influence larval movements. Among the most basic and widely used of these dispersal distance estimators is life-history, in particular the amount of time a species spends in the free-moving larval, or pelagic, stage (Grantham *et al.* 2003; Holmes *et al.* 2004; Todd 1998). The time an organism spends in the pelagic stage depends on the particular species. This time can range from a few minutes to a few months (Shanks *et al.* 2003). Those organisms that spend a short amount of time as free moving larvae have less time to move away from their origin, and therefore should disperse a much shorter distance than those species with a long pelagic phase.

The use of pelagic duration as an estimation of dispersal capability is an easy way to get basic estimates of dispersal behavior, and is the only estimation criteria available for most species (Grantham *et al.* 2003). However, this assumption does not always prove to be accurate. In a survey of literature for 32 taxa, Shanks *et al.* (2003) found that most estimates of larval dispersal capability, determined from a range of criteria,

followed the expected correlation. However, six cases were noted where actual distance of propagule dispersal was found to be shorter than expected. In these cases, the authors hypothesized that the discrepancy was due to other factors besides pelagic duration influencing larval dispersal, such as oceanographic features and larval behavior, both of which may have more influence on dispersal capability than is often assumed.

Besides life-history parameters, the second most used factor to indirectly determine dispersal capability is oceanographic data. Because ocean currents operate at differing intensities depending on the time of year or location where larvae are released, they have the potential to greatly affect dispersal capability (Botsford *et al.* 1994; Palumbi 2003). In most cases where oceanographic factors are considered, efforts are focused on surface-drifters, making the assumption that larvae act as passive particles moved by surface currents (Roberts 1997; Sotka *et al.* 2004). It is hoped that surface current data may, in particular, help to determine where cooperation between countries and regions would be most helpful, by determining which areas are most tightly linked as larval sources and sinks for specific populations. In addition, Roberts (1997) asserts that surface current data may help to determine which locations have little to no currents moving larvae to or from the area, and therefore those that do not need to focus as greatly on international cooperation or reserve networks.

However, Largier (2003) argues that oceanographic phenomena, as they relate to larval dispersal, are much more complex than surface currents alone. Indeed, other scientists agree that current data cannot be effectively used to estimate dispersal if the complexity of ocean currents, both temporally and spatially, is not taken into account

(Botsford *et al.* 1994; Cowen *et al.* 2006). Largier (2003) states that there are three oceanographic phenomena that are particularly important to consider when predicting larval dispersal: topographic eddies, the coastal boundary layer, and vertical shear.

Topographic eddies are geographic formations, such as rock outcroppings or bays along a coast, that disrupt the large-scale flow of currents (Largier 2003; Strub *et al.* 1991). These eddies, which can exhibit velocities much higher than the general current flow (Korso *et al.* 1991; Swenson *et al.* 1992), often cause water to recirculate and thus to retain larvae, enhancing both local recruitment and recruitment of nearby areas (Cowen *et al.* 2006). In addition, the disruption in current systems that eddies create may serve as boundaries to larval dispersal. For example, a strong eddy at Point Conception, California has been found to be a barrier to dispersal of several benthic invertebrate species in the vicinity (Burton 1998; Dawson 2001; Wares *et al.* 2001).

The affects that eddies can have on local retention of larvae and their ability to act as barriers to dispersal are complicated and can vary greatly, depending on eddy size, the time scale of the current driving it, the time at which larvae are released, and the duration of larval dispersal. For example, if larvae are released at a time of year when the current is not operating, or during a year in which the current is weak, the influence of the eddy on larval dispersal will be low. However, if the larvae of a species are released when the current is strong, and larval duration is shorter than the time the current is operating on the eddy, the larvae have the potential of being completely retained (Largier 2003).

A second oceanographic phenomena that Largier (2003) claims to be significant to larval dispersal, is the coastal boundary layer. This is the term given to the slow

movement of water, sometimes called "sticky water" (the term coined by Wolanski 1994), close to the coast caused by drag from the bottom and a jagged coastline interrupting current flow. This boundary of "sticky" water serves as a barrier by slowing down larvae's ability to reach the fast-moving offshore currents, those with the ability to carry larvae great distances. In essence, "larvae must go offshore to get alongshore" (Largier 2003). The coastal boundary layer differs in size and strength along coastal regions, and thus differs in its ability to retain larvae. Again, duration of the pelagic phase will greatly affect the power of the boundary over larval dispersal. For example, if the larvae of a species have a short dispersal time, and are released in an area with a wide, strong coastal boundary layer, all larvae may be retained in the "sticky water," never even reaching the strong offshore currents, and so may only be able to disperse a short distance. However, if the larvae have a long pelagic phase and the strength of the boundary is weak, the larvae may pass the surf zone very quickly, and thus will potentially be swept long distances by fast-moving offshore currents.

Largier (2003) claims that a third important factor in determining dispersal potential of larvae is vertical shear. Vertical shear is the term given to differences in current direction and strength at differing depths in the water column. These differences in currents can greatly affect larval dispersal capabilities. For example, currents near the bottom of the water column are usually slower than those near the top. Therefore, if larvae have a negative buoyancy and remain near the bottom of the water column, those larvae will have a shorter potential dispersal distance than those near the top. In addition, vertical shear becomes even more complicated in areas of upwelling, or in eddies, where

a combination of currents of differing directions may occur. Thus, in these areas, if larvae move up and down in the water column, they have the capability of being retained in one area (Hardy 1935).

While studies of oceanographic data may provide important insights into larval dispersal capabilities, just as with pelagic duration, it is oversimplifying things to use this data as the only predictor of dispersal. Many other factors, although less prevalent in the literature, have been implied to affect the dispersal of larvae, including larval behavior, timing of spawning, adult life expectancy, and natural selection. While many studies involving larval dispersal predictions from pelagic duration and oceanographic data assume that larvae act as passive particles in the ocean, it is now suggested that this is usually untrue in actuality. It has been shown that larval swimming behavior can play a role in increasing larval retention, especially when working in correlation with vertical shear, as discussed above. Because of this, when larval behavior is not taken into account, the result is often an overestimate of dispersal capabilities (Palumbi 2003; Roberts 1997; Shanks *et al.* 2003). On the other hand, if larval swimming behavior moves across the coastal boundary layer, or in the same direction as offshore ocean currents, swimming behavior can, theoretically, serve to actually increase dispersal capabilities (Largier 2003). Indeed, when Cowen *et al.* (2006) simulated the amount of connectivity between populations of reef fishes in the Caribbean, they found that active movement increased the amount of exchange of larvae between sites.

Duration of spawning and life expectancy of adults can affect larval dispersal as well. This is because the more larvae that an individual produces in a season or

throughout its lifetime, the greater the chance that some larvae will be produced when oceanographic factors, such as eddies, coastal boundaries, or vertical shear, are weak. Thus there is a greater chance that some larvae will encounter strong offshore currents and be dispersed long distances (Largier 2003).

Another factor that is often ignored when estimating larval dispersal capabilities is the influence of natural selection. Grantham *et al.* (2003) claim that the type of environment that an organism lives in may be used to predict dispersal patterns. For example, if an organism lives in isolated patches separated by uninhabitable area, it may be to the advantage of the organism to disperse short distances and remain in its habitable patch; those that disperse out of the patch will be less likely to encounter suitable habitat and thus will be less likely to survive to reproduce. In an interestingly related point, it has been suggested that marine reserves, as isolated patches of protection in a sea of inhospitable fished area, may themselves present a similar selection pressure for short dispersal distances (Botsford *et al.* 2001). On the other hand, if an organism lives in an area of frequent unpredictable disturbance, it may be more favorable for it to evolve a long distance dispersal, thereby making the offspring more likely to survive in other areas, even if one particular location is made completely uninhabitable (Grantham *et al.* 2003).

Natural selection has the ability to affect dispersal patterns in other ways. Many estimations of dispersal capabilities fail to take into account differences in recruitment and survival success from different populations (Largier 2003). Even if certain larvae make it to a population, those larvae will not necessarily survive to take part in the local

gene pool (Todd 1998). If an individual is transported a long distance, it is more likely that that individual will encounter an environment that is very different from the one it came from, and thus will be likely to have different selection factors acting on it than those the individual is adapted to. For these reasons, post-settlement mortality may play a more important role in the sustainability of adult populations and gene flow than often assumed.

The role of genetic data in determining larval dispersal

Estimating larval dispersal is not simple, and is made even more complicated by the large variety of pre-recruitment, recruitment, and post-recruitment processes that have the potential of affecting gene flow and the sustainability of a population (Table 1). In addition, while some models used to estimate dispersal have attempted to take all of these varying criteria into account (Cowen *et al.* 2006), most of the time few criteria are used when making assumptions of dispersal capabilities, leading to oversimplified estimates. Even when multiple criteria are used, scientists are often limited by the amount of accurate data available on the species in question, making it difficult to determine which factor is the most influential in determining dispersal distances (Shanks *et al.* 2003). A neglect to take additional factors affecting dispersal into account, underestimating a factor, or overestimating the importance of a factor can easily lead to a false assumption of dispersal, and thus an ineffective reserve design (Largier 2003).

Table 1. Summary of factors with the potential to impact community structure, gene flow, and sustainability (modified from Todd 1998).

PRE-SETTLEMENT PROCESSES and FACTORS [Seconds-Months]

1. Predation on larvae (in the water column)
2. Oceanographic influences (e.g. directional currents, upwelling, eddies, coastal boundary layer, and vertical shear)
3. Local hydrographic effects (e.g. tidal current variation, residual drift, hyposaline runoff)
4. Larval 'quality' (are all larvae equal?)
5. Larval behavior (e.g. vertical migration, responses to salinity, larval swimming)
6. Substratum electivity
7. Benthic predation

SETTLEMENT and RECRUITMENT PROCESSES and FACTORS [Minutes-Hours]

1. Successful encounter of, or attachment to, favorable habitat
2. Responses to substratum cues to recruit and/or attach
3. Detection of conspecifics or founders
4. Detection/avoidance of superior competitors or predators
5. Responses to allelochemical agents
6. Successful completion of metamorphosis

POST-SETTLEMENT PROCESSES [Seconds-Decades]

1. Intra-specific competition
2. Inter-specific competition
3. Biotic interactions (predation, grazing, inadvertent killing by consumers)
4. Parasitism and disease
5. Physical disturbances
6. Reproductive performance and success
7. Immigration/emigration of juveniles and/or adults

Genetic data may serve to reduce the uncertainty of dispersal estimation by directly detecting the amount of gene flow between populations. While most estimates of larval dispersal depend on criteria that can potentially *cause* dispersal and gene flow, genetic studies have the ability to measure the *effects* of this dispersal, the real focus of marine reserve design. If larvae are moving between populations, this should be reflected in the amount of gene flow seen between these populations. For example, in the stepping stone model (Fig. 3), populations near one another are predicted to exchange more individuals than distant populations. Thus, if we study the genetics of these populations,

we should see that close populations are more genetically homogenous than distant ones, which should be found to be more distinct from each other (Palumbi 2003). In this way, genetics can be used to test the validity of outcomes predicted by various dispersal factors, and thus support or refute their use in marine reserve design for a particular species (Roberts 1997).

The use of genetic data to test the predictions made by dispersal factors may result in an affirmation of assumptions. When this is the case, we can feel more confident about our predictions made according to various commonly accepted dispersal parameters. For example, as was discussed earlier, the use of pelagic phase duration is a factor commonly used to predict dispersal capabilities. Based on this assumption, we should observe those organisms with a long pelagic phase (with more time to disperse) to be genetically homogeneous along their range, as we assume these organisms to disperse over long distances. In many genetic studies, this assumption has held true (Todd 1998). For example, Hellberg (1996) measured allozyme variation in two solitary corals occurring along the same range of California coast, with very different larval durations. His results agreed with predictions: The species that lacked a free-swimming larval stage was found to have a much higher amount of genetic differentiation between populations than the species with a long free-swimming stage.

The predictions made by pelagic phase duration or other factors do not always hold true, however, when genetic data is examined. When this happens, genetic studies have the potential to give us a clue as to what primary forces, some of which may be completely unexpected, are actually driving gene flow (Palumbi 2003). For example,

through genetic studies it has been suggested that paleogeographic history may play a larger role in determining the genetic structure observed today than previously believed. Barber *et al.* (2000) found a much higher amount of genetic structure between mantis shrimp (*Haptosquilla pulchella*) populations than that predicted by pelagic duration or ocean current drifter data. They concluded that the genetic separation observed reflects the separation of the Indian and Pacific Ocean basins caused by low-level seas during the Pleistocene, 6,000-10,000 year ago. These data suggest that processes occurring thousands of years ago have the potential to affect genetic connectivity today, and so should be taken into account when designing reserves.

In a similar study, Sotka *et al.* (2004) studied the population genetics of the acorn barnacle (*Balanus glandula*) along the western coast of the United States. For most of the species range, mitochondrial and nuclear DNA sequences indicated broad dispersal capabilities, as predicted by a long pelagic phase and surface current drifter data. However, a steep genetic cline across 475 km was located in Central California, indicating an unexpected restriction of gene flow in this area. The researchers concluded that this separation reflects a historical separation of barnacle subpopulations by glacial ice during the Pleistocene. In addition, Sotka *et al.* (2004) suggest that the genetic cline has been maintained over thousands of years by a combination of selection (northern and southern areas of the California coast vary greatly in environmental conditions, especially in air and water temperature) and current-restricted dispersal.

Other genetic studies suggest that selection may play a bigger role in gene flow that often assumed. For example, Drouin *et al.* (2002) studied the population genetics

and planktonic larvae distribution of two adjacent populations of the barnacle *Semibalanus balanoides* in the Gulf of St Lawrence, to determine whether gene flow between them was restricted by larval dispersal. As predicted by the unidirectional current in the area, genetic and larval movement results indicated a north to south gene flow. However, some unusual results were found as well. A high frequency of a certain allele in the GPI locus found among larvae and recruits was not found in adults from either population. Since the GPI locus is related to glucolysis and gluconeogenesis, and properties of this protein are known to vary with temperature, the authors suggested that temperature experienced during emersion after recruitment could likely be acting on this gene as a strong selection factor. In addition, stronger genetic differentiation than expected between the two populations supports the idea that a selective force is maintaining genetic structure, even though larvae are dispersing between the populations. This suggests once again an important fact that can be ignored in the estimation of dispersal capabilities: just because larvae can get there, it does not mean they will survive to reproduce. As an intriguing alternative hypothesis, Drouin *et al.* (2002) suggested that the unique allele found among larvae may have originated from a larval source that is not intuitive based on ocean current data.

Genetic studies have uncovered other unusual mechanisms of gene flow, not necessarily intuitive at first glance. Holmes *et al.* (2004), for example, conducted RAPD analysis on populations of the bivalve mollusk *Abra tenuis* in the Dutch Western Wadden Sea to determine if population genetic data agreed with the predictions made by larval dispersal potential. *A. tenuis* produces directly developing larvae. That is, the larvae do

not have a mobile stage and so are expected to be found in genetically distinct patches with no gene flow between them. Based on this assumption and according to the current applications of larval dispersal to conservation, all patches of *A. tenuis* should be managed separately without consideration to reserve networks. However, genetic analysis indicates that there is some mixing between populations, supported by that fact that occasional decimations of populations due to stochastic events were followed by rapid recovery and recolonization. Holmes *et al.* (2004) determined that instead of larval dispersal, this unexpected gene flow was probably due to the transport of small juveniles through the digestive systems of waterfowl or through ocean currents. Other studies have demonstrated dispersal of marine organisms beyond the larval stage. Collin (2001), for example, observed adult gastropods *Crepidula* riding on the carapaces of horseshoe crabs. While the genetic affects of this dispersal habit could not be measured, it does have the potential of affecting gene flow beyond the intuitive mechanisms.

Dungeness reproduction, development, and dispersal

The reproductive and developmental cycles of the Dungeness crab have been studied in detail. In California, mating occurs March through May between recently-molted, soft-shelled females and hard-shelled males (Program Staff 1983). To prevent sperm competition, males produce sperm plugs that remain in the female's vaginal tract throughout the mating season (Oh and Hankin 2004). Female crabs store sperm internally until eggs are extruded and fertilization occurs, between the months of October

and November (Program Staff 1983). Extrusion and fertilization usually take place annually in California, but may occur up to two and a half years after mating in northern locations (Hankin *et al.* 1989; Swiney and Shirley 2001; Swiney *et al.* 2003). Females can extrude up to two million eggs, which are carried on the female's abdomen until winter, when most hatching takes place within two weeks (Higgins *et al.* 1997; Program Staff 1983). This reproductive cycle is similar for all Dungeness crabs. However, timing of stages is shifted later in the year for crabs in colder, northern locations, where development is slower (Shirley *et al.* 1987).

Larvae are released between December and May, depending upon latitude; larval release is earlier in the south and later in the north. After hatching, larval Dungeness crabs go through five zoeal stages and one megalopal stage, a transformation that takes two and a half to five months to complete, depending upon ocean temperature (Reilly 1983a; Shirley *et al.* 1987). Because zoeae spend relatively more time at the surface, due to diel migration, early stage zoeae are transported offshore by seaward-moving surface waters (Reilly 1983a).

Once larvae have molted into megalopae, they return back to the coast and settle on the floor of bays, inlets, and estuaries, usually in waters less than 20 meters deep (Jamieson *et al.* 1988; McConnaughey *et al.* 1992; Roegner *et al.* 2003). Megalopae choose settlement sites based on structural complexity (allowing more places to hide from predators, Fernandez *et al.* 1993; Hora 1999) and lack of predators and competitors that can be detected chemically (Banks and Dinnel 2000). After settling, megalopae undergo a series of six molts over the course of 4-12 months. Adult crabs reach maturity

after about two years, and become marketable size between two and five years of age (Wild *et al.* 1983a). Maximum adult size (in carapace width) ranges from 170 mm for females (Hart 1982), to 254 mm for males (Cleaver 1949), and Dungeness crabs can live up to ten years (Williams 1979).

While adult Dungeness crabs have the capability to move up to 100 miles along the coast, it has been shown that significant movement of adults is unusual (Soule and Tasto 1983; Stone and O'Clair 2002); as with many benthic marine invertebrates, the majority of dispersal is believed to occur during the pelagic phase. Since *C. magister* has such a long pelagic phase (2.5- 5 months), based on traditional assumptions we would assume the species to have high dispersal capabilities, with genetic homogeneity throughout its range. Indeed, morphological similarities between adult crabs from all along the coast suggest that Dungeness crabs from different locations could be significantly similar genetically (Soule and Tasto 1983). (Although morphological differences have been noted between Dungeness crab larvae from northern and southern locations (Shirley *et al.* 1987).) However, without genetic analysis it is uncertain whether the actual dispersal and gene flow of the species agree with predictions.

There are several reasons to believe that assumptions of Dungeness crab larval dispersal may not agree with actual gene flow, if one considers potential influences of ocean currents, behavior, and selection. Currents and other oceanographic phenomena, varying in strength and direction both temporally and geographically along the Pacific Coast of North America, especially have the potential to greatly affect dispersal distances. For example, most Dungeness crab larvae in central California are released

when the Davidson Current is the primary determinant of coastal water movement (Reilly 1983a). The Davidson Current moves parallel to the coast, at speeds up to 150 miles per month, in a northward direction (Lough 1976). However, the currents that are of primary strength in California during the pelagic phase of Dungeness crab larvae do not operate at the same intensities or at the same distance from the coast in more northern locations (Reilly 1983a). Differences in the influences on *C. magister* settlement can even be seen within California. For example, Wing *et al.* (1995) found that settlement of megalopae to the north of Point Reyes, California seemed to occur primarily during relaxation events, whereas little correlation between settlement and upwelling was seen in the south. On a longer decadal scale, in association with the El Niño/ Southern Oscillation (ENSO), it has been suggested that the pole-ward moving California Current may have more influence on dispersal and recruitment (Botsford 2001).

In addition, oceanographic phenomena such as embayments and eddies, especially in the southern half of *C. magister*'s range (Wing *et al.* 1998) and in Glacier Bay, Alaska (Taggart *et al.* 2003) may retain larvae, thus limiting dispersal capabilities in those locations. For example, a relatively strong eddy is located to the south of Point Reyes, California. The circulation of this eddy appears to retain larvae during periods of upwelling, acting perhaps as somewhat of a dispersal barrier, but sends larvae poleward and shoreward during periods of relaxation (Botsford 2001).

Dungeness crabs may also affect their own dispersal through their behavior. Dungeness crab megalopae are strong swimmers and it has been suggested that they migrate vertically in the water column on a diel basis (Fernandez *et al.* 1994; Reilly

1983a). By swimming up and down in the water column, moving between currents of differing direction, megalopae may effectively limit their dispersal (Botsford *et al.* 1994). Megalopae are known to aggregate in discrete patches at sea (Shenker 1988). In addition, Stone and O'Clair (2002) demonstrated that adult female Dungeness crabs in Southeastern Alaska show site fidelity to brooding sites. If larvae released from a single female remain together at sea and return together, perhaps to approximately the same location they were hatched, this has the potential to greatly limit gene flow between geographic locations.

Selection on Dungeness crabs, both pre- and post-settlement, may also limit the effective gene flow between geographic regions. Rate of development and survival of Dungeness crab larvae are affected by both temperature and salinity on inter-annual, intra-annual, and latitudinal scales (Botsford *et al.* 1994, 1998). Differences in temperature can affect the mortality of crabs in several ways. Colder temperatures cause larvae to develop more slowly, leaving them more vulnerable to predators for longer periods of time at northern latitudes (Botsford *et al.* 1998; Taggart *et al.* 2003). In addition, extremes in both cold and warm temperatures at the ends of the species' tolerance range have been shown to increase mortality at all stages of the Dungeness crab life cycle, especially in juveniles (Sulkin *et al.* 1996; Taggart *et al.* 2003; Tasto 1979; Wild 1983b, c).

Temperature may affect the extent of Dungeness crab larval dispersal in other ways. Because of the variation in larval duration caused by temperature, larvae potentially have more time to disperse greater distances in the colder northern part of

their range than in the south. The differences in temperature also cause larvae to be released at slightly different times during the year, causing different populations to experience not only geographic differences in ocean currents and other environmental factors, but also temporal differences (Botsford *et al.* 1994, 1998). Selection for this difference in release and settlement times of Dungeness larvae has been proposed as a possible mechanism of isolation between northern and southern populations (Botsford *et al.* 1998).

Population genetics of the Dungeness crab

As with other species, genetic analysis has the ability to give us a better idea as to what the actual dispersal capabilities of the Dungeness crab are. Biologists have recognized the importance of understanding the genetic structure of *C. magister* from the time that population genetic analytical techniques were only beginning to be developed. Soule and Tasto (1983) conducted the first published attempt to investigate possible genetic and geographic variation of Dungeness crabs throughout their range in the late 1970's. Initial electrophoretic analysis of proteins from approximately 20 loci in 300 adult male crabs from Alaska to Morro Bay, California, found low levels of genetic variation between populations, suggesting high dispersal capabilities. However, when Soule and Tasto (1983) increased the sample size to 1600 animals, and analyzed San Francisco and Eureka populations in greater detail, results were inconclusive, leading the

researchers to conclude that electrophoretic markers were not useful to distinguish population structure of the Dungeness crab.

With the advent of more advanced techniques in genetic analysis in the 1990's, however, biologists began to revisit the examination of dispersal capabilities of *C. magister* through genetic variation. Toonen and Grosberg (2003) examined six microsatellite loci in Dungeness crabs from 12 sites throughout most of the species range. Their data revealed slight, but statistically significant, population differentiation between states, leading them to conclude that larval dispersal of *C. magister* was far-reaching, but not effective enough for complete genetic homogenization of the species. Toonen and Grosberg (2003) suggested that the differentiation seen was most probably due to a combination of temporal variation in the source of larval recruits, variation in individual reproductive success, and spatially and temporally variable natural selection. They found no evidence for major barriers to Dungeness crab dispersal.

The Parr Lab at San Jose Sate University began an extensive study of the genetic structure of *C. magister* in 1997, by sequencing the Cytochrome *c* Oxidase subunit I (COI) mitochondrial gene from Dungeness crabs in various life stages throughout the species' range. Results found by Dedhia (2005), indicate strong genetic differentiation between Alaska, Oregon, and California. In fact, she found no shared haplotypes between the three states. However, sample sizes from Alaska (n=5) and California (n=16) were very small, and so results were not statistically significant. Further examination into the mitochondrial and nuclear genetic variability of Dungeness crabs in

California and Alaska is needed if we are to accurately understand the population genetic structure of *C. magister*, and thus make effective recommendations for reserve design.

Goals of this study

There are three objectives of the current study:

1. Determine mitochondrial and nuclear genetic variability of *C. magister* throughout California.
2. Assess wide-range genetic structure of *C. magister* by comparing mtDNA sequences from crabs in California to those from Oregon, Washington, and Alaska.
3. Use genetic information to assess larval dispersal capabilities of *C. magister,* and make recommendations for marine reserves and overall management strategies for the species.

MATERIALS AND METHODS

Sample collection and DNA extraction

Legs and claws from adult crabs were collected along the coast of California at Crescent City (n= 16), Eureka (n= 25), Fort Bragg (n= 25), San Francisco (n= 53), Half Moon Bay (n= 36), Monterey Bay (n= 40), Morro Bay (n= 6), and Port San Luis/Avila Bay (n= 29) (Fig. 4) between January and June 2005, during the California commercial crabbing season. Samples were collected opportunistically and were donated by local commercial fishermen, seafood markets/distributors, and scientists. All crabs were caught with standard circular Dungeness crab traps (Fig. 2). When possible, a single leg was removed from whole live or dead individual crabs. In the case of live crabs, non-lethal sampling was employed. Legs were removed from live crabs by firmly holding one of the fifth walking legs until the crab autotomized it. This minimized the risk of the crab bleeding to death from the lost leg after release.

In some cases, loose legs that had been lost by crabs while in traps or holding tanks were utilized. Commercial fishermen stated that they rarely observed crabs missing more than one leg. However, in order to minimize sample duplication, legs from the same source were sorted, and only those legs of the same size, from the same side of the body, with obvious differentiation in pigmentation were used. In one case where over 200 legs and claws were donated from a single commercial fishing company in San

Francisco, a random sample of 50 was taken by using a random number generator. All samples were stored frozen at –20°C.

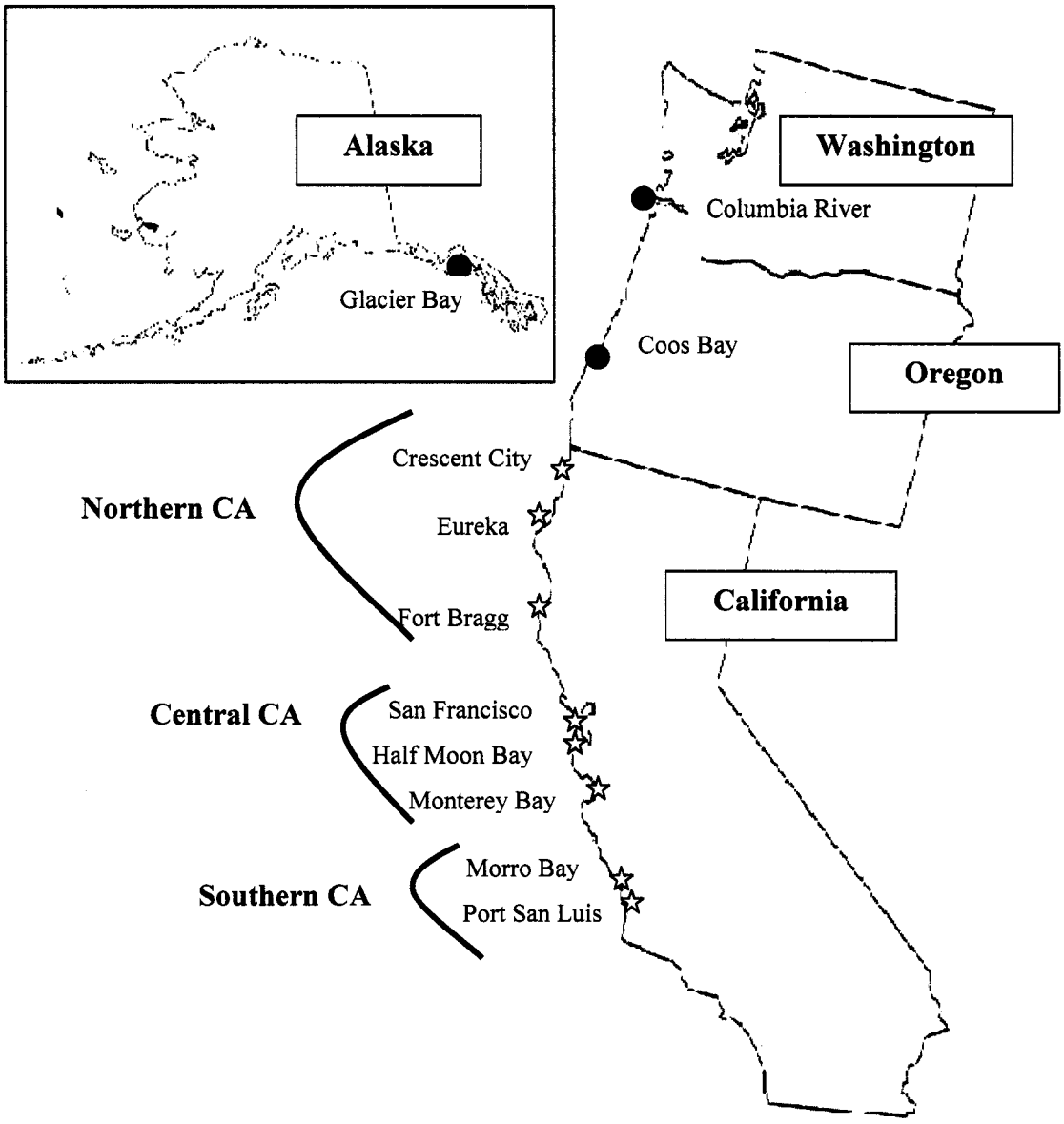

Figure 4. Sample collection map. Yellow stars indicate collection sites used in this study. Pink dots indicate sample sites used for COI data comparison.

To avoid cross-contamination, muscle tissue was removed from the innermost part of each crab leg. DNA was extracted from this tissue using the Wizard Genomic Purification Kit (Promega), following the manufacturer's instructions. Rehydrated DNA was stored at –20°C.

PCR analysis and sequencing

A ~415 base-pair region of the Cytochrome *c* Oxidase subunit I (COI) mitochondrial gene was amplified by the polymerase chain reaction (PCR) from all samples using the following primers, designed based on the sequence published by Harrison and Crespi (1999): COI_for 5' GGAGGATTTGGAAATTGATT 3' and COI_rev 5' GTACAGGAAGGGATAGTAGT 3'. PCR was performed in a 25 µl reaction containing 2 µl DNA extract and the following final concentrations: 1X PCR Buffer, 0.2 mM dNTPs, 0.4 µM forward and reverse primers, 0.4 mg/ml Bovine Serum Albumin (BSA), 4.5 mM $MgCl_2$, and 1 unit Taq Polymerase. BSA was added in order to counteract the PCR-inhibiting affects of seawater (Kreader 1996). The following thermal cycler reaction was run on a Personal Thermal Mastercycler (Eppendorf): 5 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 45 sec at 50°C, and 1 min at 72°C, and a final extension of 72°C for 10 min. Following amplification, PCR products were run on a 2% agarose gel to check for specificity of product and size of fragment, and sent to Gene Gateway, LLC, Hayward, CA, a private biotechnology company, for forward sequencing. Sequences were obtained through the use of the ABI Prism Model 377 (version 3.2)

automated sequencer (Smith *et al.* 1986) using Big Dye chemistry. Questionable sequences were verified by reverse sequencing.

In addition, a ~487 base-pair fragment of the nuclear first Elongation Factor α subunit (EF1α) was amplified with PCR from a subset of the total samples from California using the primers: EF1_for 5' ACGGCGACAACATGCTGGAGA 3' and Ef1_rev 5' CGGGGTGGTTCAGGACGATGA 3' (Sotka *et al.* 2004). PCR was performed in a 50 μl reaction containing 4 μl DNA extract and the same final concentrations of reagents as used in COI PCR reactions. The same thermal cycler program as used for COI PCR was run on a Personal Thermal Mastercycler (Eppendorf), with an increase in the annealing temperature to 60°C, and an increase in the number of cycles to 35. Because of monetary constraints, a subsample of approximately 30% of the total samples (following Sotka *et al.* 2004) from Eureka (n=11), Fort Bragg (n=4), San Francisco (n=11), Half Moon Bay (n=7), Monterey Bay (n=12), and Port San Luis (n=7) was used for nuclear sequence analysis. After confirmation of size and specificity of PCR products on a 2% agarose gel, all products were sequenced in forward and reverse directions to confirm heterozygotes.

The decision to use two different genetic markers, COI and EF1α, is important for several reasons. The COI gene is commonly used in studies of invertebrate population genetics. This is because it has high intraspecific variability, especially in crustaceans, and since it is a mitochondrial gene it is inherited maternally with no recombination (in most species, including *C. magister*), allowing COI to be used to track maternal lineages (Avise 1995). However, there is growing evidence that single-marker studies may be

misleading (Cronin 1993; Degnan 1993; Moritz 1994). For example, a high frequency of a particular haplotype in a population may indicate selection. However, overrepresentation of some haplotypes may also be due to a founding effect, genetic drift, or phylogeographic history. COI and EF1α are unlinked, have different modes of inheritance (as a nuclear gene, EF1α experiences recombination and is inherited biparentally), and have different mutation rates. Using the two markers together allows for greater resolution of genetic diversity, population structure, and factors affecting gene flow (France *et al.* 1999; Hellberg *et al.* 2002).

Data analysis

Sequences were edited and aligned using the computer program BioEdit v7.0 (Hall 2005). All COI and EF1α sequences generated were compared within and between locations in California, for individual sample sites and regions. California samples were grouped into regions as follows: Northern California (N_CA)= Crescent City, Eureka, and Fort Bragg; Central California (C_CA)= San Francisco, Half Moon Bay, and Monterey Bay; Southern California (S_CA)= Morro Bay and Port San Luis (Fig. 4).

In addition, regional California COI sequence data was compared to sequence data generated by other students in the Parr lab at San Jose State University for adult, juvenile, and larval Dungeness crabs from Glacier Bay, Alaska (n=106), the mouth of the Columbia River, Washington (n=128) and Coos Bay, Oregon (n=382) (Table 2 and Fig. 4) (Chockalingam 2004; Dedhia 2005; Upadhye 2005) in order to ascertain a more

complete picture of Dungeness crab population structure throughout the species' range. For these sequences used for comparison, adult samples were collected and processed as described for California. Larval crabs were collected by using light traps (Miller *et al.* 2004, Roegner *et al.* 2002), behavioral samplers that depend upon the positive phototropism of megalopae towards artificial illumination. DNA was extracted from whole body samples of megalopae and juveniles, and PCR and sequencing were conducted as described for California samples.

Table 2. Collection details of samples used for COI comparison.

Location	Year of Collection	Life Stage	Sample Size
Glacier Bay, AK	1999	Megalopae	2
	2000	Megalopae	21
	2001	Megalopae	55
	2004	Megalopae	28
Columbia River, WA	2004	Adults	24
		Juveniles	75
		Megalopae	29
Coos Bay, OR	1997	Adults	12
		Megalopae	12
	1998	Megalopae	37
	1999	Megalopae	28
	2001	Megalopae	135
	2003	Adults	15
		Megalopae	87
	2004	Megalopae	56

COI and EF1α nucleotide haplotypes were determined by DnaSP v4.0 (Rozas *et al.* 2003), and were defined as unique sequences differing by at least one nucleotide from all other sequences. Standard molecular diversity indexes were calculated, including nucleotide diversity (π= The average number of pairwise differences between all pairs of sequences in a sample), haplotype diversity (*h*= The probability that two random sequences taken from the same population will differ by at least one nucleotide), number of polymorphic (variable) sites within sequences (*S*), and mean number of pairwise differences between haplotypes (*d*) with DnaSP and Arlequin v2.0 (Schneider *et al.* 2000). Arlequin was also used to calculate the relative frequency of each haplotype in each subpopulation.

In order to test for the assumption of selective neutrality of mutations among COI nucleotide sequences, DnaSP was used to calculate Tajima's (1989) test of neutrality (*D*). Tajima's D value is based on the difference between π and θ (heterozygosity based on the number of variable positions in the aligned sample of sequences, Nei 1987). For this test, if $\pi = \theta$, then the population is at equilibrium for mutation and drift (Tajima 1989). If $\pi > \theta$, Tajima's D is positive, and an admixture between two populations or balancing selection may be acting on the population. When $\pi > \theta$, Tajima's D is negative, and a population bottleneck or a selection sweep may be the cause (Rand 1996). Selective neutrality of COI nucleotide mutations was also tested using Fu's and Li's statistical methods (Fu and Li 1993), based on the number of remote mutations versus the number of recent ones. Fu and Li's D test is based on differences between the number of singletons and number of mutations, whereas Fu and Li's F test is based on differences

between the number of singletons and average number of nucleotide differences between pairs of sequences (Rozas and Rozas 1999). For both tests, a negative value indicates an excess of more recent mutations in external branches (negative selection or a recent bottleneck may be affecting the gene), while a positive value indicates a deficiency of recent mutations (balancing selection may be acting on the population) (Fu and Li 1993).

Linkage disequilibrium was calculated between COI sequences in order to determine the amount of nonrandom association between nucleotide variants at different polymorphic sites (Rozas *et al.* 2001). A population is said to be in linkage disequilibrium when the observed frequencies of haplotypes in a population deviate from the frequencies of haplotypes calculated by multiplying the frequencies of individual genetic markers in each haplotype together. A Chi-square test was performed to determine whether the associations between polymorphic sites were or were not significant (Sokal and Rohlf 1981). The Bonferroni correction for multiple tests (Weir 1996) was used to avoid spurious rejections of the null hypothesis in multiple tests, assuming that all tests are independent.

DnaSP was used to calculate pairwise F_{ST} and G_{ST} in order to assess the degree of genetic differentiation between subpopulations. F_{ST} and G_{ST} are both values used to describe the degree of differentiation among subpopulations. While the original values of F_{ST} and G_{ST} were once used exclusively for diploid and haploid data respectively, they now generally have the same meaning and are often used interchangeably (Excoffier *et al.* 1992; Halliburton 2004; Weir and Cockerham 1984). F_{ST} (Wright 1951), the most common estimation of gene flow, is expressed in terms of the variance of allele/haplotype

frequency among subpopulations, ranging from zero to one. The closer the value is to zero, the less divergent the subpopulations are (more gene flow is occurring between populations); a value equal to zero indicates that the subpopulations are genetically identical, while a value of one indicates that there is no gene flow between subpopulations (Halliburton 2004). G_{ST} (Nei 1973), which has been used especially with animal mtDNA sequence data (Takahata and Palumbi 1985), is also based on gene diversities. Both F_{ST} and G_{ST} values for COI and EF1α were included in this thesis for comparison.

A Mantel test was run using Arlequin, plotting the geographic distance versus the genetic distance between COI sequences. In order to additionally examine the relationship between genetic and geographic distance, taking into account sample size at each location, the program Alleles in Space (AIS) (Miller 2005) was used.

Mismatch distributions, or the distributions of pairwise differences between sequences, were calculated for COI sequences with Arlequin in order to examine the pattern of distribution; a unimodal wave pattern indicates a population bottleneck followed by an expansion (Slatkin and Hudson 1991). Mismatch distributions were also used to estimate the demographic expansion parameter τ, which was used to date the onset of demographic expansion (Rogers and Harpending 1992). Using the formula $\tau=2ut$, where $u=\mu k$ (μ is the mutation rate per site per year, and k is the sequence length), the time of the recent bottleneck (t) was calculated using a mutation rate (μ) of 2.3% per million years for the COI gene, as calculated for crustaceans by Knowlton *et al.* (1993). In addition, the female effective population size (N_{ef}), or the number of female

Dungeness crabs reproducing in the population, both at the time of the bottleneck and at present was calculated using the formula $N_{ef} = \theta/(2\mu)$, where θ is the mutation parameter calculated by Arlequin based on mismatch distribution, and μ is again the mutation rate.

In order to gain more insight into the phylogeographic patterns observed in the COI nucleotide data, COI nucleotide sequences were translated into amino acid sequences with the program MEGA v3 (Kumar *et al.* 2004). Each amino acid sequence differing from all other sequences by at least one amino acid was defined as a protein haplotype. The frequency of each COI protein haplotype was calculated for each subpopulation by hand, and amino acid differences between protein haplotypes were calculated using MEGA.

To examine the evolutionary relationships between individual nucleotide and protein haplotypes, Neighbor-Joining (NJ) phylogenetic trees (Saitou and Nei 1987) were constructed with 1000 bootstrap replicates (Felsenstein 1985) using MEGA. The NJ method produces an unrooted tree because it does not require the assumption of a constant rate of evolution. This method constructs trees by successive clustering of lineages, setting branch lengths as these lineages are joined (Felsenstein 1985). The principle of the NJ method is to find neighbors, or sequences that are the most similar to one another, in a sequential manner in order to minimize the total length of the tree. A Median Joining Network (MJN), showing the number of mutational steps between nucleotide haplotypes, was also constructed for all COI haplotypes using the program Network v4.111 (Bandelt *et al.* 1999). In an MJN, the more tightly clustered the haplotypes, the fewer nucleotide differences there are between them.

RESULTS

COI nucleotide haplotype frequency and diversity

COI sequence diversity values are shown in Table 3. Out of a total of 846 sequences, 350 were found to be unique haplotypes (h= 0.94080). Within California, 60 COI haplotypes were obtained from a total of 230 individuals (h= 0.8502). 66.7% of the haplotypes in California, as well as 83.1% of all haplotypes were rare, and found in only one individual (Fig. 5). Li and Hedgecock (1998) have coined the term 'singletons' for such haplotypes. Three universal COI haplotypes (H1, H3, and H6) were found to be common among all regions sampled, with a general increase in frequency of these haplotypes as one moves southward (Fig. 5).

When all COI data were combined, 126 of 314 sites were found to be polymorphic. Forty-four of these sites were singleton variable (found only in one sequence) and 82 were parsimony informative (found in more than one individual). Seventy-eight sites had two variants per site, 34 sites had three variants per site, and 14 sites had four variants per site (all four possible nucleotides were found at those positions).

Table 3. COI sequence diversity values for all Dungeness crab populations: sample size (n), total number of haplotypes (HT), haplotype (*h*) and nucleotide (π) diversities, number of polymorphic sites (*S*), mean pairwise differences between samples (*d*), and Tajima test values of neutrality (*D*). (Significant Tajima D values at P<0.05 (*), P<0.01 (**))

Geographic Location	**n**	**HT**	***h***	**π**	***S***	***d***	***S/d***	***D***
All Samples	846	350	0.94080	0.01435	115	4.506	25.52153	-2.33046**
All California (CA)	230	71	0.85022	0.00591	51	1.8570	27.46365	-2.34459**
Northern California (N_CA)	66	29	0.92821	0.00943	26	2.93473	8.859418	-1.86289*
Central California (C_CA)	129	37	0.83781	0.00631	32	1.96451	16.28904	-2.05563*
Southern California (S_CA)	35	14	0.82353	0.00497	15	1.55966	9.617480	-1.86835*
Crescent City (CC)	16	7	0.83333	0.00494	8	1.55000	5.161290	-1.28738
Eureka (EK)	25	15	0.95333	0.00887	14	2.78667	5.023917	-0.86218
Fort Bragg (FB)	25	13	0.74359	0.00351	16	3.29667	4.853382	-1.10289
San Francisco (SF)	53	18	0.82353	0.00509	24	2.14804	11.17298	-1.97771*
Half Moon Bay (HB)	36	14	0.82222	0.00549	12	1.72381	6.961324	-1.27085
Monterey Bay (MY)	40	14	0.84615	0.00585	14	1.83846	7.615069	-1.39301
Morro Bay (MB)	6	2	0.53333	0.00170	1	0.53333	1.875012	0.85057
Port San Luis (SL)	29	14	0.85714	0.00557	15	1.74877	8.577457	-1.83885*
Glacier Bay, AK (GB)	106	61	0.96514	0.01390	31	4.36478	7.102305	-1.12349
Columbia River, WA (CR)	128	68	0.93135	0.01202	54	3.77534	14.30334	-2.08512*
Coos Bay, OR (CB)	382	188	0.97000	0.01885	90	5.91984	15.20311	-2.19735**

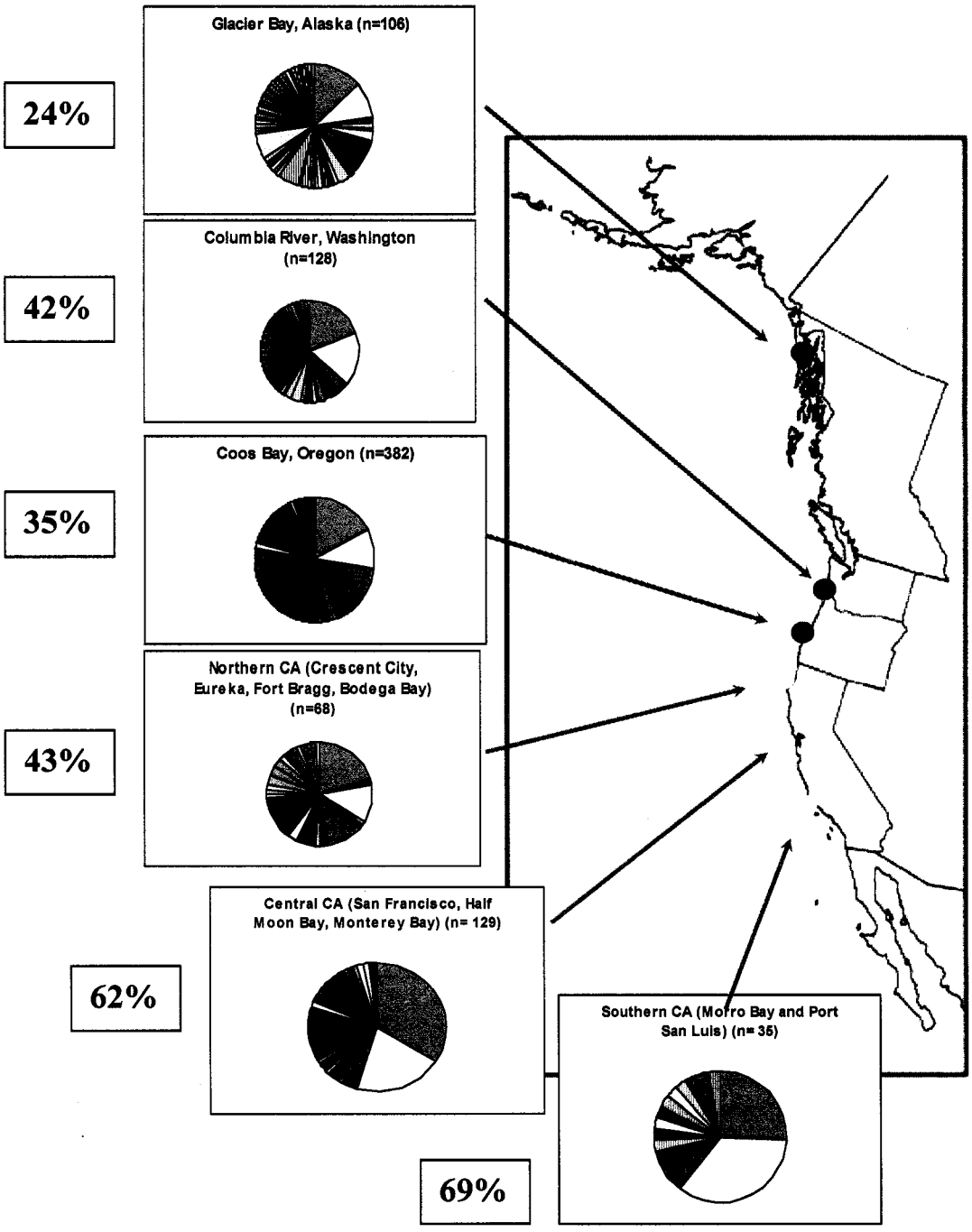

Figure 5. COI nucleotide haplotype frequencies by region. Each color represents a different haplotype. Universal haplotypes are indicated by blue (H1), light yellow (H3) and pink (H6). Percentages to the left of each graph represent frequency of combined universal haplotypes in that region.

The phylogenetic relationships between all COI nucleotide sequences are shown by an MJN (Fig. 6). An MJN indicates the number of mutational steps between haplotypes (the longer the line connecting haplotypes, the more nucleotide differences there are between sequences). In the MJN results for COI (Fig. 6), most haplotypes are tightly clustered together and therefore are very similar to one another. A NJ phylogenetic tree (Fig. 7) shows the evolutionary relationships between COI sequences in California. In this representation of differentiation between sequences, samples clustered together indicate groups of sequences that have the greatest similarity to one another. The distance of each haplotype from the base of the tree indicates the genetic divergence (relative number of nucleotide changes) from the most basal haplotype (the haplotype closest to the base of the tree). Although a phylogenetic tree including all COI sequences was drawn, due to its size that figure could not be included in this document.

In California, COI nucleotide haplotypes showed a diverse mixture of closely related maternal lineages, as indicated by the long shallow phylogenetic trees (Fig. 7). COI haplotypes clustered into two main clades: Clades I and II (Fig. 7). Haplotypes within Clade I were mixed throughout California, while haplotypes within Clade II (H20, H22, and H30) were found exclusively in Northern California. Universal haplotypes (those haplotypes found in every region of California, plus Glacier Bay, AK, Columbia River, WA, and Coos Bay, OR) were found within Clade I.

In all combined COI sequences, 189 pairs of nucleotide sites were found to have significant linkage disequilibrium (the association between nucleotides at these sites was nonrandom, i.e. nucleotides at these pairs of sites seemed to be inherited together). In

California, 50 pairs of sites were found to have significant linkage disequilibrium by Chi-Square Test (36 of those were also significant by Bonferroni procedure) (Table 4).

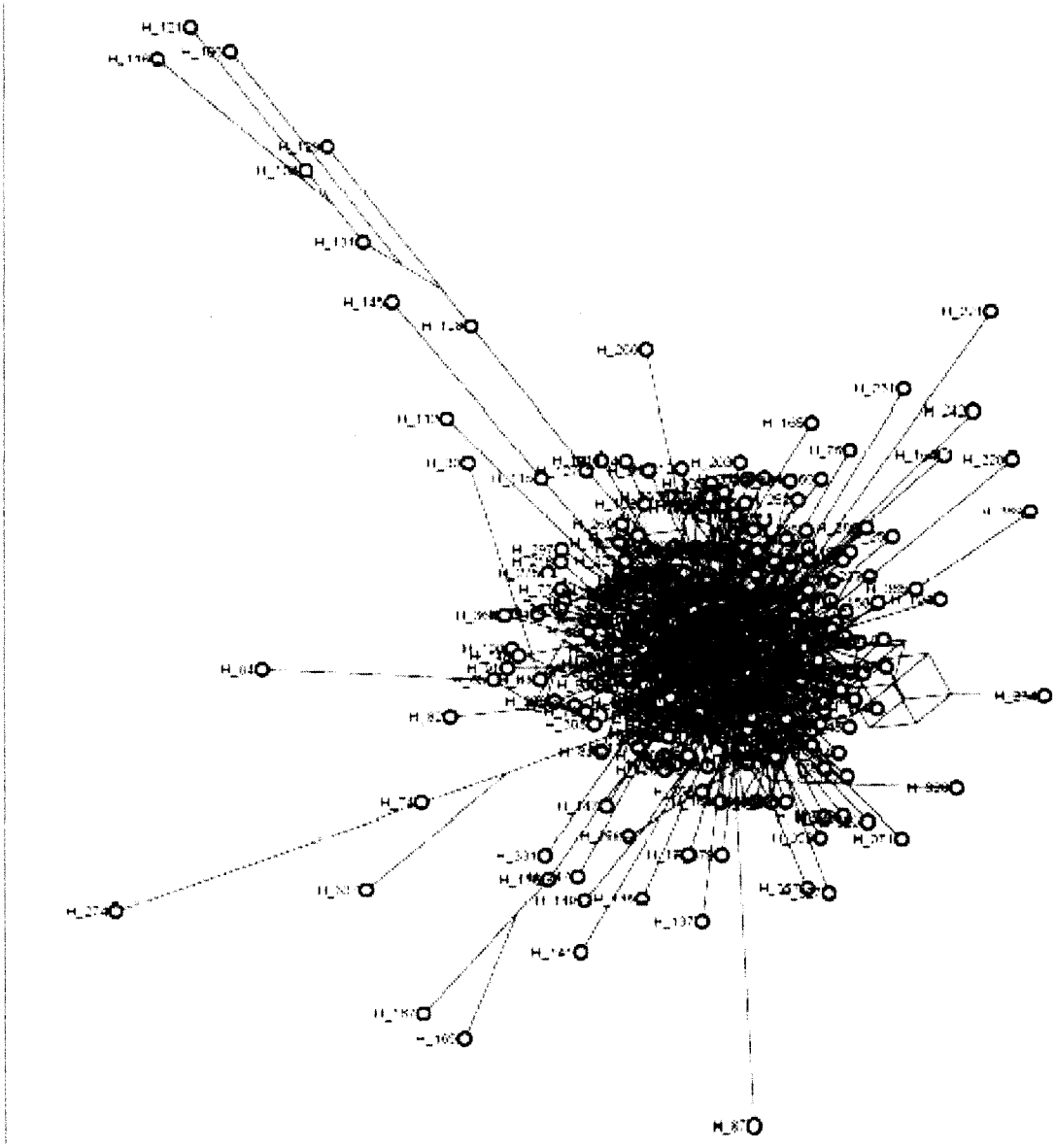

Figure 6. Median-Jointing Network (MJN) of all COI nucleotide haplotypes. Each yellow dot represents a unique haplotype. The length of each line connecting two haplotypes indicates the number of mutational steps between connected haplotypes.

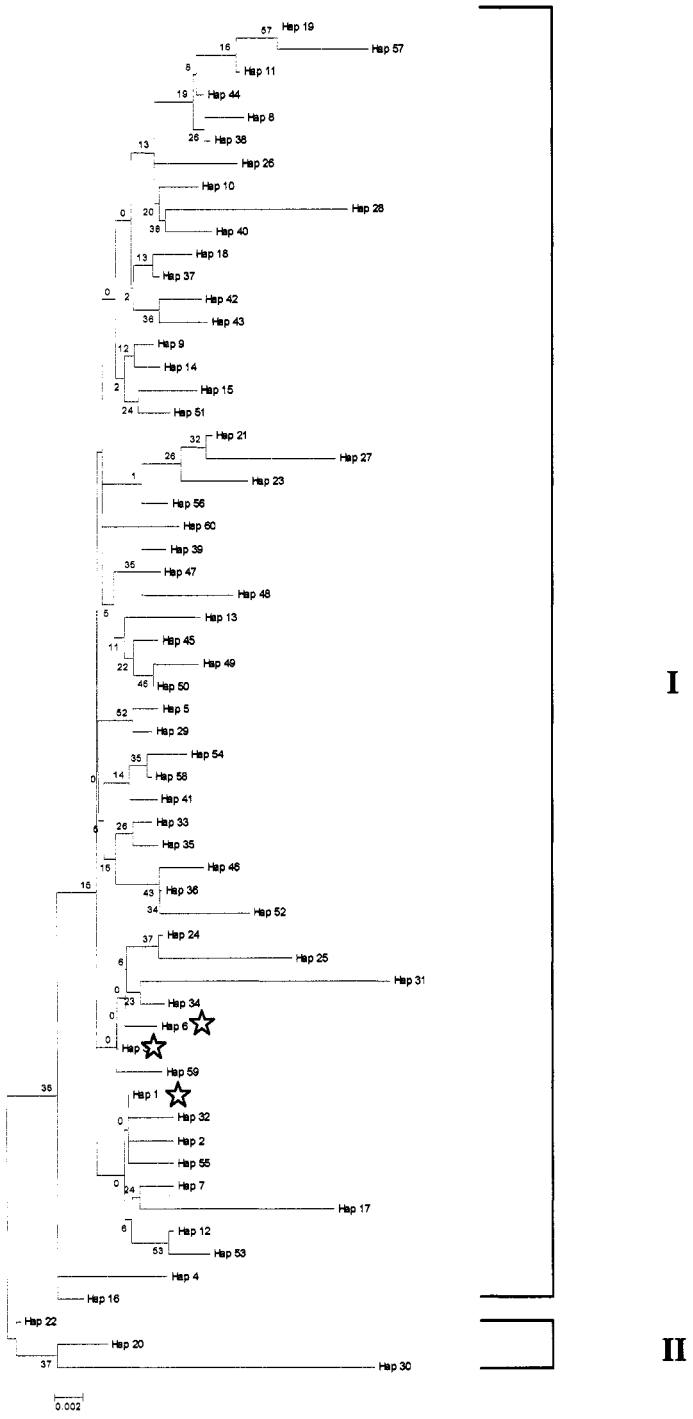

Figure 7. MEGA NJ phylogenetic tree relating COI haplotypes found in California. Numbers at specific nodes represent bootstrap values after 1000 replicates. The scale bar represents divergence using Tamura-Nei correction. Stars indicate haplotypes found in all locations (universal haplotypes).

Table 4. Site pairs in California COI sequences with significant linkage disequilibrium. (* 0.01<P<0.05, ** 0.001<P<0.01, *** P<0.001, B= Significant by the Bonferroni procedure)

Site 1	Site 2	Dist	Chi-sq
13	24	11	15.496***
13	55	42	15.496***
13	112	99	15.496***
13	118	105	58.009***B
13	125	112	15.496***
13	136	123	70.497***B
24	55	31	230.000***B
28	85	57	45.197***B
28	259	231	45.197***B
40	58	18	37.163***B
40	93	53	114.498***B
40	109	69	114.498***B
40	146	106	114.498***B
40	168	128	27.498***B
40	205	165	56.496***B
40	214	174	230.000***B
40	223	183	27.498***B
58	117	59	24.222***B
58	146	88	75.997***B
58	168	110	74.984***B
58	214	156	37.163***B
58	223	165	17.755***B
85	259	174	7.637**
93	109	16	230.000***B
93	205	112	114.498**B
93	214	121	114.498***B
101	115	14	230.000***B
101	136	35	12.584***
109	205	96	114.498***B
109	214	105	114.498***B
112	118	6	13.433***
112	136	24	12.584***
112	202	90	56.747***B
115	136	21	12.584***
117	168	51	17.755***B
117	205	88	37.163***B
118	136	18	33.211***B

118	202	84	11.652***
136	223	87	10.797**
146	168	22	56.747***B
146	214	68	114.498***B
146	223	77	56.747***B
154	232	78	27.734***B
168	214	46	27.498***B
168	223	55	12.889***
168	232	64	4.680*
205	214	9	56.496***B
214	223	9	27.498***B
232	259	27	5.875*
298	301	3	230.000***B

Fu's and Li's statistics were used to test predictions made by the neutral theory of evolution (Kimura 1983). Fu's and Li's statistics for all COI nucleotide sequences are indicated in Table 5. Values for all regions of California, San Francisco, Columbia River, and Coos Bay were found to be negative and statistically significant, indicating an excess of recent mutations in the COI gene of *C. magister*. The large number of recent mutations may have been caused by a recent expansion of the population, or negative selection acting on the gene (Fu and Li 1993).

Mismatch distribution analysis of COI data resulted in a wave-shaped graph for all subpopulations (data not shown), consistent with the sudden expansion model (mismatch observed mean = 4.158 and mismatch observed variance = 10.835). Because of this, the demographic expansion parameter (τ) could be estimated as 1.196 (Rogers and Harpending 1992), resulting in estimated time of expansion of 78,000-146,000 years before present (YBP). In addition, the female effective population size (N_{ef}) at the time

of the population bottleneck was calculated to be approximately 21,000 individuals, compared to a present N_{ef} of 2.78 million to 4.92 million individuals.

Table 5. Fu's and Li's statistical values for COI nucleotide sequences. (* P<0.05 ** P<0.02)

Group	Fu's and Li's D	Fu's and Li's F	Fu's Fs
All CA	-4.70462**	-4.36668**	-106.603
N_CA	-3.25751*	-3.26930**	-22.358
C_CA	-2.81321*	-3.00831**	-38.911
S_CA	-2.58830*	-2.77307*	-9.602
CC	-1.28054	-1.47328	-2.530
EK	-1.61949	-1.62304	-8.726
FB	-0.96587	-1.17883	-4.632
SF	-3.28737*	-3.35719**	-10.316
HB	-0.50664	-0.88204	-8.584
MY	-2.10207	-2.20441	-7.507
MB	1.05247	1.02905	0.625
SL	-2.35414	-2.57141	-9.789
GB	-1.48740	-1.60944	-71.000
CR	-3.21672*	-3.28429**	-88.847
CB	-4.12298**	-3.77299**	-353.546

COI protein haplotype frequency and diversity

When COI nucleotide haplotypes were translated into amino acid sequences, the 60 nucleotide haplotypes in California collapsed into 20 unique amino acid sequences (protein haplotypes). Seventeen out of 104 total amino acid sites were found to be variable in California, with seven being parsimony informative and 10 being singleton sites (amino acid only differed from the consensus in one haplotype). Overall, 384

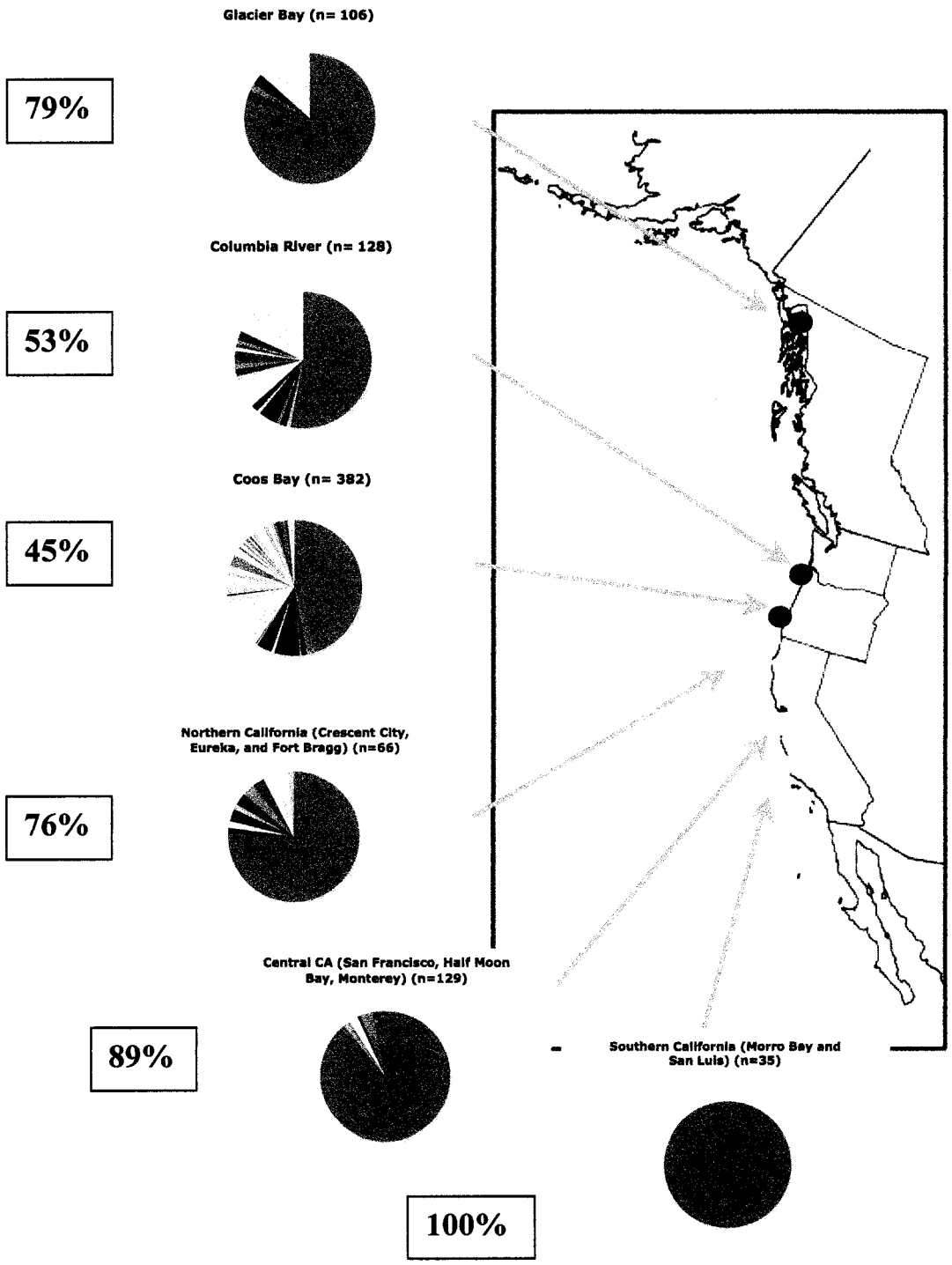

Figure 8. COI protein haplotype frequencies by region. Each color represents the frequency of a different haplotype. The universal haplotype (P1) is represented by bright blue. Percentages to the left of each graph represent frequency of P1 in that region.

nucleotide haplotypes translated into 194 unique protein haplotypes, with 62 out of 104 amino acid sites being variable. Thirty-nine of these variable sites were parsimony informative, while 23 were singleton sites. Twenty-three percent of all samples and 58% of Californian samples were found to possess the same protein haplotype (P1). The frequency of each protein haplotype by region is shown in Figure 8.

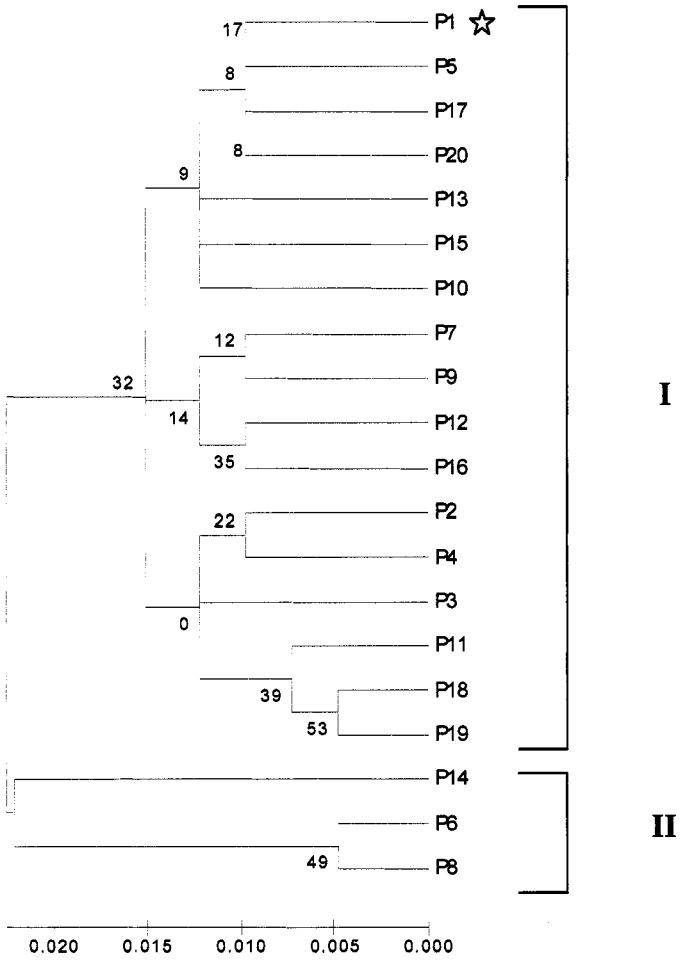

Figure 9. MEGA NJ phylogenetic tree showing the relationships between COI protein haplotypes found in California. Numbers at specific nodes represent bootstrap values after 1000 replicates. The scale bar represents divergence using Tamura-Nei correction. Star indicates haplotype found in all locations (universal haplotype).

The phylogenetic relationships between COI protein haplotypes are represented by a Bootstrap NJ phylogenetic tree (Fig. 9). COI protein haplotypes clustered into two main Clades, Clades I and II, which corresponded to Clades I and II of the nucleotide haplotypes (Fig. 7). COI protein haplotypes within Clade I were mixed throughout Northern and Central California, while haplotypes within Clade II (P6, P8, and P14) were found exclusively in Northern California. The universal protein haplotype (P1) was the only protein haplotype found in Southern California (Fig. 8). The average pairwise distance (*d*) between COI protein haplotypes in California was 0.0336.

When amino acid differences between individual haplotypes were compared, it was found that most differences were the result of "silent" amino acid substitution. That is, a hydrophobic amino acid was replaced with another hydrophobic amino acid, or a hydrophilic amino acid was replaced with another hydrophilic amino acid. These types of changes would have little to no effect on the shape of the complete protein. However, several amino acid changes were observed between COI protein haplotypes in Columbia River, WA and Coos Bay, OR that have the potential to cause changes in protein structure. That is, for some protein haplotypes in these two locations, a hydrophobic amino acid was replaced by a hydrophilic amino acid, or vice versa.

EF1α haplotype frequency and diversity

EF1α sequence diversity values are shown in Table 6. Out of 52 total sequences, 10 unique haplotypes were identified, none of which were singletons. The frequency of

each EF1α nucleotide haplotype per region is shown in Fig. 10. Four haplotypes (H1, H3, H4, and H5) were found to be common among all regions sampled in California. Slight clinal variation was observed in H1 and H5, decreasing and increasing, respectively, in frequency as one moves southward. While no EF1 haplotype was found to be unique to Northern California, three (H7, H8, and H9) and one (H10) were unique to Central and Southern California respectively.

Table 6. EF1α sequence diversity values for all Dungeness crab sample sites and regions in California: sample size (n), total number of haplotypes (HT), haplotype (*h*) and nucleotide (π) diversities, number of polymorphic sites (*S*), mean pairwise differences between samples (*d*), and Tajima test values of neutrality (*D*).

Grouping	n	HT	*h*	π	*S*	*d*	*D*
All California	52	10	0.83869	0.00376	5	1.949	0.36742
N_CA	15	6	0.80000	0.00267	3	1.789	1.14728
C_CA	30	9	0.83164	0.00263	5	2.079	0.06665
S_CA	7	5	0.84615	0.00242	2	1.549	1.69598
EK	11	6	0.79221	0.00246	3	1.667	0.69248
FB	4	4	0.82143	0.00329	3	2.250	0.83870
SF	11	8	0.85714	0.00254	4	2.381	-0.0828
HB	7	5	0.82418	0.00253	3	1.593	0.39778
MY	12	7	0.82609	0.00277	4	2.119	0.23461
SL	7	5	0.84615	0.00243	2	1.549	1.69598

Out of a total of 426 nucleotide sites in each EF1α sequence, 11 were variable with one of two different possible nucleotides at each site. Of these variable sites, four changes were transitions (e.g. a purine base changing to another purine, or a pyrimidine base changing to another pyrimidine), one was a transversion (e.g. a purine base substituted for a pyrimidine), and six were indels (insertions or deletions). The number

of nucleotide differences between haplotypes ranged from one to four, and averaged 1.600, indicating a high amount of similarity between sequences.

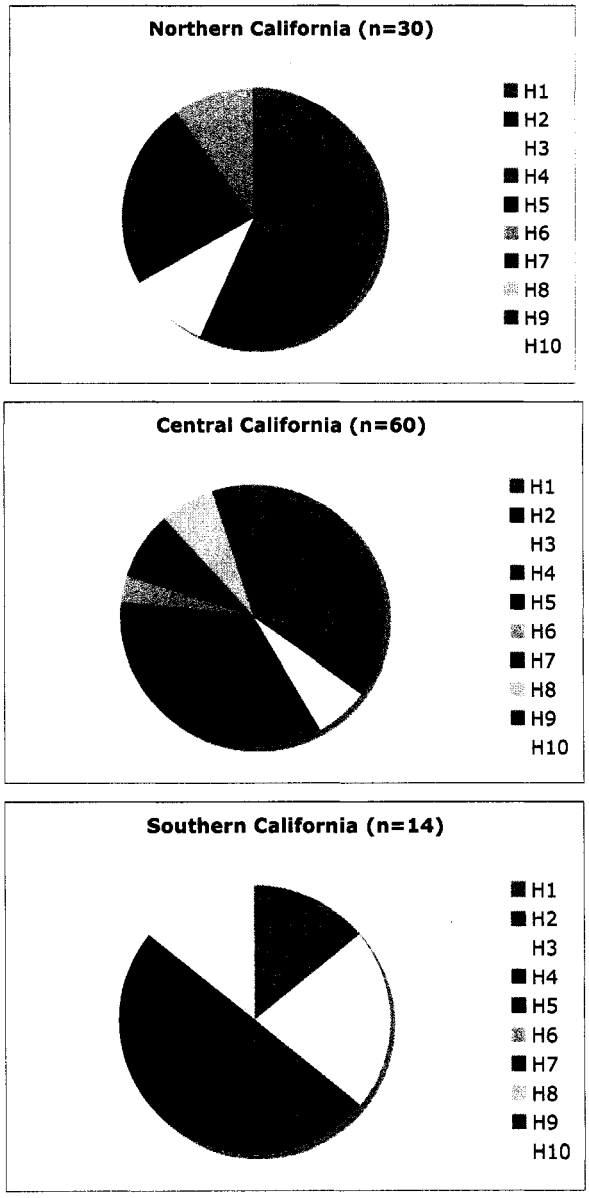

Figure 10. EF1α nucleotide haplotype frequencies in California by region. Each color represents a different haplotype.

Two phylogenetic clades, Clades I and II, were observed within the EF1α haplotype data (Fig. 11). Haplotypes within Clades I and II were independent of COI nucleotide and protein Clades I and II, and were mixed throughout all three regions of California (Fig. 10).

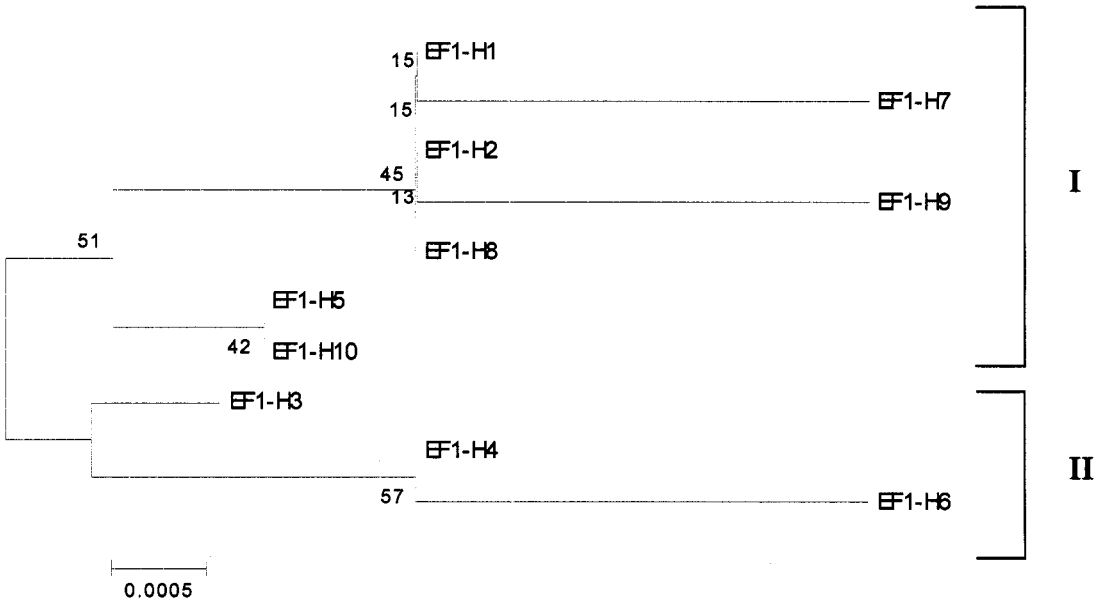

Figure 11. MEGA NJ phylogenetic tree showing the relationships between EF1α haplotypes found in California. Numbers at specific nodes represent bootstrap values after 1000 replicates. The scale bar represents divergence using Tamura-Nei correction.

Population differentiation

Pairwise F_{ST} and G_{ST} values for both COI and EF1α sequences are given in Tables 7 and 8. As discussed earlier, F_{ST} and G_{ST} both show the amount of genetic

differentiation between populations, with a number closer to zero indicating relatively high amounts of gene flow between populations, and a number closer to one indicating a lack of gene flow between populations.

Table 7. Pairwise F_{ST} and G_{ST} estimates for COI (below diagonal) and EF1α (above diagonal) sequence data between sample sites in California.

		CA	CC	EK	FB	SF	HB	MY	MB	SL
CA	G_{ST} F_{ST}	---	---	0.00614 -0.00848	0.02727 -0.02641	0.00119 -0.00812	0.00988 -0.01303	0.00301 -0.00996	---	0.01306 0.01654
CC	G_{ST} F_{ST}	0.01060 0.02264	---	---	---	---	---	---	---	---
EK	G_{ST} F_{ST}	0.01500 0.09936	0.02509 0.17239	---	0.00919 -0.04444	-0.00522 -0.00982	0.02035 0.01171	0.01359 0.00837	---	0.02849 0.04015
FB	G_{ST} F_{ST}	0.01517 -0.00258	-0.01484 0.00048	0.04085 0.16291	---	0.02511 0.01086	0.02244 -0.00575	0.03921 0.0598	---	0.03042 -0.07459
SF	G_{ST} F_{ST}	0.00247 0.00731	0.00504 0.05036	0.03272 0.11762	0.00992 0.02733	---	-0.00576 -0.00999	-0.00490 -0.01032	---	0.01337 0.06880
HB	G_{ST} F_{ST}	0.00192 0.00395	-0.00638 0.00752	0.02797 0.14932	-0.00297 -0.00512	0.00042 0.03165	---	-0.00013 -0.00935	---	0.00094 0.00645
MY	G_{ST} F_{ST}	0.00319 0.00419	-0.00237 0.02927	0.02389 0.13225	0.00285 -0.00087	0.00819 0.03576	-0.00381 0.00123	---	---	0.00371 0.02777
MB	G_{ST} F_{ST}	0.03867 0.01732	0.01384 -0.04167	0.07634 0.20701	-0.00650 0.00313	0.03142 0.03000	0.02641 0.00094	0.03485 0.03846	---	---
SL	G_{ST} F_{ST}	0.00742 0.00868	0.00871 0.05749	0.02219 0.14057	0.01654 -0.00463	0.00738 0.02326	0.00600 0.02017	0.01644 0.01766	0.04603 0.05456	---

Table 8. Pairwise F_{ST} and G_{ST} estimates for COI (below diagonal) and EF1α (above diagonal) sequence data between California regions, Alaska, Columbia River, and Coos Bay.

		GB	CR	CB	N_CA	C_CA	S_CA
GB	G_{ST} F_{ST}	——	——	——	——	——	——
CR	G_{ST} F_{ST}	0.00412 0.14260	——	——	——	——	——
CB	G_{ST} F_{ST}	0.00268 0.15303	0.00269 0.02811	——	——	——	——
N_CA	G_{ST} F_{ST}	0.00557 0.10200	0.00237 0.03116	0.00352 0.06196	——	0.01486 0.01504	0.02839 0.01825
C_CA	G_{ST} F_{ST}	0.01722 0.15478	0.00615 0.03408	0.01243 0.06318	0.00655 0.02377	——	0.01286 0.04392
S_CA	G_{ST} F_{ST}	0.01715 0.17956	0.00616 0.04785	0.01150 0.07438	0.01053 0.03602	0.00488 0.00627	——

The relationship between COI nucleotide haplotype genetic and geographic distances is shown in figures 12 and 13. In the AIS analysis, sample size and genetic distance are plotted against geographic location, with the size and position of peaks determined by sample size and genetic diversity. The MANTEL test shows a simple x-y plot with geographic distance plotted against genetic distance.

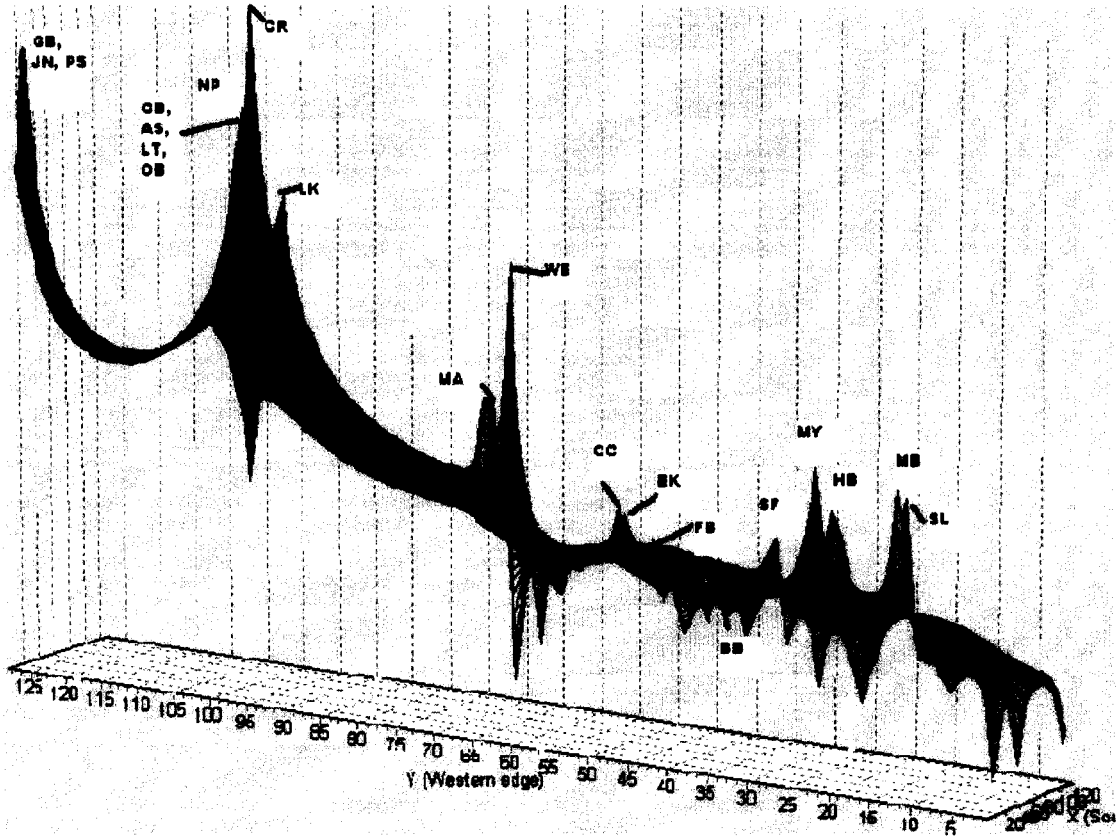

Figure 12. Results of "Alleles in Space" analysis. Placement of peaks relevant to one another is a function of geographic location. The size of each peak is determined by the sample size, while the relevant heights of each peak is indicative of genetic distance.

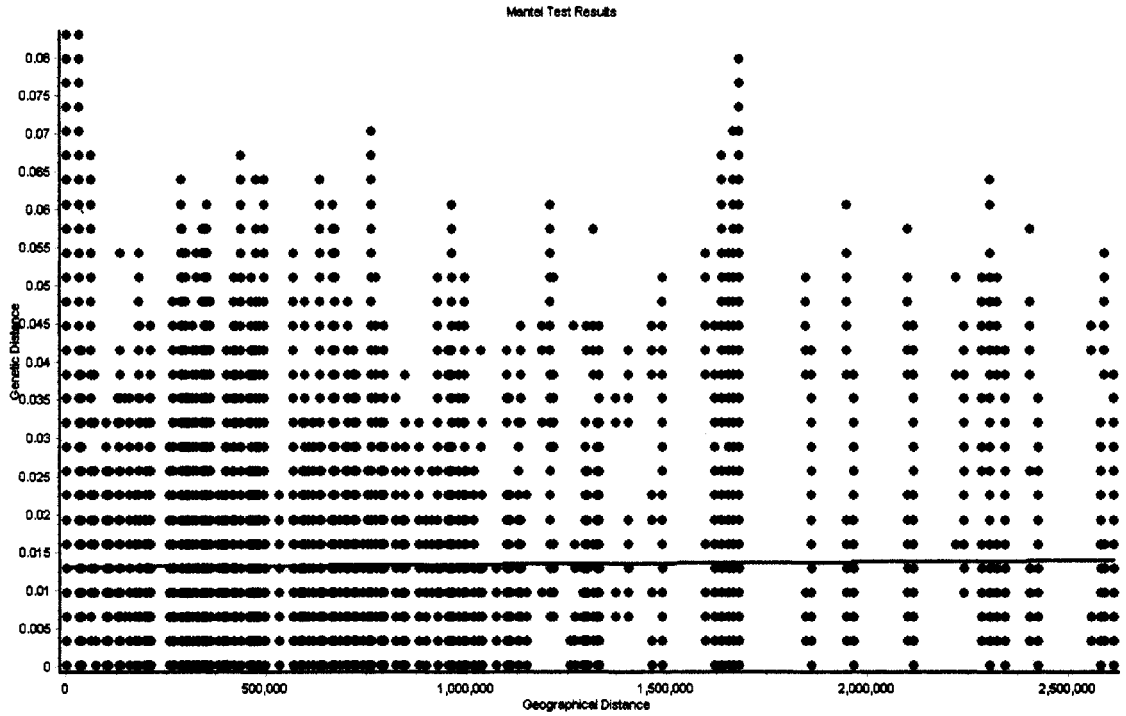

Figure 13. Mantel Test results showing the relationship between genetic distance (x-axis) and geographic distance (y-axis). Black line indicates correlation (r= 0.021029 and P= 0.782218).

DISCUSSION

Gene flow of *C. magister*

Analysis of sequence data revealed an absence of population structure, suggesting a high level of gene flow over the range of *C. magister*. This high level of gene flow is consistent with a high potential for larval dispersal, resulting in genetic homogeneity along the species' range. This is indicated by the number of universal haplotypes observed in all regions sampled, and by the lack of geographic structure observed in the sampled locations.

A large amount of mixing between populations of the Dungeness crab is supported by the incidence of universal haplotypes found in all regions. Three universal haplotypes were observed for COI (H1, H3, and H6), while four (40% of all observed) EF1α haplotypes (H1, H3, H4, and H5) were found to be common among all three regions in California. These common haplotypes indicate that individuals from the same lineages are spread among, and theoretically dispersing between, all geographic regions studied. In addition, COI haplotypes within Clade I in California (Fig. 7 and 9), and EF1α haplotypes in both Clades I and II (Fig. 11) were found to be mixed throughout California. This indicates that closely related haplotypes are found in different locations throughout California, and is again consistent with gene flow between populations.

Based on analysis of population differentiation and gene flow, there seem to be no observable barriers to the dispersal of *C. magister* within the study area of the species'

range. F_{ST} and G_{ST} values were low for both COI and EF1α, suggesting little geographic structure of the species (Tables 7 and 8). This may indicate a large amount of gene flow between populations of *C. magister* throughout their range, but not necessarily. Studies have shown that, while organisms may be physically capable of dispersing long distances, they usually do not (Halliburton 2004). Rather, only a few individuals might actually make it to distant populations, and even fewer will survive to reproduce. However, it has been shown that a very small amount of gene flow between populations (only a few immigrants per generation, if they survive and reproduce) can be enough to prevent genetic differentiation between the populations (Halliburton 2004). On the other hand, while small amounts of gene flow may be all that is needed to maintain genetic homogeneity, recent evidence suggests that much higher levels of recruitment are needed to maintain population size (Cowen *et al.* 2006). This is especially important in areas where Dungeness crabs are intensely fished, where marine reserves would be the most important.

While most groupings showed low F_{ST} and G_{ST} values, there were a few exceptions. Eureka (F_{ST}= 0.099 - 0.207), Coos Bay (F_{ST}= 0.028 – 0.153), and Glacier Bay (F_{ST}= 0.102 – 0.179) were found to have moderate F_{ST} values for COI, indicating that they are somewhat genetically distinct from the other populations. This may indicate some restricted gene flow to and from these populations, possibly the result of topology of these specific geographic areas. Humbolt Bay (Eureka), Coos Bay, and Glacier Bay all have narrow, restricted openings, which may result in the restriction of megalopae entering and/or leaving the bays. In addition, moderate genetic differentiation of these

three areas may be the result of post-settlement selection acting on crabs differently within each area.

Results of the MANTEL and AIS tests also show little geographic structure to the *C. magister* population. The AIS graph (Fig. 12) shows an apparent slight decrease in genetic diversity as one moves southward. This is in agreement with the dramatically smaller number of Dungeness crabs found in the southern end of their range as compared to the larger population size found in the northern end of the study area. This apparent decrease in genetic diversity, however, may be a result of the larger sample sizes collected from northern locations and/or the frequency of megalopae and juveniles sampled in the North, artificially increasing the genetic diversity slightly in these regions as compared to California (Toonen and Grosberg 2003). The slope of the line of the MANTEL graph (Fig. 13), was nearly flat (r=0.021029), and showed little to no isolation by distance pattern. That is, crabs that are geographically close to one another are no more genetically similar than those that are far apart.

A mixing of populations and a high mutation rate are both supported by the large number of site pairs within COI sequences found to have significant linkage disequilibrium (Table 4). A high level of linkage disequilibrium is usually a result of recombination. However, because the mitochondrial genome does not recombine, the high level of linkage disequilibrium seen in the Dungeness crab's mitochondrial COI gene indicates high levels of mutation and gene flow between populations.

Genetic Diversity of *C. magister*

The overall sequence diversity observed was high for both COI (Table 3) and EF1α (Table 6) data, with a high frequency of singletons observed for COI in both California, and in the overall sequence set. Coos Bay (h= 0.970), Glacier Bay (h= 0.965), and Eureka (h= 0.953) were found to have the highest levels of COI genetic diversity. All locations sampled in California seemed to have a similar EF1α haplotype diversity of about 0.8. The high levels of genetic diversity may support a large amount of mixing between source populations and/or a high mutation rate. Indeed, because the COI gene is maternally inherited without recombination, the high levels of haplotype diversity observed for the gene in this study indicate a diverse mixture of maternal lineages along *C. magister*'s range, as well as within subpopulations.

The phylogenetic relationships between different COI and EF1α haplotypes are consistent with a diverse mixture of maternal lineages throughout California. The COI nucleotide NJ tree (Fig. 7) was divided into two major branches, or clades: Clade I and Clade II. While the three COI haplotypes in Clade II (H20, H22, and H30) were found exclusively in Northern California, the haplotypes in Clade I were mixed throughout all regions of California. In addition, while the phylogenetic tree of EF1α (Fig. 11) was divided into two major clades like the COI tree, unlike COI, EF1α haplotypes within both Clades I and II were found to be mixed throughout California.

Although the high levels of genetic diversity observed may be due to mixing, they may also be the result of rapid mutation. COI haplotype diversity, ranging from 97.0% to

74.4% (excluding Morro Bay which had a significantly lower number of samples), was slightly higher than that of EF1α, which ranged from 84.6% to 79.2%. In addition, while 51 nucleotide sites were found to be polymorphic in COI sequences from California, only five polymorphic sites were observed among EF1α sequences, in spite of the fact that the EF1α fragment sequenced in this study was longer than the COI fragment. This is not entirely surprising, as COI is a mitochondrial gene, and animal mtDNA usually has a higher mutation rate than nuclear genes such as EF1α (Nei and Li 1979; Vawter and Brown 1986).

It is important to note that the differences observed in COI genetic diversity between regions may also have been influenced by the sampling methods employed in this study. Although all samples from California were of adult crabs, samples from Alaska, Oregon, and Washington used for COI comparison came from crabs in a mixture of life stages (Table 2). It has been suggested that populations of megalopae have higher levels of genetic diversity than juveniles, which have a higher genetic diversity than adult crabs (Toonen and Grosberg 2003). This is because older individuals have been exposed for more time to a wider array of selective pressures. We have tried to overcome this potential problem with a large number of samples. The fact that two of the highest COI haplotype diversities were seen in Columbia River (93.1%), where samples were collected from megalopae, juveniles, and adults (Table 2), and Eureka (95.3%) where all samples were from adults, suggests that the results of this study are not dominated by this life history effect.

Selection and phylogeographic history

Although no isolation by distance pattern or distinct geographic structure was seen in the data, nucleotide haplotype frequency data by region for both COI and EF1α does reveal slight clinal variation. For COI, we observed a general increase in the combined frequency of universal haplotypes from north to south (Fig. 5). This increase in frequency may be due to founding effect, or a stepping stone model of dispersal/ range expansion from the original populations. The genus *Cancer* originated in the North Pacific in the early Miocene (Harrison and Crespi 1999). It could be that, as *C. magister* traveled down the Pacific coast of North America, those individuals with the common COI haplotypes were more likely to settle in the new locations. Additionally, because the northern populations are older, they have had more time to accumulate mutations, and thus have a wider variety of haplotypes besides the universal ones. As an alternate hypothesis, the clinal pattern observed may be due to an expansion, not from original populations, but from refugia populations resulting from a population bottleneck, which may have occurred sometime later in the history of the species.

Selection may also play a role in causing the clinal variation observed. As non-neutral genes, COI (a gene coding for a respiratory protein) and EF1α (a gene coding for a protein involved in mRNA translation) could be under selective pressure. There are distinct environmental differences as one moves southward along *C. magister*'s range, from Alaska to California, especially with regard to ocean and air temperatures. Both of

these environmental factors affect respiration, a vital cellular function in which the COI protein is vital. A change in frequency of an allele, or cline, along an environmental gradient such as seen along the Pacific Coast of North America, is perpetuated by a balance between selection and gene flow (Brown *et al.* 2001). In other words, while gene flow may be acting to homogenize *C. magister*, a clinal shift, such as the one observed in our COI nucleotide data, could be due to the universal COI haplotypes being selected for in warmer southern regions of the species' range.

The idea that selection may be acting on the COI gene in the Dungeness crab population is also supported by the high amount of linkage disequilibrium (Table 4), significant Fu's and Li's values (Table 5) (Fu 1997), and the negative Tajima's D statistics (Table 3) observed for COI nucleotide sequence data. According to Rand (1996), negative Tajima's D values may be caused by a recent population bottleneck and/or a selection sweep. If the sole cause of the negative COI values in California is a bottleneck, we should also see negative Tajima's D values in our EF1α data, since a bottleneck should act on all loci in a similar way (Rand 1996). However, because we see conflicting patterns of our Tajima's D values for EF1α and COI (while values for COI were negative, those for EF1α were found to be positive), the clinal pattern is probably caused, not only by a bottleneck, but selection factors as well.

Additional data support the influence of a population bottleneck on the clinal pattern observed. For example, while Tajima's D values were found to be positive for EF1α data, none of those values were statistically significant. On the other hand, Tajima's D was found to be significant at the $p < 0.01$ level for all COI sequences (D=

-2.33046, $p<0.01$) and within California (D= -2.34459, $p<0.01$), strongly suggesting the influence of a bottleneck. In fact, the shallow phylogenetic tree (Fig. 9) and tightly clustered MJN (Fig. 6) observed for COI nucleotide haplotypes indicate that most of the variation observed was recently derived from a common ancestor, which also supports a recent bottleneck. A recent bottleneck is also supported by a smooth, unimodal mismatch distribution seen for COI pairwise differences, consistent with a bottleneck followed by an expansion, as well as Fu's F statistic, which, according to Ramos-Onsins and Rozas (2002) is the most powerful test for detecting populations growth when sample size is large.

In the case of EF1α common haplotypes, because some increase in frequency while others decrease as one moves southward (Fig. 10), factors may be acting on individual haplotypes differently. However, because EF1α data has only been collected for a limited number of crabs in California, where environmental differences are less pronounced than for the whole range, it is difficult to draw substantial conclusions as to how selection or other factors may be affecting this gene. Positive Tajima's D values observed for EF1α in most locations in California (Table 6) do indicate a possible admixture of two different populations or balancing selection (Rand 1996).

In order to more closely examine the effects of selection on the COI gene in *C. magister*, the amino acid sequences were examined; selection acts on phenotype (proteins), not genotype (DNA). By translating COI nucleotide haplotypes into amino acid sequence haplotypes, background "noise" caused by the excess of singletons was reduced, and structural variation in the COI protein investigated. One universal COI

protein haplotype (P1) was observed at a relatively high frequency in all subpopulations (Fig. 8). While high frequencies of this haplotype were seen in Alaska (79%) and California (76%-100%), only about half of the crabs sampled in the Columbia River (53%) and Coos Bay (45%) were found to possess the P1 haplotype. These differences in frequency observed at the ends of *C. magister*'s range as opposed to the center, may be due to selection and/or phylogeographic history.

Extremes in temperature, both cold and warm, have been found to have similarly negative impacts on the reproductive success and survival of Dungeness crabs (Gutermuth and Armstrong 1989; Moloney *et al.* 1994; Shirley *et al.* 1987; Sulkin *et al.* 1996; Taggart *et al.* 2003; Tasto 1979; Wild 1983b, c). Therefore, it seems reasonable that both environmental extremes could put similar selective pressures, or physiological stresses, on the population. Indeed, the high frequency of the universal COI protein haplotype P1 that we see at the extreme northern and southern ends of *C. magister*'s range, indicate that this version of the protein may be selected for at the extreme temperature tolerances of the species. P1 may be the most thermodynamically stable version of the protein, thereby making it more effective at temperature extremes than mutant versions. This scenario is supported by the fact that, when the differences between COI protein haplotypes were analyzed, no substantial structural differences were seen between haplotypes in Glacier Bay or California. That is, every amino acid substitution in these subpopulations was the result of a hydrophobic amino acid replacing another hydrophobic amino acid, or a hydrophilic amino acid replacing a hydrophilic amino acid. The only subpopulations in which structural changes were seen (a

hydrophobic change to a hydrophilic or vice-versa) were Columbia River and Coos Bay. It may be that, because these two locations are in the central, more temperate area of *C. magister*'s range, the COI protein is less constrained by the effects of temperature, thus allowing more tolerance of structural mutations caused by mutational drift.

The observed COI protein haplotype frequency patterns may also be the result of the phylogeographic history of *C. magister*. Mismatch distribution graphs show a wave pattern, indicative of a recent bottleneck followed by an expansion of the Dungeness crab population. Negative Fu's and Li's statistics (Table 5) also indicate an excess of recent mutations and a population expansion, as do Fu's F statistic (Table 5) (Ramos-Onsins and Rozas 2002), and significantly negative Tajima's D values for COI (Table 3) (Rand 1996). According to calculations, the estimated N_{ef} at the time of the bottleneck was about 21,000- 37,000, as compared to and estimated 2.78- 4.92 million at present. In other words, when the Dungeness crab population was at its smallest in recent history, there were between 132 and 234 times fewer females participating in the gene pool than there are at present.

The time of this severe bottleneck was calculated to be about 78,000-146,000 years before present (YBP). This period of time corresponds to the Pleistocene epoch, and spans the end of the Illinoian glacial period (~125-200K YBP), through the Sangamon interglacial period (~110-130K YBP), to the beginning of the Wisconsinan glacial period (~15-80K YBP) (Killey 1998). This suggests that the bottleneck may have been caused by effects of the Illinoian glacier, which would have caused much of the northern areas of the species' range to be covered in ice, thus making them uninhabitable

for *C. magister*. In addition, the glacier would have caused sea levels to be much lower than they are today (Pielou 1991), perhaps exposing areas of the continental shelf in some areas in the southern end of *C. magister*'s range. Because *C. magister* requires shallow water for survival, this would have rendered some areas of central and southern California uninhabitable for the species. Thus, during the time of maximum glaciation, the area in the vicinity of Oregon and Washington could have acted as an ice-free refuge for the species. Indeed, our COI data indicates that crabs from these areas possessed the highest genetic diversities, indicative of them being the oldest populations. Once the glacier began to recede and ocean levels began to rise, during the Sangamon interglacial period, the area of habitable coastline for *C. magister* would have expanded, thus allowing the population to expand northward and southward as well. This direction of expansion is supported by out COI phylogenetic trees for California. The most basal COI nucleotide and protein haplotypes (H20, H22, and H30, respectively corresponding to P6, P8, and P14) in California (Fig. 7 and 9) were only seen in the northern region of California, suggesting that the population in this region is the oldest in California.

It is important to note that the vicinity of Oregon and Washington may not have been the only area of refuge for *C. magister*. Indeed, high levels of genetic diversity found in Alaska indicate that a refuge may have been present in this area as well. Marko *et al.* (2004) suggest that glacial refugia were present as far north as Ketichan in southeastern Alaska. Areas of refuge may also have been present in British Columbia, Canada (Pielou 1991), however data from this region will need to be collected in order for this to be determined.

A glacial event would not only have affected sea level, but it would have also affected sea surface temperature (SST). Herbert *et al.* (2001) found that a time series of alkenone unsaturation indices in sea floor sediment cores indicated a fluctuation of 4-10 °C between glacial and interglacial periods over the last 550,000 years. At the time of maximum cooling during past periods of glaciation, the SST off of Northern California was 10 °C cooler than it is today, and 8 °C cooler in Southern California (Herbert *et al.* 2001). Since *C. magister* has a limited tolerance to cold water (Gutermuth and Armstrong 1989; Shirley *et al.* 1987; Taggart *et al.* 2003), this shift in SST could have acted to restrict the species' range at the time of glaciation, thus contributing to the bottleneck observed for the species. In addition, in each glacial cycle, maximum cooling of SST precedes maximum glaciation by 5,000-10,000 years (Herbert *et al.* 2001). So, while the bottleneck in the *C. magister* was probably influenced by the glacial cycle, the bottleneck did not necessarily coincide with maximum glaciation.

Fluctuations in SST between glacial and interglacial periods could have affected *C. magister* in other ways. Herbert *et al.* (2001) claim that during each glacial period over the last 550,000 years, the growth of the North American ice sheet caused a shift in the wind system that drives the cold waters of the California Current towards the Equator. As a result, it is likely that the California Current did not operate with contemporary force, velocity, and direction during times of glaciation. Because *C. magister* depends on the upwelling of the California Current to deliver megalopae to shore, this breakdown of the system would have greatly affected the species at the time of glaciation, and could very well have contributed to the bottleneck in the population.

The timing of the bottleneck calculated for *C. magister* is similar to that recently calculated (33,000-111,000 YBP) for the swimming crab (*Callinectes bellicosus*), a species of crab also found along the west coast of North America, in northern Mexico (Pfeiler *et al.* 2005). The bottleneck time is also similar to that calculated for the blue crab (*Callienectes sapidus*) (McMillen-Jackson and Bert 2004) and the brown shrimp (*Farfantepenaeus aztecus*) (74,000 YBP) (McMillen-Jackson and Bert 2003) in the Atlantic and Gulf of Mexico. This indicates that the Illinoian glacier may have an important affect on the phylogeographic history of multiple species of crustacea along the coasts of North America.

The Illinoian glacial period may have not been the only one to affect the phylogenetic patterns observed in *C. magister* today. Both COI and EF1α phylogenetic trees (Fig. 7, 9, and 11) show two closely related but separate lineages coexisting in the same geographic locations. Other mtDNA studies of marine invertebrates with a high potential for larval dispersal have produced similar results (Chu *et al.* 1999; Luttikhuizen *et al.* 2003; McMillen-Jackson and Bert 2003; Sotka *et al.* 2004). The occurrence of two coexisting lineages is often attributed to a Pleistocene vicariance event during the Wisconsin glaciation associated with sea level and temperature changes (Haq *et al.* 1987, Herbert *et al.* 2001), which is believed to have separated several marine species into two or more refugia populations (Palumbi 1994). While subpopulations were isolated from one another, gene flow was restricted and subpopulations began to genetically diverge from one another. When the glaciers melted and sea levels rose at the end of the Pleistocene, the subpopulations reconnected, resulting in sympatric lineages.

IMPLICATIONS FOR MANAGEMENT AND FUTURE RESEARCH

The high genetic diversities observed for *C. magister* are a positive sign for the future sustainability of the species. That is, because the Dungeness crab seems to have a healthy genetic diversity, any recent population crashes among the areas sampled are probably not due to negative effects associated with low genetic diversity, such as inbreeding depression.

Another positive sign in favor of future sustainability of the species is the apparent gene flow between distant subpopulations of *C. magister*, which should help to sustain the high levels of genetic diversity observed for the species (as long as the path between subpopulations is not blocked by anthropogenic activities). Unfortunately, while the exchange of individuals between subpopulations is apparently enough to sustain genetic diversity, it is not necessarily enough to maintain population size (Cowen *et al.* 2006). That is, fishing regulators should not depend upon the natural dispersal of *C. magister* to replenish areas that are depleted of crabs due to overfishing. So, while establishing marine reserves may be an important step towards sustaining the Dungeness crab over the long term, it should not be expected that these reserves will necessarily act as replenishing sources for nearby populations whose numbers are severely depleted. Instead, a marine reserve strategy should be coupled with a continued restriction on the size and sex of harvestable crabs, and perhaps a restriction on the number of crabs that may be annually harvested as well.

Because of the high genetic diversity and apparent long-distance dispersal capabilities of *C. magister* implied by this study, it seems that the first steps in implementing a reserve plan to aid the future sustainability of the species would be to establish a few strategically placed marine reserves along the species' range. While this may not necessarily sustain the size of the Dungeness crab population, it should help to maintain the high levels of genetic diversity currently observed for the species. Based on this first look at the genetic characterization of *C. magister*, it is recommend that at least three reserves be placed in the vicinity of the following locations: Glacier Bay, AK, Coos Bay, OR, and Eureka/Northern, CA. Based on the high genetic diversity and moderate F_{ST} values observed in these locations, moderate levels of genetic distinctness seem to be seen here, as compared to other areas of the species' range. In addition, as Coos Bay was found to have the highest COI protein diversity, including evidence of structural changes in the shape of the COI protein within this population (a "hot spot" of some kind for the evolution of the species), it seems especially important that this area be protected.

It is crucial to keep in mind, however, as mentioned earlier, that this is only the *first* substantial broad-scale look into the population genetic structure of *C. magister*. In order to make the best, most well informed decisions possible for long-term management of the species, the genetics of this organism must be studied further, including a wider range of locations (especially British Columbia, Canada), a larger number of samples, and an increased number of genes. In addition, in order to understand the role of selection in the dynamics of Dungeness crab population structure, a closer look must be taken at the genetic differences within and between various life stages at particular

geographic locations. Only when all of this information is in place, will confident and comprehensible recommendations be able to be made for the sustainability of *C. magister* and the industries that it supports.

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