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A TALE OF TWO MORPHS: GENETIC AND GENOTYPIC STRUCTURE BETWEEN *MACROCYSTIS PYRIFERA* AND *MACROCYSTIS INTEGRIFOLIA*

by

Heidi L Hargarten

A Thesis Submitted in

Partial Fulfillment of the

Requirements for the Degree of

Master of Science

in Biological Sciences

at

The University of Wisconsin-Milwaukee

August 2015

ABSTRACT

A TALE OF TWO MORPHS: GENETIC AND GENOTYPIC STRUCTURE BETWEEN *MACROCYSTIS PYRIFERA* AND *MACROCYSTIS INTEGRIFOLIA*

by

Heidi L Hargarten

The University of Wisconsin-Milwaukee, 2015
Under the Supervision of Professor Filipe Alberto

Organisms living along environmental gradients often utilize phenotypic plasticity to maximize their survival across a range of conditions. Wherever gradients occur, there is potential for divergence through isolation-by-adaptation (IBA) to build-up between genotypes experiencing different selective pressures. Plasticity in traits pertaining to mating systems in particular are likely to constitute an interesting and revealing model for the study of the underlying mechanisms behind parapatric speciation. Giant kelp, *Macrocystis spp*., shows striking plasticity in holdfast morphology and reproductive strategy when colonizing intertidal (*M. integrifolia* morph) versus subtidal (*M. pyrifera* morph) areas along temperate rocky coastlines of the eastern Pacific Ocean. In the intertidal, high photosynthetically-active radiation (PAR) and UV radiation limit development of spores, recruitment of microscopic gametophytes, and growth and survival of embryonic sporophytes of *M. pyrifera*. Although depth of parent sporophytes influences spore survival in irradiance-stressed environments, few studies have examined the effects of irradiance stress on *M. integrifolia*'s developmental stages. This study focuses on understanding the roles of IBA and plasticity in maintaining *Macrocystis* morphs along the California coastline. To test for genetic isolation caused by ecological

divergence in the intertidal, we performed fine scale spatial sampling and molecular analysis of parapatric intertidal and subtidal populations off of the Central Californian coast. Using seven microsatellite markers, we compared genetic differentiation between morphs within sites and among morphs across different sites. Furthermore, we identified the presence of clonal replicates in intertidal populations. Results show higher differentiation between adjacent subtidal and intertidal morphs than between the same morph at larger spatial scales, suggesting isolation-by-adaptation. Several potential mechanisms could explain this result: assortative or other non-random mating, longer generation times promoted by asexual growth (intertidal morph), and differential mortality due to early adaptive divergence. Spatial analyses of clonal structure do not indicate asexual reproduction as the dominant strategy in the intertidal. To explore the hypothesis of differential mortality due to adaptive divergence, we will experimentally test assortative mating at different early development stages using controlled crosses of the two morphs under different treatments of irradiance (PAR and UV) stress. Surviving embryonic sporophytes will be genotyped and a paternity analysis will be conducted. Specifically, we hypothesize offspring from *M. pyrifera* parents will experience higher than expected mortality under irradiance stress, such that paternity analyses will reveal lower than expected numbers of *M. pyrifera* offspring among surviving embryonic sporophytes.

The overarching goal of this research program is to determine if phenotypic plasticity in mating system traits observed in giant kelp may be facilitating incipient parapatric speciation in the intertidal zone. This thesis will consist of three chapters. The first will concentrate on understanding the impact of alternative methods *M. pyrifera*

utilizes for dispersal at both ecological and evolutionary scales. The second will focus on characterizing genetic differentiation and structure patterns between adjacent populations of each morph. The third will develop additional hypotheses focused on understanding differential mortality between morphs under stress.

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"The edge of the sea is a strange and beautiful place… Only the most hardy and adaptable can survive in a region so mutable, yet the area between the tide lines is crowded with plants and animals. In this difficult world of the shore, life displays its enormous toughness and vitality by occupying almost every conceivable niche." - Rachel Carson, The Edge of the Sea

Chapter 1: Comparative population genetics in the sea: can we disentangle the dispersal role of kelp rafts?

Introduction

Dispersal is the universal mechanism by which organisms achieve gene flow and population connectivity. Although several different dispersal mechanisms have evolved in the ocean, the primary strategy employed by species with sessile adult forms is the production of planktonic propagules that that are passively dispersed locally or over long distances by ocean currents (Siegel *et al.* 2003; Gaylord *et al.* 2006; Cowen & Sponaugle 2009). Conventional theory predicts that organisms having long-lived planktonic stages have high levels of gene flow, while organisms with shorter-lived duration have lower levels of gene flow, leading to greater genetic differentiation and more structured populations (Siegel *et al.* 2003; Weersing & Toonen 2009; Selkoe & Toonen 2011). However, this is not always the case due to cryptic ocean barriers, temporal oscillations in oceanographic patterns, and environmental gradients, resulting in asymmetrical or reduced gene flow. These factors can create unexpected patterns of connectivity and genetic structure across various spatial distances (Gilg & Hilbish 2003; Johansson *et al.* 2008; Alberto *et al.* 2011; Treml *et al.* 2012; Liggins *et al.* 2013; DeFaveri *et al.* 2013). These effects depend on population history (Nesbø *et al.* 2000; Liu *et al.* 2006; Pelc *et al.* 2009), demography (Dawson *et al.* 2002), life history (Shulman & Bermingham 1995a; Sponaugle & Cowen 1997; Turner & Trexler 1998), and propagule behavior (Paris *et al.* 2007; Woodson & McManus 2007; Pringle & Wares 2007; Cowen & Sponaugle 2009; Morgan & Fisher 2010; Pineda *et al.* 2010). Comparisons between species or across

different studies, or community level predictions of gene flow patterns are therefore challenging (Bird *et al.* 2007; Liggins *et al.* 2013; Sexton *et al.* 2014). When trying to address this problem, it is important to control as many variables as possible when comparing across taxa, so that genetic differences can be linked to environmental or species-specific variables that remain dissimilar.

Here we use a comparative population genetics (CPG) approach to compare the dispersal of two sympatric species of macroalgae. We define comparative population genetics as the study of genetic differentiation of two or more taxa that share many life history traits and demographic history in a restricted geographic area. Constraining the spatial range of the study differentiates our approach from comparative phylogeography (sensu Avise 1992), which is rooted in evolutionary processes. The underlying idea in CPG studies is to reduce the dissimilarity in life history traits and the variability in demographic history across taxa being compared, thereby reducing the number of potential hypotheses explaining the observed differences in genetic differentiation patterns. Several studies that fit our CPG definition have been published for marine and aquatic biological models, which have examined larval strategies (Lambert *et al.* 2003; Watts & Thorpe 2006; Barbosa *et al.* 2013), life history strategies (Criscione & Blouin 2004), and habitat types (White *et al.* 2011; DeFaveri *et al.* 2012). Moreover, comparing genetic differentiation between populations of sympatric species with similar life history traits over a limited geographical area has been used to make inferences about dispersal patterns for each species (Shulman & Bermingham 1995b; Sponaugle & Cowen 1997; Turner & Trexler 1998; Dawson *et al.* 2002).

Comparative population genetic studies using kelps (large brown algae) can be particularly insightful for understanding patterns of gene flow and maintenance of kelp forest communities (Schiel & Foster 2006). Kelps have a heteromorphic life history; large, stationary diploid sporophytes produce microscopic haploid spores that are released into the water column and disperse passively via oceanic currents. These spores settle and develop into male and female gametophytes which produce gametes that fertilize and grow into the adult sporophytes. While tracking microscopic spores through the ocean is virtually impossible, genetic studies of adult populations can give great insight into patterns of gene flow when coupled with known information on spore biology (Amsler & Neushul 1989; Clayton 1992), physical and biological processes that affect dispersal (Reed *et al.* 1988, 1991, 1992; Vadas *et al.* 1992; Dayton *et al.* 1999; Steneck *et al.* 2003; Gaylord *et al.* 2004, 2006; Graham 2007; Collens 2009; Alberto *et al.* 2010, 2011), the effects of inbreeding mortality (Raimondi *et al.* 2004; Johansson *et al.* 2013), and a variety of demographic characters (Schiel & Foster 2006).

In the northeast Pacific, the kelps *Macrocystis pyrifera* and *Pterygophora californica* commonly co-occur in kelp forests from British Columbia, Canada to Baja California Mexico (Abbott and Hollenberg 1976). *M. pyrifera* is a foundational species, with buoyant fronds that grow from the seafloor to sea surface, where it produces a floating canopy, while *P. californica* is a shorter understory species that carpets the ocean floor with stands of palm-like sporophytes. These two species co-occur in the same rocky habitat at similar depth ranges, experience many of the same biotic and abiotic pressures, and also share the same basic life history. Additionally, these two species release their spores at a similar height in the water column and their spores have very similar physical

sinking properties and survival time in the water column (Amsler & Neushul 1991; Reed *et al.* 1992). When comparing across different species, it is critical to identify the defining differences as well. Perhaps the most important difference in this study pertains to dispersal (other differences discussed below); only *M. pyrifera* is positively buoyant and intact plants that are dislodged by large waves create floating rafts that are dispersed by winds and currents. Because detached individuals can continue to grow and release spores for weeks to months while adrift, they have the potential to promote dispersal between populations that are tens or hundreds of kilometers apart from one another (Hobday 2000; Macaya *et al.* 2005; Hernández-Carmona *et al.* 2006; Graham 2007; Gaylord *et al.* 2012).

Taking advantage of this key distinction in dispersal, we employed a comparative population genetic study to infer the contribution that these drifters may have in *M. pyrifera* gene flow. In order to do this, we first completed a microsatellite-based population genetics analysis of a set of *P. californica* sites that had been previously analyzed for *M. pyrifera* (Alberto *et al.* 2010, 2011). These complementary studies of the two kelp species allow us to compare not only the levels of genetic connectivity, but how the impact of specific drivers of connectivity, namely oceanographic transport, habitat continuity, and geographic distance, may vary between the two species. The goal of this project was to determine genetic connectivity in *P. californica* along California's Santa Barbara channel coastline and compare it to previous studies on *M. pyrifera* in the same region (Alberto *et al.* 2010, 2011). We hypothesized that if drifters play an important role in *M. pyrifera* dispersal then we should see much higher genetic connectivity in this species than in *P. californica* where this type of dispersal vector is absent.

Materials and Methods

Field Sampling and Genetic Differentiation Analyses

In order to conduct a population genetic study comparable with previous *M. pyrifera* studies (Alberto *et al.* 2010, 2011), samples from approximately 50 individuals per site of *P. californica* were collected at the nine previously-sampled sites along the mainland coast of the Santa Barbara Channel, California (Table 1.1); Bullito (Bul), Arroyo Hondo (AH), Arroyo Quemado (AQ), Naples Reef (NP), Isla Vista (IV), Goleta Bay (GB), Arroyo Burro Reef (AB), Mohawk (Mk), Carpinteria (Carp).

Sampling was conducted by removing a single blade from each individual via scuba diving. Tissue was preserved and stored in silica gel for DNA extraction. Genomic DNA was extracted using the Nucleospin 96 Plant Kit (Macherey-Nagel, Germany). All collected specimens were genotyped using seven microsatellite loci previously designed for *P. californica* (Appendix A, B). PCRs were performed in 15 µl reactions and contained ±20 ng of DNA, 0.1 µM of each primer, 0.8 mM of dNTPs (Bioline), 2.0 or 2.5 mM of MgCl2 (for individual locus PCR conditions see Supplementary Tables), 3.0 µl of 5x PCR Buffer and 0.4 U of GoTaq Polymerase (Promega, Madison, WI). Cycling conditions consisted of an initial denaturing step of 5 min at 95ºC, followed by 35 cycles of 30 s at 95ºC, 30 s at annealing temperature (Appendix A, B), 45 s at 72ºC, and a final elongation step at 72ºC for 20 minutes. All PCR reactions were performed on a GeneAmp 9700 thermocycler (PE Applied Biosystems). An ABI PRISM 3130xl DNA analyzer was used to analyze fragment length using the GeneScan Liz 500 size standard

(Applied Biosystems). Raw allele sizes were scored with STRand (http://www.vgl.ucdavis.edu/informatics/STRand), binned and reviewed for ambiguities using the R package MsatAllele (Alberto 2009).

Populations were checked for the presence of null alleles using Micro-Checker (Van Oosterhout *et al.* 2004). Excess homozygosity was identified at three loci (Pc-10, Pc-14, Pc-17). In order to detect if these loci were affecting pairwise F_{ST} calculations, the program FreeNA (Chapuis & Arnaud-Haond 2007) was used to determine if null alleles needed to be eliminated from the data set, by comparing F_{ST} values of data with null alleles versus data without null alleles. FreeNA corrects for the positive bias induced by the presence of null alleles on F_{ST}, providing a more accurate estimation of F_{ST} in the presence of null alleles. Global F_{ST} estimates using the "eliminate null alleles" correction method (F_{ST}=0.0605) were roughly similar to uncorrected values (F_{ST}=0.0629). A paired t-test between all pairwise population corrected and uncorrected F_{ST} values revealed that the difference was not significant (t=1.179, df=35, p=0.247). Therefore, analyses were run with non-corrected F_{ST} values.

To estimate genetic diversity in *P. californica* populations, allelic richness, standardized for 30 samples per population, was calculated using the R package 'standArich' (Alberto *et al.* 2006) and significant deviations from Hardy Weinberg Equilibrium were determined using Genepop v.4 (Rousset 2008). Inbreeding coefficients (F_{IS}) per population were calculated using ARLEQUIN version 3.5 (Excoffier & Lischer 2010). To maintain convention in population genetic studies, pairwise genetic differentiation between populations was also calculated using the program Genepop v.4 (Rousset 2008), and converted using the formula $F_{ST}/(1-F_{ST})$ to linearize genetic

differentiation for use in our models (Rousset 1997). Significant genetic differences between pairwise F_{ST} values were determined using FSTAT, version 2.9.3.2 (Goudet 1995). In addition Wright's F-statistics, Jost's D_{EST} estimator of genetic differentiation was used for comparisons between the two species and calculated using the R package 'diveRsity' (Keenan *et al.* 2013). Jost's D_{EST} provides an estimate of among-population genetic diversity that is not affected by the within-population levels of diversity (heterozygosity). This statistic is more suitable for inter-specific comparisons, because it accounts for differing allelic richness and heterozygosity across species (Jost 2008). Global D_{EST}, mean global D_{EST}, and pairwise population D_{EST} were calculated for both *M. pyrifera* and *P. californica*. Confidence intervals for both F_{ST} and Jost's D_{EST} were determined using 1,000 bootstrapped replicates. To account for the inherent bias in bootstrapping measures of genetic differentiation (Keenan et al. 2013), we used the bias-corrected values for D_{EST} and 95% confidence intervals in pairwise population comparisons.

Due to the expected conflicting results when using D_{EST} versus F_{ST} as a measure of genetic variance between the two species, we were interested in comparing how our two measures of genetic differentiation were associated with different putative drivers of gene flow. We ran individual simple linear regression analyses with each of the following predictors: geographic distance, habitat continuity, and oceanographic transport with either F_{ST} or Jost's D_{EST} as dependent variables (Figure 1.1, Table 1.2). Due to the fact that regression models identified different monthly transport times in the models with the highest goodness of fit in each species (June for *M. pyrifera* and April for *P. californica*), we used the spring oceanographic transport times to compare between these two kelps.

We compared the slopes of these regressions to determine if there was any evidence for differences in genetic differentiation between the two species based on our predictor variables. A difference in slopes would indicate different rates of change in genetic differentiation per focus predictor between the two species.

Transport time and directionality

To understand how oceanographic transport might explain genetic differentiation as compared to geographic distance, we used an available Lagrangian particle simulation model (Mitarai *et al.* 2009). This study simulated the dispersal trajectories of over 50 million passive Lagrangian particles in the southern California Bight (SCB) domain over the period from 1 January 1996 to 31 December 2002, with 135 uniformly-distributed, near-shore circular patches (5 km in radius) as release sites (see Mitarai et al. 2009 for more information on the Lagrangian particle simulations). Average transport times were calculated in days for particles to travel between each pairwise cell in the model and averaged again across the seven years simulated. However, these are not a measure of velocity and do not account for the specific pattern or path particles travel. These are fundamentally oceanographic distances, which have proven to be a better predictor of genetic connectivity than models based simply on Euclidian distance, in several marine systems (Weersing & Toonen 2009; White *et al.* 2010; Alberto *et al.* 2011). An important property of Lagrangian particle simulations is the inherent asymmetry in transport times between populations; particles travelling from site *i* to *j* might have a different mean transport time than particles travelling in the opposite direction, from *j* to *i*. Therefore, we used the shortest of the two transport times between pairwise populations to account for

this asymmetry, hereafter referred to as the minimum oceanographic transport time (Alberto et al. 2011). We calculated this time for each month, season (spring, summer, fall, winter), and the annual average. Two pairs of populations occur within the same five kilometer oceanographic cell used to estimate transport times, AH and AQ, and AB and Mk. This prohibits measures of oceanographic connectivity from being estimated for those pairwise comparisons, thus, sites AH and AB were removed from regression analyses using oceanographic transport (Alberto *et al.* 2011).

Modelling Population Genetic Differentiation

We used multiple linear regression to model genetic differentiation between populations. Our predictor variables included pairwise measures of habitat continuity, geographic distance, and the minimum oceanographic transport time (TT). We used the Akaike information criterion (AIC) to compare models with different combinations of predictor variables. All regression analyses were done in R (R Core Team 2014). Co-occurrence of these kelp species along rocky coastal areas enabled us to collect samples for both kelps at the same sampling coordinates, and to use the same measures of habitat continuity (HabCont) and geographic distance (GeoDist) from Alberto et al. (2011), allowing direct comparison between the two studies. These two measures were characterized using the California Department of Fish and Game kelp cover GIS layer (http://www.dfg.ca.gov/marine/gis/natural- resource.asp), and a composite of annual cover data from 1988 to 2003 was used. Geographic distance was estimated using the shortest ocean distance (quasi-Euclidean or straight-line distance without crossing land) between two sites.

Both single and multiple regression models were used to estimate the association of our three predictors, GeoDist, HabCont, and TT, with genetic differentiation. The effect of oceanographic transport time variability along the year was investigated by averaging the seven years of simulated data in different time intervals (monthly, quarterly, and annually). We used both the stepwise removal and addition processes to find the best fitting multiple regression model. Transport times during the months of June, July, August, September, and October were removed from our models, because *P. californica* does not produce spores during this time; including them would have led to biologically irrelevant interpretations.

We also investigated if seasonal oceanographic connectivities associated with the timing of reproduction and reproductive effort in *P. californica* could better explain the genetic differentiation estimated here. To do this, we extracted data from Reed et al. (1996) describing monthly variation in reproductive allocation for *P. californica*, and used it as a proxy for overall reproductive activity in *P. californica* in the study area. We then compared the goodness of fit (AIC values) of regression models using oceanographic transport times for different months with the period of reproductive activity for those months. As response variable for all these models we used both pairwise $F_{ST}/(1-F_{ST})$ and Jost's D_{EST}. To determine if there was a significant difference between the slopes of the regression lines and intercepts between the two species we used an ANCOVA.

Results

Comparative population genetics of M. pyrifera *and* P. californica

Microsatellite data indicates moderate genetic diversity in *P. californica* (allelic richness range 5.95 to 8.35) across all populations. *P. californica* allelic diversity was comparatively lower than corresponding *M. pyrifera* populations, (*M. pyrifera* allelic richness 11.22 to 13.46) (Table 1.1), indicating lower genetic diversity in *P. californica* compared to *M. pyrifera* in this region. Additionally, there were no deviations from Hardy-Weinberg Equilibrium, indicating most *P. californica* populations are experiencing migration from other patches. All populations were significantly differentiated from one another except for the neighboring populations Isla Vista (IV) and Goleta Bay (GB) (p=0.0153) (Appendix C).

In order to compare the level of gene flow among the two kelps, we calculated both global and pairwise measures of genetic differentiation within kelp species, and compared. Global genetic differentiation among all *M. pyrifera* populations (global D_{EST}=0.0736 and global F_{ST}=0.0237) was lower than genetic differentiation found among all *P. californica* populations (global D_{EST}=0.0911 and global F_{ST}=0.0667). However, confidence intervals for global mean D_{EST} overlapped between *M. pyrifera* (global mean D_{EST}=0.1108, LCI=0.0942, UCI=0.1309) and *P. californica* (global mean D_{EST}=0.1056, LCI=0.0947, UCI=0.1172). A paired t-test used to determine if pairwise population D_{EST} differed between the two species confirmed the difference was not significant (t=-1.7641, df=35, p=0.08644). Since pairwise and global D_{EST} values do not differ, dispersal of the two kelps is roughly the same in the Santa Barbara Channel. Results from comparing global F_{ST} were not congruent with D_{EST} measurements, as *M. pyrifera* global mean F_{ST} was significantly lower (global mean F_{ST}= 0.03734, LCI=0.03199, UCI=0.04330) than *P.*

californica (global mean F_{ST} =0.08307, LCI=0.07262, UCI=0.09517). Additionally, the difference in pairwise population F_{ST} between the two species was significant (t=-4.4848, df=35, p-value=7.522×10^{-5}). Discrepancies between these two measures of genetic differentiation are likely due to differences in heterozygosity and genetic diversity observed between the two kelps (Table 1.1). Higher heterozygosity and higher genetic diversity observed in *M. pyrifera* suggests gene flow among populations both within and outside of the study area.

We observed that slopes of F_{ST} on geographic distance, habitat continuity and oceanographic distance were all steeper for *P. californica* than for *M. pyrifera* suggesting larger dispersal distance in *M. pyrifera* (Table 1.3, significant interaction) However, when using D_{EST} to control for different level of within-species genetic diversity, the differences between species in slope and intercept were not significantly different, although the intercept was still higher for *P. californica* (Figure 1.3).

Modelling genetic connectivity in P. californica

Single regression models identified oceanographic transport time during the month of May as the best predictor of genetic differentiation (F_{ST}) in *P. californica* (Table 1.4). In simple linear regressions, habitat continuity was a better predictor of genetic differentiation than geographic distance. However, when combined with oceanographic transport in a two-predictor multiple regression models this was not always the case, especially during the spring months (Table 1.4). The multiple regression model with highest goodness of fit included habitat continuity, geographic distance, and transport

times during the month of April ($p=0.002$, $R^2= 0.503$, AIC=-125.04) and was considered the best overall model (Figure 1.2).

Oceanographic Transport and gene flow in P. californica

Figure 1.3 shows the variation throughout the year in *P. californica's* reproductive allocation (Reed 1996, sori area measurements) and range in pair-wise oceanographic transport time between populations. The goodness of (AIC) fit of models predicting genetic differentiation, differing by monthly oceanographic transport time used, is also shown for the months when the species is reproductive. During the winter months, oceanographic transport times were much longer than in the spring months, with May having the fastest overall transport between populations (Figure 1.3). Spore production and release (as measured by sorus area) increases during the winter, when oceanographic current velocity is slow. When currents speed up, a decrease in sorus area associated with spore release without new sporangial tissue production is observed. The best model predicting genetic differentiation was observed during this period of declining sorus area and fast oceanographic transport (AIC, broken grey line, Figure 1.3). Due to slow transport times, none of our sampled populations were predicted to have been directly connected via spore dispersal during the portion of the reproductive period that extended from November through February.

Discussion

Comparative population genetics of M. pyrifera *and* P. californica

Our comparative population genetics study focused on the regional levels of genetic differentiation between *Pterygophora californica* and *Macrocystis pyrifera.* Given the number of life history similarities between these kelps, we tested the hypothesis that gene flow was much higher in *M. pyrifera* due to the role kelp rafts may play as dispersal vectors for *M. pyrifera,* a trait absent in *P californica*. We first conducted a population genetic analysis of *P. californica* to quantify the associations between distance, oceanographic transport and habitat continuity with genetic differentiation, and compare them with available data on *M. pyrifera.* Linear models based on F_{ST} genetic differentiation estimates found that geographic distance, habitat continuity, and oceanographic distance had steeper rates of change for *P. californica* compared to *M. pyrifera*. This interaction between species was not found when D_{EST} was used to control for different levels of within-species genetic diversity. Second, we determined the level of among population (global) genetic differentiation and between population (pair-wise) genetic differentiation for both species, and then compare those measures between the two species. Both global and pairwise genetic differentiation indicated larger genetic distances among and between *P. californica* patches than among and between *M. pyrifera* when using F_{ST}. Again, when D_{EST} was used the differences were not only smaller between species, but also non-significant. The disparity between two different measures of genetic variation, F_{ST} and D_{EST}, highlights the importance of using directly comparable genetic measurements in order to produce meaningful data in cross-species comparative studies. Additionally, it provides an example of the use of Jost's D_{EST} in studies of isolation by distance, and other genetic differentiation drivers, and exemplifies its usefulness when compared with traditional measures of genetic

variation. Thus, these findings do not support the hypothesis that rafting sporophytes play an important role in extending the dispersal of *M. pyrifera* at the geographic scale of this study.

While we consider the positive buoyancy of dislodged *M. pyrifera* sporophytes carrying viable sporophylls to be the key life history difference between *M. pyrifera* and *P. californica*, there are other differences that could also play a role explaining genetic differentiation in these kelps. *M. pyrifera*, reproduces throughout the year, with seasonal peaks occurring twice annually during early winter and late spring (DeWreede 1986; Reed 1990; Reed *et al.* 1996, 1997). Continuous spore release year round maximizes dispersal potential during periods of high advective flow, which we would expect to decrease genetic differences between and among *M. pyrifera* patches. *P. californica*, meanwhile, has a strict reproductive window with highly synchronous spore release from November to April (DeWreede 1986; Reed 1990; Reed *et al.* 1996, 1997). Synchronization in spore release is predicted to promote gene flow by increasing the spore cloud that is available to disperse per unit of time. These periods also coincide with favorable conditions for not only recruitment (Reed & Foster 1984; Deysher & Dean 1986; Reed 1990; Reed *et al.* 1996), but extended dispersal distances as well. The combination of these conditions would lead to lower levels of genetic differentiation in *P. californica*.

In addition to the different strategies utilized for spore release, the age structure of kelp patches, regardless of species, might also have an effect on gene flow and the genetic makeup of patches across a region. *P. californica* sporophytes tend to live longer than *M. pyrifera* sporophytes (Rosenthal *et al.* 1974; Hymanson *et al.* 1990). The

difference in lifespan creates different generation times between the kelp species. *P. californica* has a longer generation time, and thus slower genetic turnover among its populations compared to *M. pyrifera*. These longer generation times would increase the amount of shared alleles between populations over time, diluting the effects of genetic drift and promoting higher connectivity between populations of *P. californica*. The genetic effects of longer generation times could balance the effect of floating rafts enhancing the dispersal potential of *M. pyrifera*, and could also lend an explanation as to why we see similar levels of genetic differentiation between *P. californica* and *M. pyrifera.* Along with these life history differences, the properties of some of our predictor variables may also influence our ability to compare connectivity patterns between these two species. Specifically, habitat continuity for both species was estimated using satellite remote sensing of *M. pyrifera* canopy cover, which is highly correlated to rocky habitat (Cavanaugh *et al.* 2010, 2013, 2014). This could lead to biased estimates of *P. californica* in areas where the two species do not co-exist and thus introduce error to our models estimating the influence of habitat continuity in genetic connectivity. Despite these life history differences, results from this and other studies support the notion that rafting sporophytes do not play a large role in connectivity among *M. pyrifera* patches.

Our results using a comparative genetic approach agree with previous studies analyzing the effects of kelp rafts on patch dynamics. For example, Reed et al. (2004) found no correlation between the size of the spore source provided by *M. pyrifera* rafts and the density of new *M. pyrifera* recruits on a large artificial reef, which instead was positively correlated with distance from the nearest standing population of *M. pyrifera* . This observation indicated that distant spore dispersal from extant populations rather than

more local spore dispersal from drifting rafts were much more likely to be the source of new recruits that initially colonized the artificial reef. Alberto et al. (2011) found that oceanographic connectivity for late spring (June) had the best fit predicting genetic differentiation in *M. pyrifera*. However, this time period is when sporophyte dislodgement is minimal (Reed *et al.* 2008). The near absence of rafters during periods of environmental conditions that produce and optimal setting for extending dispersal distances further indicates that such floating sporophytes are a negligible component of gene flow among *M. pyrifera* patches.

Although evidence argues that floating rafts contribute little to population dynamics of *M. pyrifera* , they may contribute to infrequent but nonetheless important episodes of gene flow that maintain genetic connectivity across greater geographic distances and longer temporal scales (Gillespie *et al.* 2012; Saunders 2014). Infrequent contributions from drifters may still be adequate to supply the 'one migrant per generation' needed to maintain gene flow and dilute the effects of genetic drift that create high levels of differentiation among distant populations in the absence of long-range dispersal capabilities. Genetic studies that target longer temporal scales and done across its global range bolster this argument for *M. pyrifera*, (Coyer *et al.* 2001; Macaya & Zuccarello 2010c; Astorga & Hernández 2012), and could partially help explain why we observe low genetic differentiation between, but high allelic richness within *M. pyrifera* patches.

Drivers of P. californica *genetic connectivity*

Oceanographic distance, habitat continuity, and geographic distance all explained genetic differentiation of *P. californica* in the Santa Barbara Channels similar to that previously found for *M. pyrifera* in this region (Alberto et al 2011). Oceanographic transport during spring months explain most of the variance in genetic differentiation for both *P. californica* (April) and *M. pyrifera* (May). Spring time in the Pacific Ocean is characterized by upwelling, where cold, nutrient rich water is brought from the deep ocean into shallower coastal areas (Bograd *et al.* 2009). Additionally, current speeds increase, creating an environment that is optimal for both extending dispersal distances and promoting survival for new recruits (Santelices 1990, Dayton et al. 1999, Lynn et al. 2003, Kerswell 2006).

The months where slow transport times occur correspond to periods of high reproductive effort. As transport times become faster, we see a reduction in sorus area per sporophyll which represents spore release during winter and early spring months (Reed et al 1996). This pattern reveals an interesting association between the seasonal reproductive cycle and synchronous spore release of *P. californica*, with conditions that are optimal for extending dispersal distances. During winter and spring, sea surface temperatures are at a minimum, infusing kelp forests with cold, nutrient rich water. Ocean temperature plays a critical role in marine dispersal (O'Connor *et al.* 2007), and the cold water during this time of year could allow spores to survive longer in the water column during the spring. Coupled with the increase in current speeds, these conditions appear to be optimal for extending dispersal distances as well as survival and growth of early developmental stages. This is likely to explain why we found the oceanographic connectivity matrix for this period to best fit genetic differentiation between populations.

Dispersing during these periods maximizes survivability, which is a key component in the evolutionary trade-off between dispersal range and cost of dispersal, resulting in larger dispersal distances for the same cost. Our work alone can't elucidate if this association is an adaptive strategy or simply a spurious correlation, as we have not measured actual costs and benefits to such a strategy.

Dispersal affects both the population dynamics and population genetics of species. Equally, the dynamics and genetics of populations dictate dispersal behavior. Many forces select for higher dispersal probability, such as temporal variability in habitat (Van Valen 1971), mechanisms for inbreeding depression (Bengtsson, 1978), and kin competition (Hamilton 1964; Hamilton & May 1977). The evolution of dispersal mechanisms driven by these forces can be seen as a balance between the costs of increased mortality during dispersal, or during the settlement in novel habitats, pooled together simply as the cost of dispersal. Future research could be directed towards discerning the effects of environmental variability, inbreeding depression avoidance and kin competition as putative drivers of selection for increased dispersal distance. The first two elements (environmental variability and inbreeding depression) are generally considered important factors driving the life-history of many kelps (Raimondi et al. 2004, Graham et al. 2007, Bell et al. *in press*). A simple start to such research program would be to first look for similar associations between transport speed, temperature and reproductive allocation in other areas of *P. californica* distribution. In addition to oceanographic conditions, the availability of habitat may also be playing a role in the degree of connectivity among *P. californica* patches.

The role habitat continuity plays in determining levels of gene flow in both sessile and mobile marine species has been demonstrated in numerous previous studies (Pielou 1978; Johnson & Black 1991; Riginos & Nachman 2001; Johansson *et al.* 2008; Alberto *et al.* 2010, 2011; Tarnowska *et al.* 2012; D'Aloia *et al.* 2014). Contrary to the oceanographic transport, habitat continuity was not modeled using seasonal variation, but using a composite of species cover across several years. This composite of kelp cover does not show fluctuations in the amount of *M. pyrifera* kelp cover biomass across months or years. However, such variation does occur, and years with lower kelp cover may indicate higher habitat availability for new recruitment to occur (Cavanaugh *et al.* 2010). Fluctuations in habitat availability can occur after severe winter storms, predation, and death. Recruitment occurs soon after habitat becomes available in kelp forests, mostly from remaining nearby adults in the kelp bed (Raimondi *et al.* 2004; Reed *et al.* 2004). Annual or decadal fluctuations in the availability of habitat may partially dictate how many recruits can settle and survive to adulthood, thus play a role in age structure and the longevity of certain genotypes within patches. The long-lived sporophyte stage of *P. californica* may be more affected by decadal trends in habitat fluctuation. This potential variability in habitat continuity would therefore have an influence on gene flow and potentially change our interpretation of genetic differentiation in *P. californica.* Future studies could focus on identifying how habitat availability may fluctuate on a monthly or annual basis, and in what capacity such variance may contribute to connectivity patterns. In fact, demographic connectivity incorporating this variation has been shown to predict patch occupancy well in Macrocystis pyrifera (Castorani et al. in press).

In summary, our results, coupled with results from Alberto et al (2011), provide strong evidence for several factors driving genetic structure in kelp forests: oceanographic transport, geographic distance, and habitat continuity. These drivers can be evaluated and used in other studies of marine organisms to more fully understand dispersal and connectivity within and among marine populations. The ability to estimate genetic connectivity among marine organisms remain critical for our understanding of gene flow and population connectivity in marine systems (Palumbi 1994; Valero *et al.* 2001; Levin 2006; Weersing & Toonen 2009; White *et al.* 2010; Selkoe & Toonen 2011; Sotka 2012; Liggins *et al.* 2013), and vital to the implementation of effective management and conservation efforts (Manel *et al.* 2003; Shanks *et al.* 2003; Lilley & Schiel 2006; Planes *et al.* 2009; Gaines *et al.* 2010), and garner a greater understanding of population dynamics in marine systems.

Chapter 2. Genic and genetic differentiation between *Macrocystis spp.* morphs

Introduction

One of the great debates in biology is what constitutes a species, and how new species form. Historically, speciation has been said to be driven by a restriction in gene flow over time via some sort of geographic separation or barrier between populations, leading to reproductive isolation, and the inability to mate if subsequent contact occurs (Mayr 1942, 1963, Coyne & Orr 2004). The myriad of exceptions found for allopatric speciation has created a diverse and ever growing body of research aimed at identifying how and when speciation can occur (Coyne & Orr 2004). One particular branch of research is focused on understanding how speciation can occur in parapatry, without barriers, in the presence of gene flow (Servedio & Noor 2003; Bolnick & Fitzpatrick 2007; Smadja & Butlin 2011). This scenario of speciation with gene flow can occur along environmental clines or gradients, where there are changes in environment conditions across a landscape. These gradients in environmental conditions can be gradual or steep, and can induce a multitude of phenotypic differences between populations along clines (Case & Taper 2000; Doebeli & Dieckmann 2003; Doebeli *et al.* 2005; Miner *et al.* 2005; Pfennig & Pfennig 2012; Savolainen *et al.* 2013). These varying phenotypes, and the underlying genetic diversity on which plasticity functions, can be subject to selection based on different environments encountered along a gradient (Johnston *et al.* 2001; Schmidt *et al.* 2008; Prada *et al.* 2008; Moczek *et al.* 2011). This can result in local adaptation (Pfennig & Murphy 2002a; DeWitt & Scheiner 2004; Doebeli *et al.* 2005; West-Eberhard 2005; Martin & Pfennig 2010b; Pfennig *et al.* 2010; Fitzpatrick 2012),

even in the presence of gene flow (Bricker *et al.* 2011; White *et al.* 2011; Pespeni *et al.* 2013; Bourret *et al.* 2013; DeFaveri *et al.* 2013; Gould *et al.* 2014; Nanninga *et al.* 2014)

Depending on its strength and frequency, gene flow can have a multitude of effects on the ability for populations to locally adapt along a gradient (Garant *et al.* 2007; Gavrilets & Vose 2007; Gavrilets *et al.* 2007; Thibert-Plante & Hendry 2009; Smadja & Butlin 2011; Sexton *et al.* 2014). There are two primary forces that work against each other when gene flow occurs between two populations. The first is the antagonizing force of recombination. Recombination breaks apart advantageous gene combinations that would otherwise promote fitness and divergence. Along gradients, this can slow the adaptive process by creating a fitness cline of partially unfit hybrids along the gradient (Felsenstein 1981). More importantly, recombination will break apart associations between traits that could lead to reproductive isolation, a necessary component to complete the speciation process (Felsenstein 1981; Servedio 2009; Smadja & Butlin 2011). Recent advances in genomics have identified specific regions of genomes that may be less subject to recombination than others due to chromosomal inversions that can suppress recombination (Kirkpatrick & Barton 2006; Hoffmann & Rieseberg 2008; Twyford & Friedman 2015). Such inversions can lead to a buildup of divergence in only key, specific regions within the genome, advancing adaptation even in the face of recombination (Gavrilets 2004, Doebeli *et al.* 2005; Via & West 2008; Feder & Nosil 2010; Servedio *et al.* 2011; De Wit *et al.* 2012; Via 2012; Nosil & Feder 2012; Feder *et al.* 2012). The alternative force of divergent natural selection via reinforcement can overcome the effects of such genetic homogenization (Abbott *et al.* 2013; Barton 2013).

Reinforcement is the increase in pre-zygotic isolation between two populations in response to selection against hybrid offspring, often due to critical phenotypic differences and allele mismatches between genetic material from diverging parental populations (Kirkpatrick & Ravigné 2002; Servedio & Noor 2003; Butlin 2005; Ortiz-Barrientos *et al.* 2009; Arnegard *et al.* 2014). Such incompatibles arise between combinations of loci that produce low quality offspring, called the Dobzhansky-Muller incompatibilities, and can occur even when selection against hybrids is weak (Dobzhansky 1937, Muller 1942, Servedio & Kirkpatrick 1997; Servedio 2000; Nosil *et al.* 2005). These hybrid individuals are then removed from the population due to natural selection and being outcompeted by their respective parent populations in the different environments. Reinforcement depends upon the level of gene flow that occurs between populations, and the strength of the selection against hybrids (Servedio & Kirkpatrick 1997; Case & Taper 2000; Nosil *et al.* 2003). Many of the studies considering reinforcement as a driver of speciation are concerned with empirical evidence using mate preference and sexual selection (Seehausen & van Alphen 1999; Kirkpatrick & Ravigné 2002; Rundle & Nosil 2005; Hey 2006; Vergara *et al.* 2012). However, not all organisms undergoing adaptation along gradients utilize mate selection. Mating system variation can come in many forms, such as differences in flowering time in angiosperms along latitudinal gradients, or plasticity between sexual and asexual reproduction from ideal to edge habitat. As the divergence between traits related to gene flow can accelerate the adaptive process (Nosil *et al.* 2005), selection on mating system traits could further promote divergence along an environmental gradient.

Such variation in mating systems can decrease the amount of gene flow between conspecific populations or among closely related species (West-Eberhard 2005; Antonovics 2006; Zardi *et al.* 2011). Low levels of gene flow, coupled with natural selection pressures on certain phenotypes, may work together to maintain distinct genetic taxa in areas where environmental gradients exist (Case & Taper 2000; Doebeli & Dieckmann 2003; DeWitt & Scheiner 2004; West-Eberhard 2005; Thibert-Plante & Hendry 2011) as evidenced by field studies (Keddy 1981, 1982; Billard *et al.* 2010; Zardi *et al.* 2011; Bricker *et al.* 2011). Based on the potential for mating systems to promote differentiation among populations, plasticity in traits within mating systems represent a potential model for the study of the underlying mechanisms behind divergence along environmental gradients and parapatric speciation (Hendry *et al.* 2007; Schluter 2009).

An ideal area to test how variation in mating systems affects population along environmental gradients is the rocky intertidal zone. The intertidal zones of temperate reefs are characterized by steep environmental gradients of immersion time and desiccation, temperature, irradiance and salinity. Intertidal zones constitute a well-known example of vertical community structure characterized by high species diversity (Dayton 1971; Lubchenco 1978; Sousa 1979), the envelope of each species being controlled by both biotic and abiotic factors such as wave exposure (Lewis 1964, Evans 1947; Stephenson & Stephenson 1961), temperature (Wethey 1983), light and photosynthesis (Johnson *et al.* 1974), salinity (Druehl 1967), and predation and competition (Connell 1961). Many studies have examined the different mating system strategies that have evolved in this turbulent, constantly fluctuating ecosystem (Coyer *et al.* 2001; Billingham *et al.* 2003; Moola & Vasseur 2009; Becheler *et al.* 2010; Demes & Graham 2011). One

strategy that is thought to be utilized at the upper limits of a species zonation in the intertidal is asexual reproduction (West *et al.* 2001; Tatarenkov *et al.* 2005; Demes & Graham 2011; Oppliger *et al.* 2014).

Asexual reproduction, or clonality, has been cited to be an adaptation for organisms to recover after a disturbance, in stressful environments, or at their geographic limits (Dorken & Eckert 2001; Dethier *et al.* 2005; Moola & Vasseur 2009). Clonal succession, as opposed to sexual succession, has been shown in the field to be the primary driver for recovery after disturbance in terrestrial systems (Silvertown 2008; Moola & Vasseur 2009). The capability to reproduce asexually and grow clonally allows organisms to quickly re-establish when broken or disturbed, and also allows successful genotypes to persist despite some disturbances (Barrett *et al.* 1993; Tatarenkov *et al.* 2005; Wright & Davis 2006). Mating system variation utilizing both sexual and asexual growth can also assist in determining the costs and benefits of such plasticity (Loehle 2013). Additionally, asexual reproduction is thought to be adaptive in stressful environments or at physiological limits. Sexual reproductive structures are often energetically costly to produce and maintain. The ability to divert that energy to maintenance and growth is a survival mechanism that doubles as plasticity in reproductive strategy (Vallejo-Marín *et al.* 2010).

The strategy for clonal reproduction has evolved multiple times independently in many different groups of algae due to limits in investment for sexual reproduction caused by costs for growth and repair (Smith *et al.* 2004; Tatarenkov *et al.* 2005; Demes & Graham 2011; Oppliger *et al.* 2014). Most kelp species exhibit either sexual or asexual reproduction to maintain populations. The giant kelp, *Macrocystis spp*, potentially has a

plastic mating system that allows it to utilize a sexual system in the subtidal (*M. pyrifera*), and both a sexual and an asexual system in the intertidal (*M. integrifolia*) where sexual reproduction is severely constrained (Demes *et al.* 2009a; Demes & Graham 2011). Since this kind of plasticity is rare in kelps, the giant kelp can be used as a model to study potential species divergence facilitated by environmentally induced phenotypic plasticity on mating system traits. Because of its ecological importance, giant kelp has been widely studied for decades, and has gone through some major classification shifts due to its highly variable morphology along the depth gradient (between the intertidal and the subtidal zones where it occurs around the globe). The *M. pyrifera* form is found in both hemispheres along the coasts of continents bordering the Pacific Ocean, as well as around the tip of South Africa. Its distribution is limited to anti-tropical areas because of the negative effect temperature has on growth (Demes & Graham 2011). The *M. integrifolia* form is only found along the Peruvian/northern Chilean coast in the southern hemisphere, and approximately northern Estero Bay in California to southeastern Alaska in the Northern Hemisphere (Coyer *et al.* 2001; Graham 2007). The two forms do not necessarily occur in sympatry together or in parapatry. For example, in Chile, the *M. integrifolia* form is found along coastlines without co-occurring *M. pyrifera* populations. Historically, morphological variation associated with the form of the holdfast had been used to classify the intertidal form, *M. integrifolia*, the subtidal form, *M. pyrifera,* and the intermediate form, *M. angustifolia*, as separate species (Graham *et al.* 2007).

Crossing experiments by Lewis and Neushul (1994) were carried out to determine if there was reproductive isolation between different forms, then considered different species, of *Macrocystis*. They used crosses between *M. integrifolia*, *M. pyrifera*, and *M.*

angustifolia, from the Pacific Northwest coast and the Tasmanian coast in Australia. All these experimental crosses yielded viable sporophytes between hybrid combinations. Lewis and Neushul concluded that even though there was no reproductive isolation between the species, they should still be considered different species based on the morphological species concept. Lewis and Neushul (1994) stated that *Macrocystis* was in an active period of speciation with both morphological and physiological speciation occurring based on differences in growth rate due to the different habitats the forms are found in. The physiological isolation may have led to the partial or complete ecological isolation seen in the different morphologies today (Lewis & Neushul 1994). Later studies testing the fertility between the three different *Macrocystis* morphotypes again demonstrated that all three taxa were able to produce viable offspring when spores from each form were crossed. The resultant offspring produced intermediate holdfast morphologies (Westermeier *et al.* 2006; Demes *et al.* 2009a). These authors concluded that all three morphotypes are a single species, *Macrocystis pyrifera,* with phenotypic variation mostly explained by environmental cues inducing phenotypic plasticity.

Further support for combining different morphotypes into a single species was obtained through transplant experiments. *M. integrifolia* individuals and their rhizomes were taken from the intertidal and transplanted into and down the subtidal depth gradient. Results yielded a switch in holdfast morphology from rhizomatic growth to the conical holdfast typical of *M. pyrifera* individuals (Demes *et al.* 2009). Thus, these different morphologies indicate phenotypic plasticity in energy allocation for vertical versus horizontal growth. Energy in *M. pyrifera* seems to be allocated for fast vertical growth and sexual reproduction, whereas in *M. integrifolia*, it seems to be allocated for

establishment, blade and holdfast repair, and horizontal growth. The need for plasticity comes from different pressures on growth experienced by the two forms. *M. pyrifera* needs to grow quickly to out-compete conspecific competitors for light resources. *M. integrifolia* needs to establish firm, robust holdfasts to survive in the harsh intertidal environment (Graham *et al.* 2007).

Recent work using genetic markers (Coyer *et al.* 2001; Alberto *et al.* 2009; Macaya & Zuccarello 2010a; c) has supported and confirmed that *M. pyrifera* and *M. integrifolia* are the same species. Concurring with these results, extensive clonality was found in disconnected holdfasts of the *M. integrifolia* form in the intertidal area across tens of meters, while in deeper areas the *M. pyrifera* form shows high sexual allocation (Alberto et al. unpublished). Samples of both morphs collected in the same site shared many of the same microsatellite alleles, in accordance with the hypothesis of the deeper form being the source of the shallow form. Before this preliminary study, and the development of these highly polymorphic *Macrocystis* microsatellites, there had been no way to measure asexual allocation in the shallow form.

Previous work has already shown that the sexual, post-settlement stages (gametophytes and embryonic sporophytes) of the giant kelp life cycle rarely, if at all, develop in the shallow area due to high irradiance (PAR) or UV light or both (Graham 1996). Post-settlement stages only develop in the intertidal within a shaded, protected area that shields them from PAR and UV. Difference in intraspecific meiospore size and in gametophyte germination rates have also been observed between shallow and deep *M. integrifolia* populations in British Columbia (Swanson & Druehl 2000). Here, shallow *M. integrifolia* had significantly larger spore sizes, and had higher where larger spore sizes

from shallow *M. integrifolia* had higher survival and were less inhibited by UV-B exposure than deeper *M. integrifolia* (Swanson & Druehl 2000). Higher survivability under UV exposuer of larger single-celled organisms has also been found in other plankton species as well (Karentz *et al.* 1991; Bothwell *et al.* 1993), indicating that there may be an adaptive mechanism for surviving exposure to UV conditions by growing larger in the shallows to reach a size where such exposure is no longer lethal. Such an adaptive advantage could potentially lead to assortative mating between phenotypes if the ability to produce larger spores by the *M. integrifolia* morph were to promote higher survivability than smaller spores from the *M. pyrifera* morph. Alternatively, these effects could be due to maternal affects and the stressful environment experienced by the parent. Since zoospore viability and survival after exposure to high PAR and UVB is related to the growth depth of the parent sporophyte (Wiencke *et al.* 2000; Swanson & Druehl 2000), the environment experienced by the parent may be dictating how well early microscopic stages are able to survive and develop.

Thus far, results suggest that upper intertidal population of the *M integrifolia* phenotype may constitute a sink, dependent on colonization from subtidal *M. pyrifera* and thus with reduced evolvability. Under this model, the subtidal *M. pyrifera* reproduces sexually via the production of spores. In the intertidal zone *M. integrifolia* reproduces primarily via clonal growth or via seeding from subtidal areas where sexual reproduction is not impeded. Never the less, *M. integrifolia* does produce sporophylls in the intertidal (Jeffries 2015). The presence of sporophylls in the intertidal, and previous success culturing sporophytes from those spores, indicates that *M. integrifolia* may potentially have the ability to successfully recruit sexually with locally produced spores in addition

to asexual reproduction. The ability to use multiple mating strategies in the intertidal, coupled with differential survival of microscopic life stages between morphs, should theoretically lead to a restriction in gene flow where the two forms co-occur. If such adaptive plasticity is in fact coupled with a restriction in gene flow between *M. integrifolia* and *M. pyrifera* there is opportunity for these different phenotypes to become fixed due to environmental differences. When there is a strong association between phenotype and its environment, rapid speciation can occur. First, due to stark environmental differences, phenotypes can become fixed. Second, divergent selection causes genetic assimilation and other adaptive modifications of each phenotype. And finally, reproductive isolation evolves due to restriction in gene flow as a result of adaptive divergence (West-Eberhard 1986, 1989, 2005). Differential mortality between *M. integrifolia* and *M. pyrifera* spores and gametophytes in the intertidal versus the subtidal could be one such factor that could lead to selection against gene flow between the two forms, eventually leading to reproductive isolation and speciation (chapter 3).

The overarching goal of this research program is to determine if phenotypic plasticity in mating system traits observed in giant kelp may be facilitating incipient parapatric speciation in the intertidal zone. This study will specifically focus on characterizing genetic differentiation and structure patterns between adjacent populations of each morph. We expect that if *M. integrifolia* populations are dependent upon genetic rescue from *M. pyrifera* to maintain intertidal populations we will observe low genetic differentiation between morphs. Patterns of genetic differentiation would look very different, however, if *M. integrifolia* is able to self-maintain its population and there is some level of localized adaptation which would lead to genetic divergence between

morphs. In order to do characterize these patterns of genetic differentiation and structure, we first (1) determine how the presence and prevalence of sexual reproduction varies across the intertidal depth gradient colonized by *Macrocystis*; (2) characterize genetic differentiation between *M. pyrifera* and *M. integrifolia* in three sites where parapatric distribution is present; (3) explore fine scale spatial distribution of genetic kinship within and between morphs. Finding high levels of genetic differentiation between the two morphs would indicate that there is restriction in gene flow between the two morphs, and further investigation into our overarching research goals will be warranted.

Methods

Collection and DNA Extraction

We sampled seven sites along the range of *M. integrifolia* and *M. pyrifera* overlap along the coast of Central California (Figure 2.1). Both morphologies occurred at three of our sampling locations, Cambria, Stillwater Cove, and Point Piños. Preselected X-Y coordinates were generated and samples were collected from individuals at each point along transects. Intertidal *M. integrifolia* samples were collected during low tide. Subtidal *M. pyrifera* samples were collected by SCUBA. Along shore dimensions of patches ranged from approximately 50-100 meters, and cross shore dimensions ranges from approximately 6-25 meters, depending on the size of the patch at each location. At Stillwater cove, a hierarchical sampling design was used for *M. integrifolia* collections. The overall scale of the design was determined based on the distance between the *M. pyrifera* patch and the *M. integrifolia* patch to reproduce sampling distances between and

within morphotype. The alongshore distance that both *M. integrifolia* and *M. pyrifera* were sampled (across morph patches) along were adjusted according to the cross shore distance (within morph patches) (Appendix D). Once this distance had been determined, using both field estimates and Google™ Earth imagery, distances between the smallest hierarchical categories were established for the *M. integrifolia* patches. Samples were collected along five meter transects. There were distances of 5, 10, and 15 meters separating these five meter sampling transects, which would allow us to estimate kinship at varying distances classes along the length of the patch (Table 2.1). During sampling, one individual was identified by the holdfast and a single, non-reproductive blade was removed. Care was taken to avoid sampling the same holdfast twice during intertidal collections. After collection, a three-centimeter cutting was removed from each blade and stored in silica gel to dry DNA extraction. DNA was extracted using the NucleoSpin® 96 Plant II Genomic DNA from Plant kit by Macherey-Nagel.

Genetic Differentiation Analyses

All samples were analyzed using seven microsatellite loci previously developed for *Macrocystis pyrifera;* BC-4, BC-8, BC-18, BC-19, BC-25, Mpy-8, Mpy-11 (Alberto *et al.* 2009). Using multilocus genotyping, microsatellite peaks were scored using the program STRand (Toonen & Hughes 2001, http://www.vgl.ucdavis.edu/STRand). The R package 'MsatAllele_1.05' (Alberto *et al.* 2009) was used to visualize and bin fragment size data into microsatellite alleles. Allelic richness for each population was calculated using R package 'standARich' (Alberto *et al.* 2006), standardized for 32 individuals per population. Observed and expected heterozygosity as well as inbreeding coefficients (F_{IS})

(100,000 bootstraps to determine 95% confidence interval) per population were calculated using Genetix v. 4.05 (Belkhir et al 2004).

We used the program FreeNA (Chapuis & Arnaud-Haond 2007) to detect if excess homozygosity or null alleles present in the data set would affect pairwise F_{ST} calculations, by comparing F_{ST} values of data with null alleles versus data without null alleles. Microsatellite null alleles are commonly found for a variety of reasons, but can lead to the overestimation of F_{ST} in populations that are highly differentiated (Chapuis & Arnaud-Haond 2007). Global F_{ST} values for comparison were calculated by resampling over all loci with 5,000 replicated runs at the 95% confidence interval. Global F_{ST} estimates using the "eliminate null alleles" correction method (F_{ST}=0.3037) were lower compared to uncorrected values (F_{ST}=0.3193). A paired t-test between all pairwise population corrected and uncorrected F_{ST} values revealed that the difference was significant (t=8.9397, df=44, p=1.88×10^{-11}). Therefore, ENA corrected F_{ST} values were used for convertion using the formula $F_{ST}/(1-F_{ST})$ to linearize genetic differentiation for use in genetic comparisons of populations (Rousset 1997). Significant genetic differentiation between all pairwise population combinations was tested using 10,000 permutations with an adjusted nominal 5% type error I for multiple comparisons of 0.000758 using the program FSTAT version 2.9.3.2 (Goudet 1995). Only one pairwise comparison was found to not differ significantly, Van Damme *M. integrifolia* and Van Damme *M. angustifolia*. These two populations were combined into one for all additional analyses.

Additionally, we calculated Jost's D_{EST} to measure genetic differentiation using the R package 'diveRsity' (Keenan *et al.* 2013). Jost's D_{EST} provides an estimate of

among-population genetic diversity that is not affected by the within-population levels of diversity (heterozygosity). This statistic accounts for differing allelic richness and heterozygosity across populations or species being compared (Jost 2008). To account for the inherent bias in bootstrapping measures of genetic differentiation (Keenan et al. 2013), we used the bias-corrected values for D_{EST} and 95% confidence intervals in pairwise population comparisons. Pairwise measures of differentiation were used to test for isolation-by-distance affects, and relationships between genetic diversity and latitude were explored as well.

Clonal Analysis

To identify the relative contribution of both sexual recruitment and asexual growth to the genetic structure of intertidal populations, we conducted a clonality assessment using the program GENCLONE 2.0 (Arnaud-Haond & Belkhir 2007). This program uses the multi-locus genotype (MLG) of each individual to determine the probability of finding a specific genetic identity, first by determining the probability any sampled genotype could be found within the population based on the sampled allelic diversity (P_{gen}). Second it calculates the probability that individuals sharing the same replicate genotype came from unique sexual recombination events (P_{sex}), as well as the probability for resampling that same genotype multiple recurring times within the population (Arnaud-Haond *et al.* 2007). P_{sex} is used to test the null hypothesis that x individuals in a sample of n sharing the same MLG are the result of x different sexual events that by chance, given the allelic frequencies of the sample, had the same exact MLG fingerprint. The rejection of this hypothesis, when $P_{sex}<5\%$, supports accepting the

alternative hypothesis that the *x* identical MLGs are clonemates resulting from the same sexual recombination. GENECLONE also allowed to estimate P_{sex} with corrections for departures from Hardy-Weinberg equilibrium.

Because P_{sex} estimation is dependent on the number of loci used and their polymorphism, failing to reject the null hypothesis of multiple sexual events may be the result of low statistical power in cases where the marker system is not polymorphic enough. For five of our sites, the ones with lowest allelic richness, we indeed failed to reject the null hypothesis for *M. integrifolia*. Therefore, *M. integrifolia* individuals with shared multilocus genotypes were amplified at an additional three highly polymorphic microsatellite loci; Mpy-7, Mpy-9, Mpy-19 (Alberto *et al.* 2009) to increase statistical power for these assessments. We calculated pairwise distances between individuals to determine the minimum and maximum distances between identified clones. Additionally, we tested for edge effects (E_e) and aggregation (A_c) using GENCLONE 2.0. The edge effect tests the null hypothesis of random distribution of clones, by estimating the effect of sampling design on the estimate of genotypic richness. In populations with clonal groups, genotypic richness can be overestimated if a clone was only sampled once, due to its presence on the edge of the sampling area (Arnaud-Haond *et al.* 2007). The aggregation index provides an estimate to determine if neighboring individuals are more likely to share the same MLG (Arnaud-Haond *et al.* 2007). Both of these statistics were estimated using 1,000 permutations.

Genetic Structure Analysis

In order to identify genetic clusters of genetic co-ancestry and classify accordingly all individuals collected across our sampling range, we used the program STRUCTURE 2.3.4 (Pritchard *et al.* 2000). Due to the large spatial scale of our data, we analyzed several spatial classes, or hierarchies, within our data after initial co-ancestry clusters were determined. The first and largest hierarchy included all seven of our sample sites. At three of these sites, both morphs were present. At these sites, we distinguished between the *M. pyrifera* and *M. integrifolia*, creating a total ten unique populations to include in the STRUCTURE analysis. We used the admixture setting with a burn in length of 50,000 and 100,000 post-burn in MCMC repetitions per value of *K* (number of potential clusters). Allele frequencies were considered to be correlated and we tested *K*=1 to 10 with 15 repetitions at each *K*. Computed log-likelihoods were used to determine the most likely co-ancestry clusters by calculating Δ*K* (Evanno *et al.* 2005).

The initial STRUCTURE run using the admixture model identified two likely co-ancestry clusters (K=2) at the highest hierarchy, one represented by populations north of the Monterey Peninsula, and another by populations on the Monterey Peninsula and south (Table 2.2, Figure 2.2). The break between these two clusters occurs at Point Piños, between the *M. pyrifera* and *M. integrifolia* patch. There was also minor support for five clusters (K=5) using the admixture model. This minor peak indicates potential, additional levels of sub-structure within each of the top level clusters. Therefore, we proceeded with a hierarchical analysis within each of the two main clusters identified, using the same settings as the first hierarchy. Finally, we were also interested to see how sample sites with parapatric patches of both morphs were clustered. At this third and smallest hierarchy, we run individual STRUCTURE analysis for each of the three sites with both

morphs (Point Piños, Stillwater Cove, and Cambria) with the same initial settings, using the admixture setting, for K=2 with 10 iterations. We considered both morphs as one population at each location for these runs.

Migration and Kinship

First generation migrants were detected using the program GeneClass2.0 (Piry *et al.* 2004). All populations were analyzed together, as well as individual populations with parapatric *M. pyrifera* and *M. integrifolia* patches. The likelihood computation used for migration detected uses the ratio of the likelihood computed from the population where the individual was sampled over the highest likelihood value among all population samples, including the population the individual was sampled in (Paetkau *et al.* 2004). The criterion for the likelihood analysis chosen was the Bayesian method developed by Rannala & Mountain (1997). In addition, the probability that an individual is a resident of its sampled population (i.e. null hypothesis of not a first generation migrant) was estimated using the resampling algorithm from Paetkau et al (2004). The number of simulated individuals was 10,000 with a type 1 error rate set to 0.05. We assigned individuals as 1st-generation migrants if the probability of an individual belonging to its sampled population was less than 0.05 (i.e. rejected the null hypothesis).

Fine scale spatial genetic structure within populations can be detected using spatial autocorrelation analyses to determine the level of coancestry between pairs of individuals within a population. Spatial genetic structure integrates migration and gene flow over multiple generations, and gives an estimate of past effective gene flow (Johansson *et al.* 2013). Spatial genetic structure can be created by a variety of factors,

such as gene flow, genetic drift, and natural selection. Under the hypothesis of no spatial genetic structure, we are able to compare the kinship coefficient to the distance between individuals in order to predict if the relationship between distance and genetic relatedness falls within this expectation. Kinship that is higher than we expect at a certain distance class could result for several different reasons: dispersal is very local (ie within the distance class), limited migration events into the patch, or asexual reproduction, and kinship Kinship that is lower than we expect can be due to high levels of admixture from nearby populations (Loiselle *et al.* 1995; Alberto *et al.* 2005, 2006; Johansson *et al.* 2013; Krueger-Hadfield *et al.* 2013a, 2014).

The fine scale spatial genetic structure was studied using a spatial autocorrelation analysis of Loiselle's measure of coancestry (Loiselle *et al.* 1995) in the program SPAGeDi v. 1.4 (Hardy & Vekemans 2002). We computed kinship between pairs of individuals within each morph at arbitrarily defined distance classes (Table 2.1), and computed 95% confidence intervals using 2,000 bootstrap permutations to test the null hypothesis of no spatial genetic structure. The presence of repeated MLGs in our intertidal samples increases the level of kinship, simply due to clonality, within an area defined as the clonal subrange that depends on the size of clones (Alberto *et al.* 2005). Thus, we also run the analysis with a single copy of each MLG found, and using the mean spatial coordinates of multiple copy MLGs, to estimate the effects of kinship un-confounded by the spatial spread of the clone.

An additional analysis was done for Stillwater Cove, where the spatial arrangement of the two morphs made it possible to sample similar spatial distances within and between morphs. This allowed comparing the rate of change in kinship at

similar for spatial scales. If indeed there are limitation of gene-flow between morphs we should observe a steeper slope of kinship with distance for that analysis than for within morphs. For between morphs kinship estimation, allele frequencies were estimated from pooling the two morphs together.

Results

Genetic Differentiation

Observed heterozygosity (adjusted R^2=0.6223, p=0.0041) and allelic richness (adjusted R^2=0.6378, p=0.0034) decreased with increasing latitude, as well as from *M. pyrifera* to *M. integrifolia* patches at each location (Table 2.3). Decreasing allelic richness and heterozygosity with an increase in latitude is indicative of northward range expansion by *Macrocystis spp.* after the recession of the last glacial maximum (Hewitt 1996; Graham 2007), and has been demonstrated in both northern (Johansson et al. *in prep*) and southern hemisphere (Macaya & Zuccarello 2010c). Analyses of genetic differentiation revealed significant differences between all pairwise populations (Table 2.4; $p<0.00111$). To compare our two measures of differentiation, F_{ST} and D_{EST}, we used a paired t-test. Differentiation using ENA corrected, linearized F_{ST} was significantly higher than differentiation using Jost's D_{EST} (t=9.1783, df=44, p=2.12×10^{-10}). Both measures indicate isolation-by-distance across the range of populations, (F_{ST} adjusted R^2=0.11, p=0.01486; D_{EST} adjusted R^2=0.1256, p=0.0097). When using only populations with parapatric *M. pyrifera* and *M. integrifolia*, the signature for isolation-by-distance

disappeared for both measures of genetic differentiation (F_{ST} adjusted R^2=-0.07251, p=0.8207; D_{EST} adjusted R^2=0.03112, p=0.25).

Clonal Analysis

Intertidal *M. integrifolia* individuals sharing the same MLG were genotyped with three additional loci to increase statistical power in P_{sex} estimation. Every intertidal population had at least one group of sample units that shared the same MLG (Table 2.5). Most of these MLGs represented in fact sample units from the same clone, hereafter called clonemates, given that their P_{sex} were lower than 0.05. However, in one population, Tomales Bay, all of the individuals that shared the same MLG probably could be explained by different sexual recombination events (P_{sex}>0.05), therefore, clonal assessment was not unambiguously identified for this population.

All aggregation indices among shared MLGs within a patch were significant, except for *M. integrifolia* found in Tomales Bay (Table 2.5), indicating that clonemates are likely to be found near one another. Minimum distances between clonemates range from 0.10m to 0.76m. The maximum distance between clonemates is more variable, from 0.10m-11.40m. Stillwater Cove had the highest number of units within a single clone, eight, as well as the lowest genotypic richness of all the intertidal populations (*R*=0.57). Even though we detected asexual reproduction in *M. integrifolia* patches genotypic richness was relatively high, ranging from 0.57 to 0.98, indicating that asexual growth may not be the primary mode of reproduction in the intertidal habitat.

Genetic Structure

Across all sampled sites and morphotypes, two main structure groups were identified with strong support (ΔK=2199.2). One group north of the Monterey Bay Peninsula, and one group south of the Monterey Bay Peninsula (Table 2.2, Figure 2.2). The break between these two groups occurs at the well-documented ocean biogeographic boundary in the Monterey Bay region (Dawson 2001; Tseng & Breaker 2007; Pelc *et al.* 2009). Hierarchical analyses within the southern cluster, admixture setting revealed the highest delta K values at K=2 and K=5 (Table 2.2, Figure 2.3). Within the northern cluster, the highest delta K values were at K=2 and K=5 (Table 2.2, Figure 2.4)). Upon analysis of individual populations at the lowest hierarchy, STRUCTURE identified complete admixture between *M. pyrifera* and *M. integrifolia* at Cambria, (Figure 2.5 c). However at Point Piños and Stillwater Cove, different co-ancestry clusters were identified as the most likely classification separating the two morphs (Figure 2.5 a, b) (Table 2.6).

Migration and Kinship

To identify if recent migration has occurred between the two morphs, we used GENECLASS2 to determine the probability of dispersal, identifying individuals who may have been 1st generation migrants to a conspecific patch at a sampling location. In each patch, there was at least one likely first generation migrant (Figure 2.6).

The spatial variation in individual genetic coancestry, within and among *Macrocystis spp.* morphs, was studied using a spatial autocorrelation of pairwise kinship. In Stillwater Cove, for the *M. integrifolia* morph, we observed significantly higher coancestry than expected at the smallest distance classes (<5m), indicating spatial genetic

structure at fine spatial scales (Figure 2.7 a). At larger distance classes (>20m), we observed individuals with lower kinship than expected. Such results indicate very localized or non-random dispersal, such as asexual growth. When all but one clone of a group are removed, there is a small, but not effective decrease in kinship levels, indicating that the sampling effects of such clones do not influence spatial genetic structure (Figure 2.7 b, c). Coancestry for *M. pyrifera* in both locations, generally indicate no spatial genetic structure present in those patches (Figure 2.7 c, f), and is consistent with fine scale spatial analyses of relatedness of *M. pyrifera* at other locations (Johansson *et al.* 2013).

At Stillwater Cove, we were able to compare the between morph kinship as well. When allelic frequencies were pooled, both within morph comparisons had higher than expected kinship across most distance classes. Our across morph comparison revealed lower kinship than expected, indicating that individuals are not as related as they should be if we assume they are in the same genetic population. However, the slope of the kinship lines do not differ at the larger distances classes when comparing among morph and between morph kinships. (Figure 2.8).

Discussion

If *M. integrifolia* is dependent upon genetic rescue from *M. pyrifera*, we would expect to see low levels of genetic differentiation at parapatric sites. At two of our three parapatric sites, *M. pyrifera* and *M. integrifolia* patches are separated by high levels of genetic differentiation, distinct coancestry groups, low numbers of migrants between

parapatric patches, and distinct spatial genetic structure at fine distance scales between parapatric patches. Three processes may provide explanations for why we observe these genetic and phenotypic distinctions between these two morphs: 1) Dispersal limitations may be associated with life in the intertidal; 2) Phenotypic plasticity present in morphology and reproductive life history is an advantageous strategy affecting generation time, effective population size and dynamics, and therefore affecting allelic frequencies changes between subtidal and intertidal; and 3) some level of localized adaptation has developed resulting in lower fitness of migrants due to dramatic variance in abiotic conditions, constituting initial steps of early ecological speciation. These processes are not mutually exclusive and the importance of each factor may vary from site to site based on local intertidal conditions and the history of each intertidal population.

Dispersal in the Intertidal

In the intertidal, we observed that *M. integrifolia* utilizes two different reproductive strategies to maintain populations within intertidal habitat, namely asexual growth and sexual reproduction. In most intertidal populations, there are only a few clonal groups which have only a couple clonemates, which are found close together. However, genotypic richness within intertidal areas is still quite high, which indicates that sexual reproduction by *M. integrifolia* occurs as well (Graham 1996; Wiencke *et al.* 2000; Navarro *et al.* 2007), and may be more prevalent within the intertidal than previously thought. Within the intertidal, the ability to reproduce sexually is likely controlled by both the available nutrients, and the physical conditions such as temperature and irradiance (Santelices 1990; Reed *et al.* 1996; Buschmann *et al.* 2004;

Demes & Graham 2011), which can vary widely within intertidal areas and their geographic location both daily and annually (Pfister *et al.* 2007; Wiencke & Amsler 2012; Mota *et al.* 2014). The occurrence of such favorable conditions, even if rarely, would reduce the cost of energy investment for sporophyll production in the intertidal. Irradiance would still impose mortality, so spores that survive may only be the ones that settle in shaded areas, which are presumably very close to the parent sporophyte and other nearby neighbors, protecting early developmental stages from harmful irradiance. However, this hypothesis does not explain why recent work by Jeffries (2015) found that in cleared intertidal areas within an *M. integrifolia* bed, there was no recolonization by sexual recruits. It may be that the temporal scope of such an experimental study may not capture yearly and decadal variability that might allow for rare colonization events of cleared intertidal areas. Perhaps newly available intertidal habitat is colonized by early successional organisms and *M. integrifolia* may only recruit successfully in the later stages of intertidal colonization, where shade is not only provided by the parent sporophyte, but a variety of other algae as well. Settlement closer to the parent plant would affects the genetic structure of intertidal patches as well. The closer microscopic spores fall and develops near a parent plant, the more likely it is to be selfed (Raimondi *et al.* 2004; Johansson *et al.* 2013). Due to the fact that *M. integrifolia* population sizes are small, such instances of selfing and asexual growth would drive kinship higher than expected at closer distances.

In Stillwater Cove, *M. integrifolia* had much higher kinship at short distance classes than expected. Such results indicate very localized or non-random dispersal. However, asexual growth is a form of dispersal which inflates kinship levels within the

clonal subrange (Alberto et al. 2005). When the analysis was repeated with a single copy of each clone, the decrease in kinship spatial autocorrelation patterns was still outside of the expected range under random distribution of spores within the spatial scale analyzed, indicating that the sampling effects of such clones do not influence much the fine scale spatial genetic structure. However, asexual reproduction is expected to impact the kinship levels indirectly as well. Large clones should have disproportionately higher reproductive success, because they produce larger number of spores. Thus the dominance of a few genotypes within the intertidal at Stillwater Cove should contribute to higher levels of kinship through indirect selfing and bi-parental inbreeding.

When coancestry was compared between morphs at Stillwater Cove, we observed higher kinship within morphs and lower kinship between morphs than expected if all morphs were a part of the same genetic population. These results reveal that in Stillwater Cove, morphologies are not related, and an individual is more related to its own morph than it is to the conspecific morph, regardless of the distance between them. Furthermore, migration in to the intertidal is inhibited due mortality from exposure to high irradiance (Graham 1996; Wiencke *et al.* 2000; Navarro *et al.* 2007). Only migrants that settle in well shaded areas may be able to survive to reproductive maturity in the intertidal. Such instances of successful migration may be very infrequent, as suggested by the absence of new recruits observed in intertidal habitat during extensive reproductive surveys (Jeffries, 2015), and the detection of few first generation migrants in genetic analyses between either morph at Point Piños and Stillwater Cove. All the assigned migrants were single copy MLGs in our sample, suggesting that these individuals are probably not the largest or oldest in the population. Such rare migration between *M. pyrifera* and *M. integrifolia*

patches may not provide enough gene flow to overcome the effects of higher reproductive success of larger clones, and other genetic contributions from within the *M. integrifolia* gene pool. Such reduction in migration could create the two distinct populations we observe in genetic cluster assignments, and large levels of pairwise genetic differentiation (Spieth 1974; Slatkin 1985, 1987; Mills & Allendorf 1996).

Another factor expected to limit dispersal in the intertidal in relation to the subtidal is reduced immersion time. Evidence for this effect has been found within other similarly distributed seaweeds (Engel *et al.* 2004; Pearson & Serrão 2006; Krueger-Hadfield *et al.* 2013a), across kelp taxa (Billard *et al.* 2010; Coyer *et al.* 2011) and for comparisons across other nearshore invertebrate species distributed over different intertidal zones (Kelly & Palumbi 2010). However, at Point Piños, we do not observe a significant pattern of spatial genetic structure within the *M. integrifolia* patch. This could be related to the fact that genotypic richness in this patch was very high, with only a couple clonal groups present reducing the effects of biparental inbreeding due to disproportionally higher reproductive success by larger clones. If this *M. integrifoli*a patch is younger than the one at Stillwater Cove, it may not have had enough time to establish a stationary phase representative of the drift– dispersal equilibrium and build up the spatial genetic structure (Rousset 1997; Vekemans & Hardy 2004). Despite these high levels of genotypic richness, the overall diversity within intertidal patches was lower than corresponding *M. pyrifera* patches. Low diversity within the intertidal could be due to founder effects by a few migrants from *M. pyrifera* patches. In this scenario, initial colonization in the intertidal would have been by *M. pyrifera* migrants that settled in shaded areas. Plasticity in holdfast morphology (Demes *et al.* 2009a) and reproductive

method based on environmental conditions (Buschmann *et al.* 2004) would then allow persistence in the intertidal Over time, genetic clusters we observe along *M .integrifolia's* range could in part be due to genetic drift or the long term effects asexual growth has on population structure as clones develop and grow within the intertidal.

Asexual growth prolongs the lifespan of genotypes in those populations. A given genotypes thus remains in an intertidal populations much longer than a genotype would in subtidal populations. Estimates based on holdfast elongation rate (Jeffries, 2015) and the maximum distance observed between clonemates in Stillwater Cove reveal some clonal lineages have been present in the intertidal for at least 400-500 years, assuming that a holdfast can't re-anchor if detached. *M. pyrifera* sporophytes are not known to survive that long in the subtidal, as patch existence and density fluctuates on decadal scales (Rosenthal *et al.* 1974; Dayton *et al.* 1984; Ladah *et al.* 1999; Cavanaugh *et al.* 2013). Thus, subtidal populations experience frequent gene flow from surrounding areas, and recombine alleles much more frequently than intertidal populations, driving higher genetic turnover and diversity intertidal populations (**S2**). Such a dramatic disparity in genetic longevity creates a large difference in generation time between parapatric subtidal and intertidal patches. These differences in generation times could drive the high levels of genetic differentiation observed between morphs around the Monterey Peninsula, as well as the low levels of differentiation between *M. pyrifera* morphs around the peninsula, even though they are much farther apart from each other. However, these genetic differences disappear between morphs at Cambria, even though asexual reproduction occurs in the intertidal there as well. At Cambria the intertidal patch and the subtidal patch are separated by about 500 meters, and the intertidal patch is along open coast line,

with few rocks acting as a wave break. Even though they are separated by a large distance, the current and tidal patterns my drive faster transport and gene flow between patches. Alternatively, given the higher hydrodynamic exposure at Cambria's intertidal it is possible that the *M. integrifolia* population goes extinct more frequently than in other two more protect sites. Faster dynamics in the extinction and subsequent recolonization from the subtidal *M. pyrifera* would constrain genetic differentiation to develop.

At Point Piños the different morphs were also separated by about 500 meters. However, at this location the California current meets the Monterey Peninsula producing very high wave energy and fast moving currents in the subtidal *M. pyrifera* area (Paduan & Rosenfeld 1996). The *M. integrifolia* patch, is located in a protected bay shaped like a bowl and sheltered from wave action. The opening to the bay is small, and does not face north, the direction the California current travels through. It is possible that this bay is may receive relatively less gene flow from *M. pyrifera* compared to the more open system at Cambria. Thus, the local intertidal spore from sexually reproducing *M. integrifolia* source may be greater than incoming *M. pyrifera* migrants. Additionally, conditions inside the intertidal are comparatively more stable and have less wave action, so there may not be available habitat for new migrants to recruit to as frequently compared Cambria. Within Stillwater Cove, kelp are even more sheltered from the open ocean the open ocean and high wave energy. Here, morphs occur right next to each other, and our sampled patches were about 50 meters apart and no physical barriers exist, other than a depth change. At this scale, there should not be any barrier to gene flow given spore dispersal distances in *Macrocystis* (Reed *et al.* 2004; Gaylord *et al.* 2006; Alberto

et al. 2010). The observation of such stark genetic differences at these small spatial scales indicate that other, non-mutually exclusive processes might be at work.

Phenotypic Plasticity

Plasticity is utilized by marine organisms with broad dispersal to maximize survival in the unpredictable and highly competitive environments propagules may settle on (Norton 1992; Vadas *et al.* 1992; Sultan 2000; Selkoe & Toonen 2011; Sotka 2012; Muth 2012). The ability for *Macrocystis* to exhibit plasticity in both its morphology and reproductive strategy in response to changing environmental conditions has been well documented (Womersley 1954; Neushul 1971; Lobban 1978; Buschmann *et al.* 2004, 2013; Graham 2007; Demes *et al.* 2009a; Schiel & Foster 2015), and is likely utilized to maximize efficiency between energy costs associated with sexual reproduction and energy needed for growth and maintenance (Eckert 2002; Honnay & Bossuyt 2005; Vallejo-Marín *et al.* 2010; Oliva *et al.* 2014) Such plasticity in reproductive method can impact the population genetics.

The ability to grow clonally affects the genetic structure of populations utilizing it in a variety of ways. Depending on the size, a single genotype could constitute a large proportion of the genetic make-up of a population, and contribute a disproportionate amount of genetic material when sexual reproduction does occur (Prati & Schmid 2000; Vallejo-Marín *et al.* 2010). In small, isolated intertidal populations, clones reduce the effective population size and may disproportionately affect allele frequencies within patches. Additionally, increased longevity of clonal groups results in overlapping generations and an increase in the potential for selfing. When sexual reproduction does

occur, higher biparental inbreeding is expected, which can explain why Stillwater Cove, which has the most clonal groups, had higher kinship at small spatial scales. New migrants, or rare genotypes would be easily eliminated in small intertidal populations by genetic drift, which can explain reduced allelic diversity in intertidal populations, especially in northern populations that do not have corresponding offshore *M. pyrifera* patches.

In these northern populations, the influx of new genetic material from southern *M. pyrifera* populations is probably very low due to the largely asymmetrical currents flowing from north to south along the northeast pacific for most of the year (Hedgecock 1994; Wares *et al.* 2001; Schoch *et al.* 2006a; Graham 2007; Saunders 2014), resulting in infrequent migrants traveling northward. These migrants are likely from rafting sporophytes traveling northward. Rafting sporophytes are able to reproductive even when dislodged, releasing viable spores as they drift with the currents (Macaya *et al.* 2005; Hernández-Carmona *et al.* 2006). These spores are presumably able to recruit in these isolated northern intertidal habitats due to their plastic phenotypes. Plasticity in phenotype to accommodate for a wide variety of environmental conditions does not appear to be limitless, however, as these intertidal populations are found in sheltered coves and small bays, where they are sheltered from high energy waves traveling down the coast. Once established, the ability to grow asexually would prolong the time founding genetic variation would remain present in northern populations. During sexual reproductive events, genetic drift would further remove allelic diversity in the absence of migration. These factors also result in lower allelic richness over time, and would explain why in two populations, Tomales Bay and Bodega Bay, we are unable to conclusively

resolve our ability to decipher between sexual and asexual origins of repeated MLGs in these populations. Local environmental forces may thus be playing a role in determining genetic differentiation and diversity not only between *M. pyrifera* and *M. integrifolia*, but within the intertidal as well.

In the greater Bodega Bay area, local oceanographic conditions may be playing a role in how much gene flow occurs into, out of, and around the bay (Morgan & Fisher 2010). Tomales Bay experiences very swift currents during tide changes, as well as dramatic changes in salinity (Hearn & Largier 1997). The population of *M. integrifolia* that lives within the bay is located on a sheltered side of an island near the middle of the bay, potentially isolating the patch from incoming migrants. Additionally, the dramatic fluctuations in salinity within Tomales Bay may induce high mortality in incoming spores and early microscopic stages, further limiting gene flow into this population. The combination of these factors result in a case of severe isolation of Tomales Bay which would lead to a drastic decrease in genetic diversity we observe. Additionally, these extreme conditions experienced within Tomales Bay indicate the importance of plasticity not only for survival of recruits in harsh conditions, but in the role it may play facilitating adaptation to intertidal conditions.

Localized adaptation

We may be observing genetic differences between different morphs at close spatial scales due to gene flow restriction for a variety of reasons, such as pre-zygotic isolation, a reduced number of migrants entering a population (Dobzhansky 1937, Mayr 1963), or immigrant and hybrid inviability (Nosil *et al.* 2005; Abbott *et al.* 2013).

Numerous studies demonstrate complete compatibility between not only *M. pyrifera* and *M. integrifolia* gametophytes (Lewis & Neushul 1994; Westermeier *et al.* 2006), but the ability of *Macrocystis spp.* to hybridize with numerous other members of the Laminariaceae family as well (Druehl *et al.* 2005), indicating few, if any pre-zygotic barriers exist between morphs. However, these experiments were run in a laboratory setting, under controlled conditions, which may have excluded any factors that may play a role in detecting ecological speciation. Additionally, when these laboratory reared crosses were transplanted into the field, there was differential success between the different hybrids, showing that even though hybridization is possible, hybrid offspring may be at a disadvantage (Druehl 1978; Lewis *et al.* 1986; Lewis & Neushul 1994). Moreover, other lab experiments have revealed differential survival under irradiance stress between offspring from sporophytes related to the parent depth (Wiencke *et al.* 2000). Such observations imply that there may be some degree of post-zygotic isolation. If such mechanisms are in play localized adaptation could lead to reduction of phenotypic plasticity and trait assimilation. This evolutionary reduction in the degree of phenotypic plasticity is expected from selection against alternative high-cost metabolic pathways, leading once environmentally-conditioned traits to become differently expressed in divergent environments (Nosil *et al.* 2005; Hendry *et al.* 2007; Schluter 2009; Thibert-Plante & Hendry 2011; Fitzpatrick 2012; Arendt 2015).

The differences in the degree of differentiation and admixture between morphs we observe at our three parapatric sites reveal we may be observing a continuum of divergence between morphs (Hendry 2009). The variation we see in divergence across their range could, in part, be due to differences in the amount of gene flow that occurs at

each location. The differences in migration divergence we observe could make it difficult to decipher between ecological divergence and genetic drift due to the use of neutral genetic markers (Thibert-Plante & Hendry 2010). Additionally, recent research has shown that ecological divergence in the presence of gene flow along environmental gradients may be driven by adaptations seen at a key functional regions across a genome, (Fitzpatrick *et al.* 2008; De Wit *et al.* 2012; Pespeni & Palumbi 2013; Yeaman 2013; Arnegard *et al.* 2014), which would not necessarily be detected by neutral markers.

Variation in genes related to survival in the intertidal versus the subtidal could be seen in a variety of phenotypic traits such as; DNA repair after irradiance damage, the ability to effectively respond to dramatic changes in salinity, temperature, and nutrient levels, the ability to efficiently change holdfast morphology, as well as the ability to effectively balance energy allocation for holdfast elongation, physiological regulation for stress response, organismal maintenance and repair, and reproduction. Localized adaptation in these traits related to survival in the intertidal would result in lower fitness of incoming migrants, and could lead to local adaptation and ecological divergence seen in other algae species (Billard *et al.* 2010; Cánovas *et al.* 2011; Zardi *et al.* 2011; Coyer *et al.* 2011; Kostamo *et al.* 2011). The persistence of successful genotypes via clonal growth could potentially be a mechanisms for promoting adaptation, by maintaining successful, well adapted genotypes within a population for many years (Tatarenkov *et al.* 2005). Clone groups with higher fitness and longevity would be able to grow large, resulting disproportionately higher reproductive success across many generations, which could speed up the adaptive process in the intertidal.

Additionally, asexual reproduction removes the risk of outcrossing with less fit genotypes. During sexual reproduction in the intertidal, the risk of such outcrossing is reduced if incoming migrants are at a disadvantage before fertilization occurs, such as death due to irradiance exposure (Graham 1996, 1997). If outcrossing does occur, these hybrid offspring may have intermediate phenotypes that are ill-adapted to intertidal conditions. These hybrids may die during the early stages of development, grow inefficiently, produce low quality sporophylls, or potentially no sporophylls at all. Such hybrid inviability would further decrease gene flow between morphs, preserve adaptive differences, and strengthen isolation and divergence between *M. integrifolia* and *M. pyrifera* where they co-occur. Over time genetic differences building up at key traits, such as reproductive success, may lead to assortative mating in the intertidal between the morphs, promoting divergence, and potentially speciation. This process may never actually occur due to constraints on population size within intertidal locations, but this system could still constitute a potential case study to focus on understanding the early stages of such ecological speciation.

The factors discussed here, dispersal limitations, phenotypic plasticity in reproductive system, and localized adaptation, may all be interacting together to drive the genetic differentiation patterns we observe between the intertidal and subtidal *Macrocystis spp.* morphs. Site specific differences indicate that there could be variation in how quickly any adaptive process may occur, display a remarkable plasticity for environmental change, and highlight the actual limitations to dispersal intertidal and how organisms accommodate for such limitations. The relative importance of each factor at

each site may vary as well, depending on the population size and history. Further research exploring these factors will focus on simulation studies and laboratory experiments.

Simulation studies could explore different demographic characteristics as the drivers of genetic differences between morphs, a one way migration model may be developed, where infrequent migrants (less than one per generation) from a source enter a sink. The source population would have a higher effective population size, and gene flow from a large source population, minimizing the potential for genetic drift to affect the genetic structure. The sink population would have a comparatively lower mutation rate, smaller effective population size, and only experience gene flow in the form of infrequent migrants from the source population. Additionally, such a model would incorporate the propensity for asexual growth in the intertidal. Experimental crosses between the two morphs under environmental conditions mimicking intertidal stress, can be used to test the hypothesis that *M. integrifolia* is adapted to the intertidal. My third and final chapter focuses on testing this hypothesis.

Chapter 3: Testing for early assortative mating between *Macrocystis pyrifera* and *Macrocystis integrifolia*

Introduction

Adaptive plasticity is the ability of genotypes to produce alternate phenotypes that enhance survival and reproduction in new environments (West-Eberhard 2005; López-Maury *et al.* 2008; Pfennig *et al.* 2010; Fitzpatrick 2012). Adaptive plasticity can lead to diversification (Pfennig & Murphy 2002b; DeWitt & Scheiner 2004; West-Eberhard 2005; Niemiller *et al.* 2008; Martin & Pfennig 2010a; Pfennig *et al.* 2010; Fitzpatrick 2012) and speciation under certain circumstances (Waddington 1942; Diekmann *et al.* 2005; Pfennig *et al.* 2010; Thibert-Plante & Hendry 2011; Moczek *et al.* 2011). The underlying cause of plasticity is genetic, and mediated by environmentally-induced differential gene expression (Gasch *et al.* 2000; West-Eberhard 2005; Scoville & Pfrender 2010; Thibert-Plante & Hendry 2011; Moczek *et al.* 2011; Leichty *et al.* 2012; Schlichting & Wund 2014). Phenotypes that accommodate environmental change along steep selective gradients can be maintained due to high selection against disadvantageous phenotypes despite high levels of migration and gene flow (Meyer 1987; Case & Taper 2000; Zardi *et al.* 2011; Chevin & Lande 2011; Gould *et al.* 2014). The maintenance of advantageous phenotypes allows distinct morphs and genetic taxa to persist in areas where these steep gradients occur (Keddy 1981, 1982; Watkinson 1985; Case & Taper 2000; Doebeli & Dieckmann 2003; DeWitt & Scheiner 2004; Kawecki & Ebert 2004;

Schoch *et al.* 2006b; Billard *et al.* 2010; Zardi *et al.* 2011; DeFaveri *et al.* 2013). Perhaps one of the most well-known examples of environmental gradients are rocky intertidal habitats. These habitats are characterized by extreme changes in immersion time, desiccation, temperature, irradiance exposure and salinity, creating stressful and selection driven environments. One of the key factors controlling the upper and lower growth limits and survival of organisms in the intertidal is irradiance.

In aquatic and marine environments, light attenuation down the water column is controlled by the optical properties of water, which scatters and absorbs light. Photosynthetically active radiation (PAR) and ultra-violet A (UVA) waves penetrate farther down the water column than ultra-violet B (UVB) (Booth & Morow 1997; Day & Neale 2002). Varied light environments are created down the tidal gradient by differences in particulate concentrations, time of day, time of year, and latitude (Hader *et al.* 1995, Diaz *et al 2000*), and create a structured and defined habitat in which organisms can adapt to. Both upper and lower levels of irradiance characterize each habitat within the tidal gradient, and survival is dictated by an organism's tolerance to both irradiance limits. In temperate waters, the intertidal is characterized by high species diversity down its light gradient due to this highly structured light environment (Goldberg & Kendrick 2004; Short *et al.* 2007; Smale *et al.* 2011).

Reactions to irradiance stress differs among species and is determined by both genetically fixed adaptation and by physiological acclimation (Bischof *et al.* 2006). Sessile organisms such, as kelps, have the ability to adapt to variation in light levels on a temporal scale (daily and seasonal) and on a spatial scale (landing of dispersal stage) (Fairhead & Cheshire 2004). Ecotype specific differences in photosynthetic capacity and

photosynthetic efficiency have been observed between populations of the kelp *Laminaria saccharina* that experienced different light environments (Gerard 1988). Zoospores from deep-water species of kelps and algae experience higher photo-inhibition and lower recovery rates after UV exposure compared shallow-water species (Bischof *et al.* 2000; Wiencke *et al.* 2000; Roleda *et al.* 2004, 2005). Kelp meiospores from adult *Macrocystis integrifolia* and *Pterygophora californica* in British Columbia that experienced high UV stress have been observed to have higher germination and survival rates than kelp meiospores from adults that experienced low UV stress, indicating potential genetic adaptations in response to environmental stress (Swanson & Druehl 2000). The kelp *Macrocystis spp.* is a temperate water kelp species that experiences drastically different light environments depending on the time of day, time of year, and where its dispersal stage lands along the irradiance gradient along tidal coastlines.

Extreme morphological plasticity associated with depth separate two giant kelp morphs, the subtidal *M. pyrifera* and the intertidal *M. integrifolia*, along the Central California coast where these two phenotypic forms co-occur (Coyer *et al.* 2001; Demes *et al.* 2009a; Macaya & Zuccarello 2010b). The *M. pyrifera* morph is found in the subtidal, has a distinct conical (mounding) holdfast and reproduces sexually through an alteration of generations. The *M. integrifolia* morph is mostly found in the intertidal, has a spreading rhizoidal holdfast, and reduced or absent sexual structures. Previously, it was thought to reproduce via asexual growth and seeding from subtidal populations. Current research shows evidence that clonal growth contributes a smaller than anticipated portion of reproduction in the intertidal (see Chapter 2). The high intensities of PAR found in the intertidal can severely limit the development of spores and gametophytes of *M. pyrifera*

and *M. integrifolia* (Graham 1996). UV radiation significantly effects gametogenesis and early nuclear division and translocation of growing *M. pyrifera* gametophytes (Huovinen *et al.* 2000). Growth rates of embryonic sporophytes of *M. pyrifera* have been found to be significantly affected by exposure to UVB irradiance (Navarro *et al.* 2007). Few studies have looked at the effects of high PAR and UVB on the *M. integrifolia* morph's early developmental stages (Graham 1996), although it has been identified that the parent environment plays an important role in determining if their spores will survive in irradiance stressed environment (Swanson & Druehl 2000). In a recent clearing experiment set to study the mechanisms utilized by *M. integrifolia* to recolonize space, no sexual recruits established successfully in cleared areas after one year (Jeffries 2015) and all growth was through rhizome extension, However, this study didn't follow recruitment under the canopy of existing kelp (which is a notoriously challenging task); where attenuated light may allow for the initial stages of sexual recruitment to develop.

Previous crossing experiments with *Macrocystis* spp. have identified that isolated gametophytes of both morphs hybridize freely and form viable sporophytes in laboratory conditions (Lewis *et al.* 1986; Lewis & Neushul 1994; Westermeier *et al.* 2006). Even though crosses produce viable offspring in controlled laboratory conditions, the addition of environmental factors or transplantation into natural conditions yields differential survival between crosses, probably due to the difference in environment between parent sporophytes and where the subsequent offspring were grown (Lewis *et al.* 1986; Westermeier *et al.* 2006, 2013). When cultured separately and exposed to high PAR stress, both morphs experience high mortality and impeded growth, although there was an observable difference between the depth limits of natural recruitment and experimental

recruitment survival (Graham 1996). Recent genetic work and transplant studies have shown that although *M. pyrifera* and *M. integrifolia* belong to the same species (Coyer *et al.* 2001; Demes *et al.* 2009b; Macaya & Zuccarello 2010b), there is evidence to suggest that *M. pyrifera* and *M. integrifolia* have strong genetic differentiation between morphotypes in two out of three sites analyzed (chapter 2). This sets the stage for further genetic divergence across their environmental gradient and adaptation under gene-flow limitation. What previous studies have not explicitly shown is if there is differential survival in the intertidal between microscopic developmental stages sired from crosses using within and between morphs parental contributions.

In this study we used a microsatellite marker assisted parental analysis of experimental crosses between and within giant kelp morphs. Our goal was to test if early assortative mating can be detected when progeny is exposed to non-lethal doses of UVB and high PAR, mimicking the intertidal environment. Our null hypothesis is that there will be no differential mortality between different developmental stages of *M. pyrifera* and *M. integrifolia*. Alternatively, we hypothesize that early developmental stages of *M. integrifolia* will have a higher survival rate under UV and high PAR conditions; such that surviving embryonic sporophytes will have higher than expected frequency for the within *M. integrifolia* parental contribution class. The presence of differential mortality between morphs will be identified during the stages of early development: pre-settlement spores, post-settlement spores, gametophytes, and post-fertilization embryonic sporophytes. The main questions we expect to answer are: 1) is there differential survival between different early developmental stages of *M. pyrifera* and *M. integrifolia* when exposed to UVB and High PAR, and 2) do offspring from *M. integrifolia* x *M. integrifolia* parents have a

higher survival rate than *M. integrifolia* x *M. pyrifera* or *M. pyrifera* x *M. pyrifera* parents when exposed to UVB and High PAR stress?

Methods

Field Collection and Shipment

Sporophylls blades from ten individuals of each morph, constituting the parental pool in our study, were collected using scuba diving from Stillwater Cove, California in April 2014. Individuals were collected a minimum of 10m apart to avoid the collection of the same genetic individual because of clonal growth in *M. integrifolia* (chapter 2). Sporophyll blades were shipped via air mail to Milwaukee, Wisconsin and released upon arrival, within 24 hours of collection. Sporophylls were layered in a sealed, Styrofoam container. Contributions from each parent were separated by several layers of cold, seawater infused paper towels. Upon arrival in lab, a three-centimeter cutting was removed from each parent blade and stored in silica gel to remove water and preserve tissue until DNA was extracted from these parental individuals.

Spores for culture

Immediately after arrival in the lab, spores from each *M. pyrifera* (Mp) and *M. integrifolia* (Mi) parent were isolated and released separately by re-immersing sporophylls from each individual in 500 mL of seawater (Instant Ocean) at 15°C for ~10 minutes. The concentration of spores from each individual were estimated using a hemocytometer (Reed *et al.* 1991; Swanson & Druehl 2000; Véliz *et al.* 2006), under

125x magnification. Flow cytometry was used to confirm spore concentration, as well as estimate average spore size for each individual as well.

Once spore density had been estimated for each individual of each morph, a 2L spore suspension with a concentration of 1,000 spores per mL^{-1} (Ray Lewis, pers comm) with equal concentrations of each parent, were combined in enriched Provasoli medium (PES) (Provasoli 1968, Mike Graham, pers. comm.). Next, 30 mL of this mixed spore solution was added to each of 64, 60mm wide x 20mm deep well petri dishes, and cultured in a plant growth chamber (Percival Scientific Inc., Perry, IA) under 40 µmol m^{-2} s^{-1} white light, at 15°C (Reed 1990; Reed *et al.* 1991). A 16hr light: 8hr dark cycle was maintained throughout the experiment (Lewis & Neushul 1994, Lewis, pers comm.) as control conditions. PES seawater solutions was changed once every two weeks.

Experimental manipulations

The control group was not exposed to any irradiance treatments and was kept in standard culture (40 µmol m^{-2} s^{-1} white light, at 15°C) conditions for the duration of the experiment. It has been previously observed that sensitivity to UV irradiation decreases with increasing age of early developmental stages (Dring *et al.* 1996). In order to assess differential effects of irradiance during the different stages of early development, four different stages of development were used: Pre-Settlement (as soon as possible after distribution into petri dishes), Post-Settlement (50% of living spores settled), Gametophytes (male and female differentiation observed), and Post Fertilization (~100 cell sporophyte blade present). We used a factorial design where each level of development was combined with the four levels of irradiance treatment. It has been

observed that high irradiance limits recruitment in the intertidal, but PAR and UVB potentially have different effects on *Macrocystis* spp. recruitment and growth (Graham 1996). In order to differentiation between the effects of high PAR (HPAR) and UVB, these factors will be tested both separately and together, resulting in four different levels of irradiance treatment: control, UV, HPAR and UV with HPAR. Experimental groups were kept under standard culture conditions (40 µmol m^{-2} s^{-1} white light, at 15ºC) until they reach their assigned developmental stage for irradiance treatment. Each irradiance treatments will consist of one 30 min. exposure at the beginning of the assigned experimental stage (Swanson & Druehl 2000). White fluorescence bulbs were used to emit light conditions for standard culture conditions under normal PAR. A UV light (UVA 340 (Q-Lab)) (Dring *et al.* 1996; Bischof *et al.* 1998; Wiencke *et al.* 2000; Rousseaux *et al.* 2004) was used to emit light waves in the 290-340 wave lengths at an intensity of 25mWatt m^{-2} (Swanson & Druehl 2000). A 3mm Lexan ™ filter sheet was used to block light waves shorter than 400nm from entering petri dishes in the control and HPAR treatment groups, as well as from the UV experimental groups before or after their respective developmental treatment level (Graham 1996; Swanson & Druehl 2000).

The HPAR treatments were run during the last hour of the dark cycle. The control (1) and UVB (2) groups were covered with a dark box that will not allow light penetration. The HPAR (4) group were covered with a 3mm clear Lexan ™ filter sheet to prevent UVB transmission, allowing only the UVB + HPAR (4) group to be exposed to UVB. High intensity of the PAR waves used the highest available setting of PAR in the growth chamber, 200 umol m^{-2} s^{-1}. Four replicates, petri dishes, were used for each combination of developmental and irradiance treatment levels in our factorial design,

totaling 64 experimental units. Before experiments started, distances below the lights were optimized so that experimental units received the most accurate intensity for each treatment level. Intensity of light was measured using a QSL-101 light meter (Biospherical Instruments Inc., San Diego, CA) and recorded at the beginning of each treatment session every day. After irradiance treatment, cultures were kept in standard culturing conditions and allowed to grow to sporophytes. Irradiance intensities and exposure times were designed to create an environment that leads to stressful growing conditions, although sublethal to avoid 100% mortality of microscopic stages which would prevent parental analysis of progeny.

Parentage Analysis

In order to determine which, if any, morph had more successful recruitment under irradiance stress, we needed to determine the paternity of each sporophyte in the offspring collected. A total of 47 embryonic sporophytes from each trial were collected one week after the irradiance treatment on the embryonic sporophyte treatment group, for a total of approximately 3,000 individual offspring collected. Collections were conducted by observing embryonic sporophytes under dissecting microscopes, individually separated and cleaned, and placed in a 96-well genotyping plate, previously prepared with 20µl of dilution buffer and kept on ice until the end of the microscope dissection. After sample collection, the 96-well plates were vortexed and spun down before incubation at room temperature for 5 min. Genotyping was performed using the Phire Plant Direct PCR Kit (#F-130, Thermo Scientific) with a few adaptations made to the manufacture's protocol. Genotyping PCRs were carried out in two multiplex reactions

(Multiplex #1 loci: –BC-18, BC-19, BC-25 and Mpy-8; Multiplex #2 loci: – BC-4 and Mpy-11) (Alberto et al, 2009). Each reaction was performed in 20µl final volume, using 10 µl of 2x Phire Buffer lysate ($MgCl_2$ and dNTPs included), 0.5µM of each primer, 0.4µL of Phire Hot Start DNA polymerase and 0.5ul of the pre-incubated DNA lysate. PCRs were performed on a 384-well block Eppendorf Thermocyler (Pro384 Mastercycler, Eppendorf) with an initial denaturation of 5 min at 98°C and followed by 40 cycles of 98°C for 5 sec, 60°C for 5 sec and 72°C for 20 sec and a final step of extension at 72°C for 1 min. The final PCR products were mixed in the proportion 1:2 (multiplex #1 : multiplex #2) and genotyped using GeneScan 500 LIZ as size standard (Applied Biosystems) on a ABI Prism 3700xl (School of Fresh Water Sciences Genomics Facility, Milwaukee, WI). Raw allele sizes were scored using STRand v.2.4.59 (Toonen & Hughes, 2001 http://www.vgl.ucdavis.edu/informatics/strand.php). The R package 'MsatAllele_1.05' (Alberto 2009) was used to visualize and bin fragment size data into microsatellite alleles.

After genotyping and scoring was completed, any offspring with null alleles was removed from our parentage analysis, due to the fact that the full offspring genotype is needed to prevent any false parentage assignment based on an offspring genotype with missing data. After removal of these offspring, we were able to use 1,795 offspring with the full 6-locus genotype to assign parentage using the program Cervus 3.0 (Kalinowski *et al.* 2007). The number of alleles and allele frequencies of both the parents and the offspring were identified at each locus, as well as expected and observed heterozygosity. The allele frequency data of the parent group was used to simulate parentage data by assessing both the power of the microsatellite markers used and the confidence in

parentage assignments. We simulated parentage data using the parent pair analysis with unknown sexes. 10,000 offspring were simulated, our estimated proportion of parental genotypes sampled was 0.996, the proportion of the loci genotyped was set at 1.0, and the proportion of mistyped loci was set at 0.01. We also simulated inbreeding, at a rate of 0.05, and allowed testing for self-fertilization.

In this simulation, the likelihood ratio was calculated using those described in (Kalinowski *et al.* 2007). For each offspring, these likelihood analyses considers two hypotheses for each candidate parent; the candidate parent is the true parent, and the candidate parent is not the true parent. The likelihood of each hypothesis, given the observed parental genotypes, is calculated using the probability of obtaining the observed offspring genotype. The likelihood of the candidate parent being the true parent is divided by the likelihood that the candidate parent is not the true parent. This likelihood is calculated for each locus, and the overall likelihood is calculated by multiplying the ratio for each locus together. Thus, the larger the likelihood ratio, the more likely the candidate parent is the true parent. After parentage simulations were run, the parentage assignments for our observed offspring was conducted. After removal of offspring whose parentage could not be ascertained due to multiple as likely parents, or the same likelihood for outcrossing between two parents and selfing, we were able to use a total of 1,727 offspring for all further analyses.

First, we estimated the expected frequencies for each parental of the three parental classes, within morphs (two classes: Mp·Mp and Mi·Mi) and between morphs (one class: Mp·Mi). We used a goodness of fit test to evaluate the null hypothesis that these observed frequencies could have been sampled from a population with expected

frequencies of 0.25, 0.25 and 0.50, respectively. These expected frequencies result from mixing equal spore numbers from each individual and having equal number of individuals from each morph. Here, we ignored specific individual parent assignments and focused on the three types of parental class excess or deficit in relation to what is expected by chance. If *M. integrifolia* microscopic stages have an adaptive advantage in stressful conditions, we should observe an excess in frequency of offspring assigned to the Mi·Mi class, and a deficit for Mp·Mp and Mp·Mi. Evidence of such differential survival based on parent morphology would indicate that there is potential for assortative mating and selection against certain genotypes of *Macrocystis spp.* during development in stressful conditions. Such observations could explain the maintenance of different morphological and reproductive strategies in the presence of gene flow between the intertidal and subtidal environments.

Selfing and outcrossing rates for each morph were also determined within each treatment group. Selfing is sometimes used as a strategy in stressful environments as a form of reproductive assurance, reducing outcrossing with potentially ill-suited genotypes in a given environment (Kalisz *et al.* 2004; Raimondi *et al.* 2004; Cánovas *et al.* 2011; Winn *et al.* 2011; Schwander *et al.* 2014). Even though mortality due to selfing is high in *M. pyrifera* (Raimondi et al. 2004, Johansson *et al.* 2013), the benefits of reproductive assurance may outweigh the costs of selfing in the intertidal. We expect that if such a strategy were being utilized, we would observe higher selfing rates from offspring sired by *M. integrifolia* versus *M. pyrifera*.

If selfing rates are elevated in the paternity assignments, we will try to discern if selfing or other modes of asexual reproduction are occurring. Indirect selfing via sexual

recombination, from two gametophytes that arise from the same sporophyte, occurs naturally in *M. pyrifera* (Raimondi *et al.* 2004; Johansson *et al.* 2013), and is a strategy for survival in stressful environments (Billingham *et al.* 2003; Billard *et al.* 2010; Barner *et al.* 2011; Zardi *et al.* 2011). There are different ways an offspring could possibly be derived from a single parent contributor. Diploid gametophytes could arise develop normally into sporophytes through the processes of apomixis and endomitosis (Simon *et al.* 2003; Koltunow & Grossniklaus 2003; Neiman *et al.* 2014; Oppliger *et al.* 2014). Apomixis, also known as parthenogenesis, is a process where meiosis is replaced by a mitotic division, creating gametophytes that are genetically identical to their sporophyte parents. A different mechanism is endomitosis, if a haploid gamete doubles chromosomes in a mitotic event, creating offspring that are diploid and homozygous at all loci (Gall *et al.* 1996; Simon *et al.* 2003). Such modes of asexual reproductions would allow beneficial alleles to remain while quickly removing deleterious mutations that may occur in a population, and could be considered an adaptive process to preserve successful genotypes, or at least out weight costs of sexual reproduction and avoid inbreeding in stressful or edge environments (Roleda *et al.* 2004; Hörandl & Hojsgaard 2012; Barcaccia & Albertini 2013; Burke *et al.* 2015).

To identify if offspring are being derived from these alternative asexual methods, we need to determine what frequencies we would expected to find such genotypes in our offspring population given the allelic frequencies of our parent population. To test for apomixis, we need to identify all the offspring that have an identical genotype to any of the parents, and determine the probability of observing such counts by chance (i.e., different sexual recombinations). This is equivalent to the test used to determine P_{gen} in

Chapter 2. If our observed number of offspring identical to a parent genotype is higher than that probability, our most logical explanation would be those offspring were derived from automixis. To test for endomitosis, we need to calculate the probability of finding six homozygous loci in an offspring, given the parent allele frequencies:

$$\prod_{l=1}^{l=n} \sum_{A=1}^{A=n} P\,A^2$$

where l indicates the locus and PA^2 indicates the frequency of the allele in the parent population. If our observed number of offspring that are homozygous at the six loci is higher than our expected probability, endomitosis may be a valid alternative to explain the observed genotypes. Pursuing the identification of different modes of selfing and asexual reproduction may prove to be informative about microscopic spore and gametophyte behavior. It has been noticed in lab studies that female *M. pyrifera* gametophytes undergo parthenogenesis when isolated from male gametophytes (Druehl *et al.* 2005, Graham, pers. comm.), and has been observed in other macroalgae as well (Lewis *et al.* 1993; Druehl *et al.* 2005; Oppliger *et al.* 2007, 2014; Krueger-Hadfield *et al.* 2013b).

Results and Discussion

Parentage Analysis

The parent population was polymorphic at all loci and did not deviate from expected heterozygosity at any loci (Table 3.1). *M. pyrifera* parents had higher allelic richness (AR) across all loci (AR=4.167), compared to *M. integrifolia* parents (AR=2.5).

Mean spore size did not differ between *M. integrifolia* and *M. pyrifera* parents (data not shown). The offspring population deviated from expected heterozygosity at all loci, and had a higher number of alleles at most loci (after checking for scoring errors in genotyping data) (Table 3.2). Additional alleles should not be present in our offspring sample, but may have occurred due to undetected scoring errors, allele dropout (Hoffman & Amos 2005), the presence of extra parental genotypes that were not genotyped represented in the sporophyll sample, because multiple genetic individuals can be present in a single holdfast (F. Alberto, personal communication), or through mutation, since exposure to high levels of irradiance damages DNA and is a known mutagen (Karentz *et al.* 1991; Bothwell *et al.* 1993; Hader *et al.* 1995; Huovinen *et al.* 2000; Tedetti & Sempéré 2006).

Offspring used in this parent contribution assessment were those where confidence in assignment was high, and individuals with mismatched loci (i.e. offspring with new alleles) were removed. Initial parentage assignments reveal that offspring sired by two *M. pyrifera* parents experienced higher than expected frequency in almost all treatment units (Table 3.3, Figure 3.1). Likewise, offspring sired by two *M. integrifolia* parents experienced lower than expected observations. Deviations from expected observations were significant in almost all treatment units as well and controls. These initial results suggest that *M. integrifolia* do not have higher fitness under stressful irradiance conditions tested here. In general, crosses involving *M. integrifolia* parents seem to do worse even in controlled conditions and independently of the development stage when stress treatment was applied.

We ran parentage assignments allowing for the possibility of self-fertilization. Selfing rates in the control treatments were similar for both *M .integrifolia* (0.31-0.47) and *M. pyrifera* (0.32-0.52). However, these selfing rates are much higher than expected selfing rates based on our parent population (0.05). Selfing rates do not appear to differ between the two morphs, neither among developmental stages, nor among irradiance groups (Table 3.4). The total number of offspring identified by Cervus 2.0 as selfed was 461. Based on the expected selfing rate and the number of offspring in our pool, we would have expected to find approximately 87 selfed offspring. This much higher than expected number of selfed progeny, lead us to probabilistically investigate other alternative forms of asexual reproduction that may be occurring.

In testing for apomixis, which should produce multilocus genotypes (MLGs) identical to one of the individuals in the parent pool, we identified 1,496 unique MLGs out of 1,796 total individuals (parents and offspring). We only considered these clonal groups to potentially be due to apomixis if there was a parent genotype included in a group of individuals sharing the same MLG. In total, five of our 20 parents sired offspring with identical genotypes, including two *M. pyrifera* parents (P05 and P08) and three *M. integrifolia* parents (I06, I07, and I08). *M. integrifolia* parent I06 had the highest number of individuals that were identical (8), and a total of 14 offspring had a genotype that was identical to a parent MLGS. There was no clear association with any irradiance treatment or developmental stage. It is unlikely that apomixis occurred in our experiment given the high number of clonal groups that don't include any individual from the parent pool. This indicates that there may be some other contributing factor, such as spore clumping during release from the same parental individual, during the experimental

crosses. However, microscopic inspections made in the culture plates after spore settlement did not show evidence of such aggregation.

In testing for endomitosis, we found 128 offspring that were homozygous at all six loci, while the expected count was approximately 12. From here, we compared the genotype of each offspring to each parent, to determine which parents could have created the offspring's genotype through meiosis followed by a mitotic duplication, i.e. a parent that had each allele found in the homozygous progeny. Out of the 128 offspring that were homozygous, we excluded 49 offspring that did not match the latter condition, leaving 79 offspring that were homozygous at all loci, and may have been created through endomitosis. The number of homozygous progeny that was excluded is also much higher than the expected number for this type of MLG. If there are non-genotyped parents in our sample these could explain some of these excluded progeny. Allele dropout can also contribute to the excess of MLGS found in this homozygous class. We also identified the specific parent contributions to the set of completely homozygous offspring. We observed a clear discrepancy between parent morphs, with *M. integrifolia* parents contributing disproportionately more to homozygous offspring than *M. pyrifera* parents (Table 3.5). This discrepancy between morphs will be the subject of further investigation with this data set to more fully understand how successful reproduction occurred in our experiment, as it may provide a small glimpse into the 'black box' of the early life history of *Macrocystis spp.* Because of the controlled nature of our study, the estimated frequencies of endomitosis need to be considered with care. If there were limitations to fertilization in the cultures due to settling density, endomitosis might be a strategy to continue the life cycle. Such strategy, where each region of the genome will be found in

the homozygous state, will result in near maximum inbreeding depression for the individual progeny (Raimondi et al. 2004). It is likely that in nature such fertilization limitation might not occur due to unlimited number of potential parents and higher spore densities, resulting in much lower rates of endomitosis. Even if the natural rates of endomitosis are similar to what is reported here, there is no evidence that this class of individuals reach the adult stage from the data collected in many giant kelp natural populations (Alberto *et al.* 2010; Carney *et al.* 2013, Johansson *et al.* in prep). This is most likely a result of the strong inbreeding depression in the species (Raimondi *et al.* 2004; Johansson *et al.* 2013). The fact that we can observe these excesses of selfing, and potentially endomitosis derived progeny, here at the developmental stage genotyped (approximately two-week old embryonic sporophytes) suggests that inbreeding depression might only act at later developmental stages. However, this conclusion might be biased by the highly controlled setting of our experiment providing ideal developmental conditions outside of irradiance stress.

Thus far, results suggest that offspring of *M. pyrifera* parents survive better than *M. integrifolia* parents under irradiance stress. *M. pyrifera* parents are likely able to produce higher quality offspring due to the fact that they are not under stress, whereas *M. integrifolia* parents are likely under physiological stress due to living in the intertidal environment causing the production of lower quality spores, which may be why we observe smaller spore sizes in *M. integrifolia*. Adult sporophytes used in this study may have had spores that had already been damaged in the field, carrying over maternal effects into our lab experiment and affecting propagule survival (Mousseau & Fox 1998). The poor condition of spores would explain why we observed a deficit in both Mi Mi and

Mi Mp parental contributions in our offspring. Another explanation for our results could be that our irradiance stress was not stressful enough. Macroalgae are able to undergo repair after DNA damage (Roleda *et al.* 2004), and in our experimental design developing offspring had much time for repair after the one time irradiance exposure. Repeated exposure across multiple developmental stages, or repeated exposures within a developmental stage, would increase damage to DNA and tissue, and thus divert energy to repair and survival instead of growth and development. If *M. integrifolia* were able to repair DNA and tissue more efficiently than *M. pyrifera* we might not see these differential effects unless stress were more sustained, or if the experiment was allowed to continue for a longer period of time. Extending the experiment over a longer period of time would allow us to see how irradiance stress affects not only microscopic stage survival, but development of young sporophytes. Our experiment also does not include any other stressors that are present in the intertidal, such as temperature, desiccation, salinity, etc. The combination of these factors may drive assortative mating in the field, and the absence of these additional sources of stress in our experiment may explain why we are unable to observe such assortative mating. The inclusion of data on of selfing and alternative asexual reproduction occurring, if any, would further our understanding of how plasticity in reproductive strategy assists spores and gametophytes in colonization and survival under stressful conditions.

Figure 1.1. Comparing different pairwise genetic differentiation metrics, D_{est} and F_{st}, association with different predictors between *Macrocystis pyrifera* (closed dots, thick regression line) and *Pterygophora californica* (open dots, thin regression lines). Predictors are of genetic differentiation, from top to bottom, are: spring oceanographic distance (days), habitat continuity (surface of kelp per km along shore), and geographic distance (km).

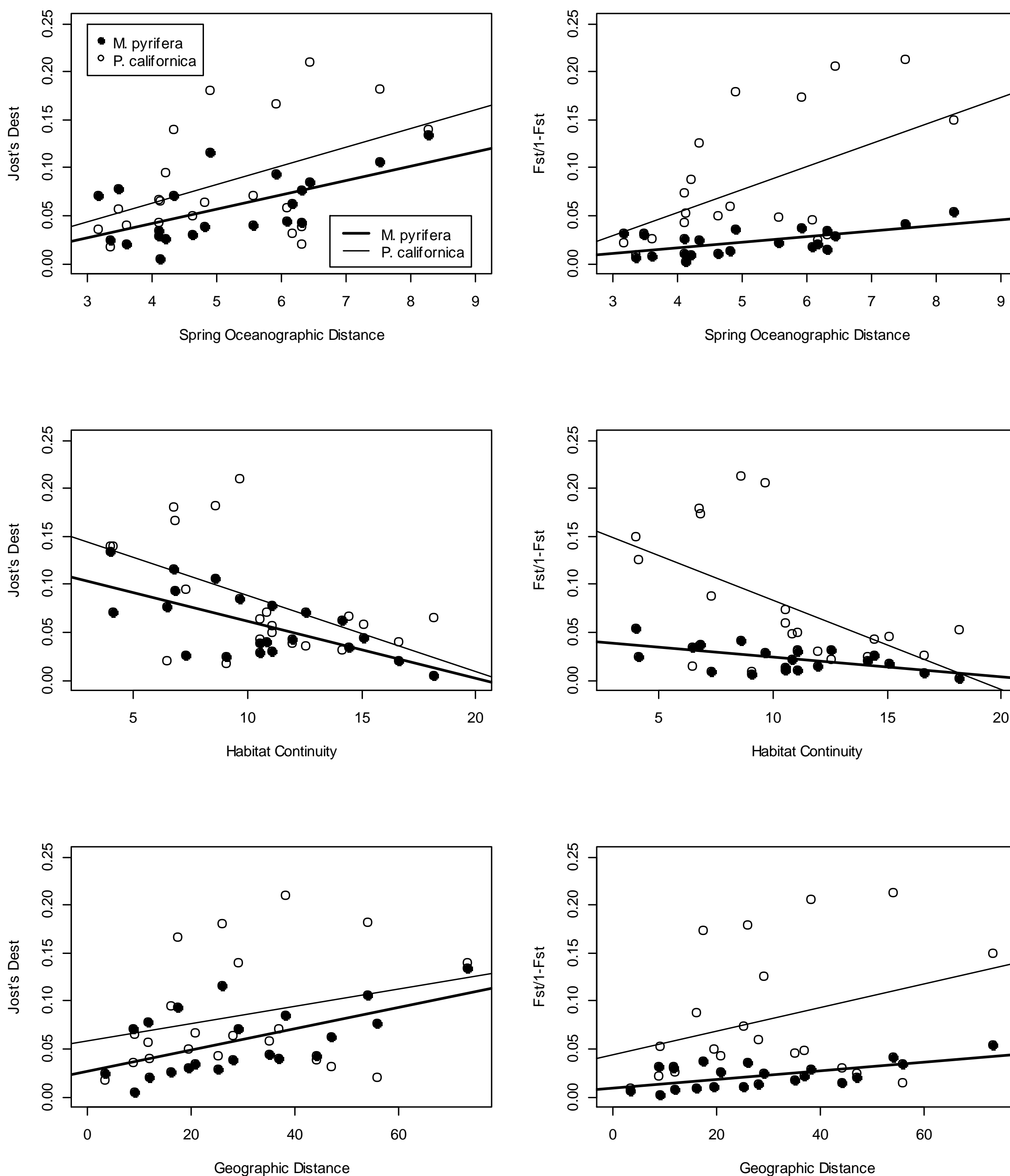

Figure 1.2. The optimized multiple regression model of pairwise genetic differentiation for *Pterygophora californica* populations in the Santa Barbara Channel, Southern California included oceanographic distance, habitat continuity, and geographic distance (p=0.0011, R2= 0.757).

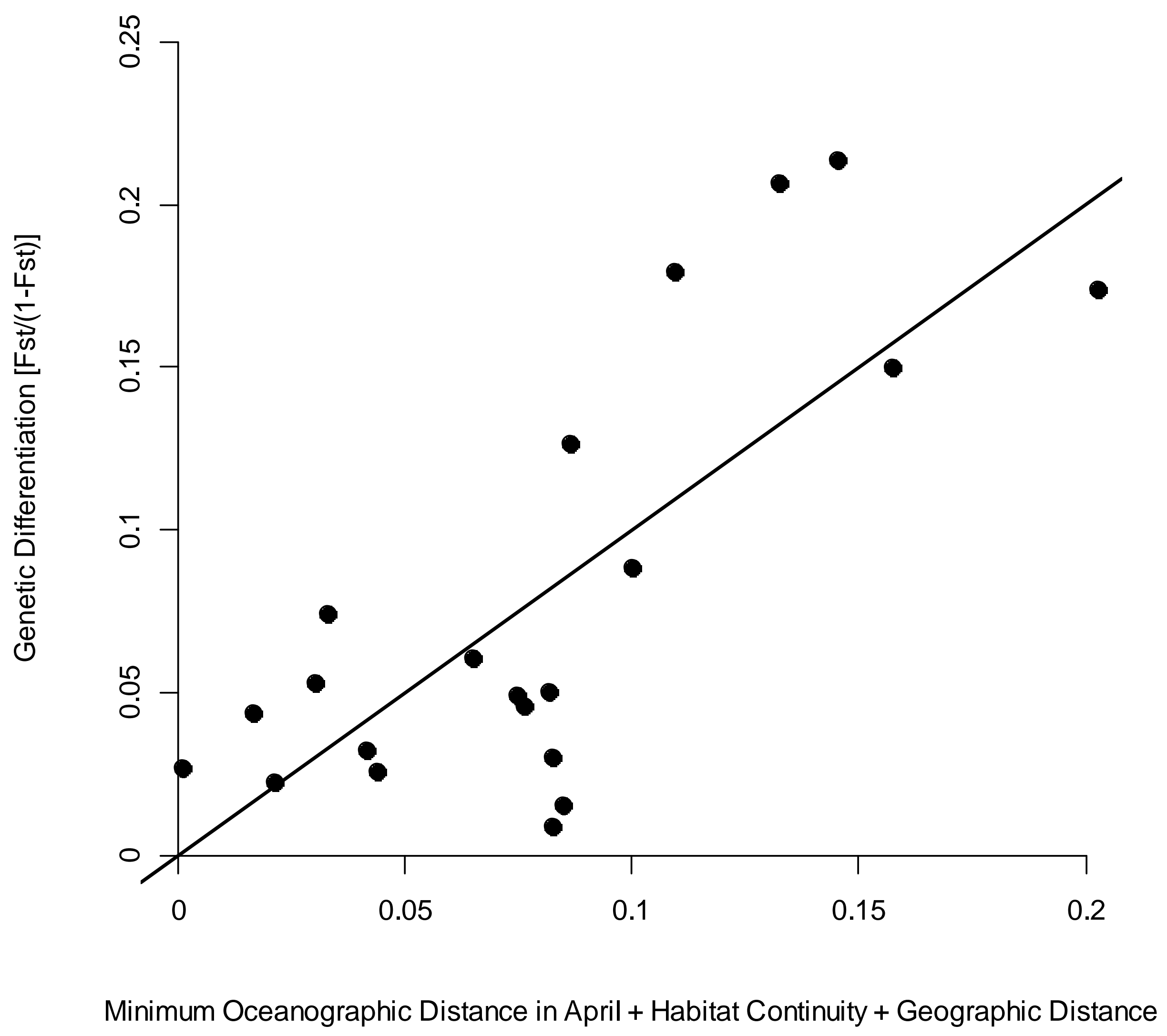

Figure 1.3. Temporal change during one year for oceanographic transport, *Pterygophora californica* reproductive effort, and *P. californica* pairwise populations' genetic differentiation in the Santa Barbara Channel. The range of oceanographic transport velocities between the sampled populations is represented by the light grey area. *P. californica* reproductive effort, measured as sorus area (from Reed et al 1996) is represented by the solid black line. The broken line in grey shows the change in goodness of fit (AIC), for multiple regression models explaining genetic differentiation, when minimum transport time for each month was used together with habitat continuity and geographic distance. Model fit for months outside the reproductive period is not shown.

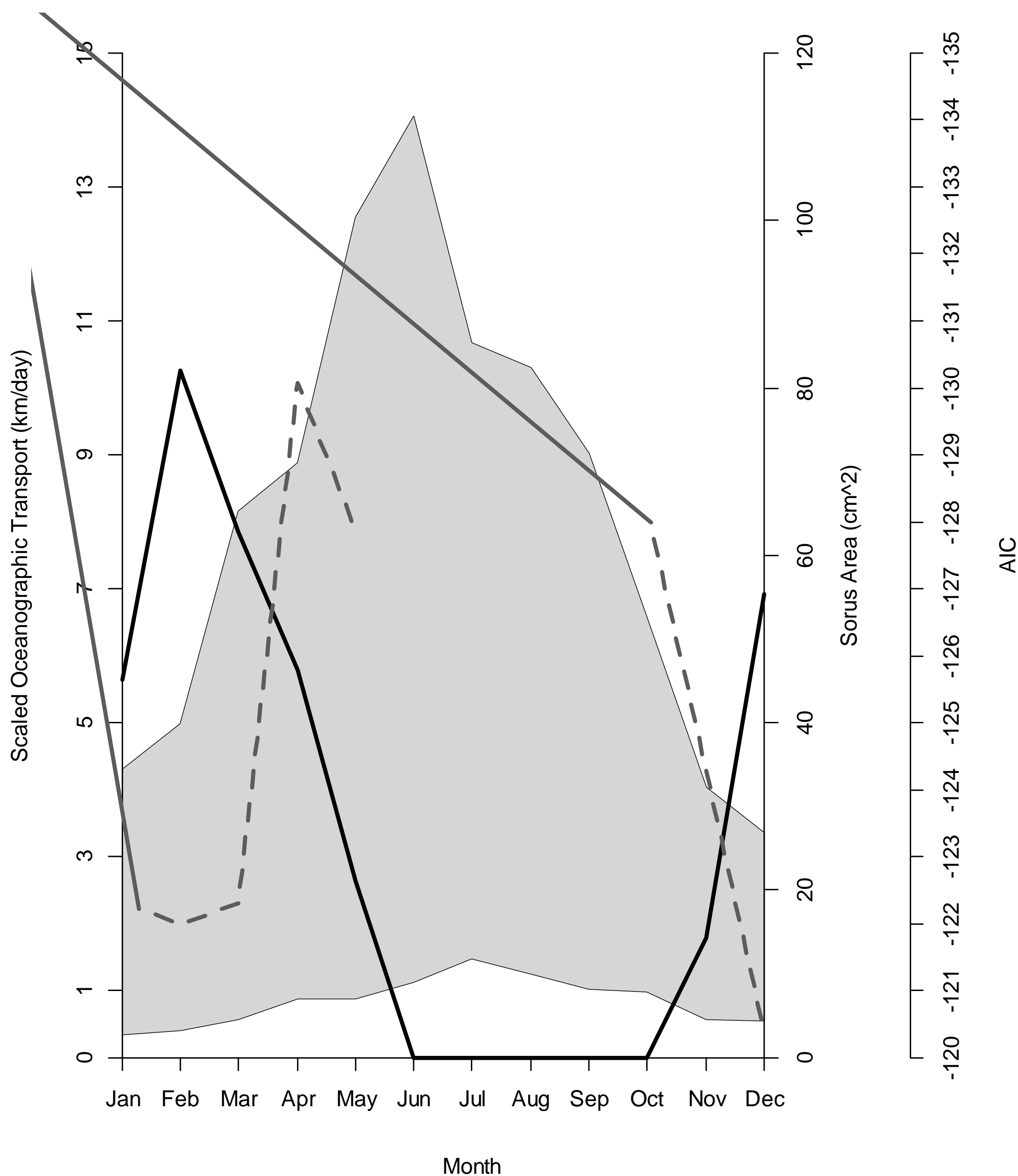

Figure 2.1. Sample locations along the coast of California. At three sites both *M. pyrifera* and *M. integrifolia* morphologies were found. At the remaining sites, only the *M. integrifolia* morph was found. We sampled at seven sites, and collected samples from 10 different *Macrocystis spp.* patches.

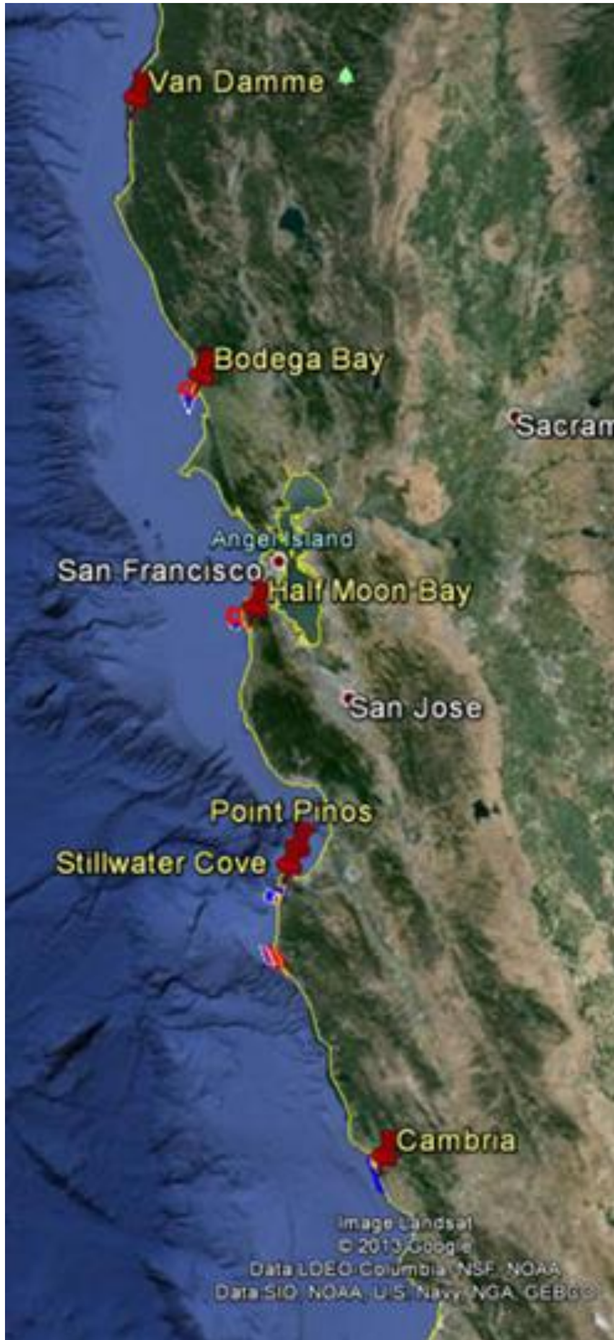

Figure 2.2. Structure output using the admixture ancestry model for all individuals in all populations. Delta K identified the best model of K=2 Populations in black are north of the Monterey Bay Peninsula, and the *M. integrifolia* population from Point Piños (Northern tip of the Monterey Bay Peninsula). Populations in gray are found on and south of the Monterey Bay Peninsula.

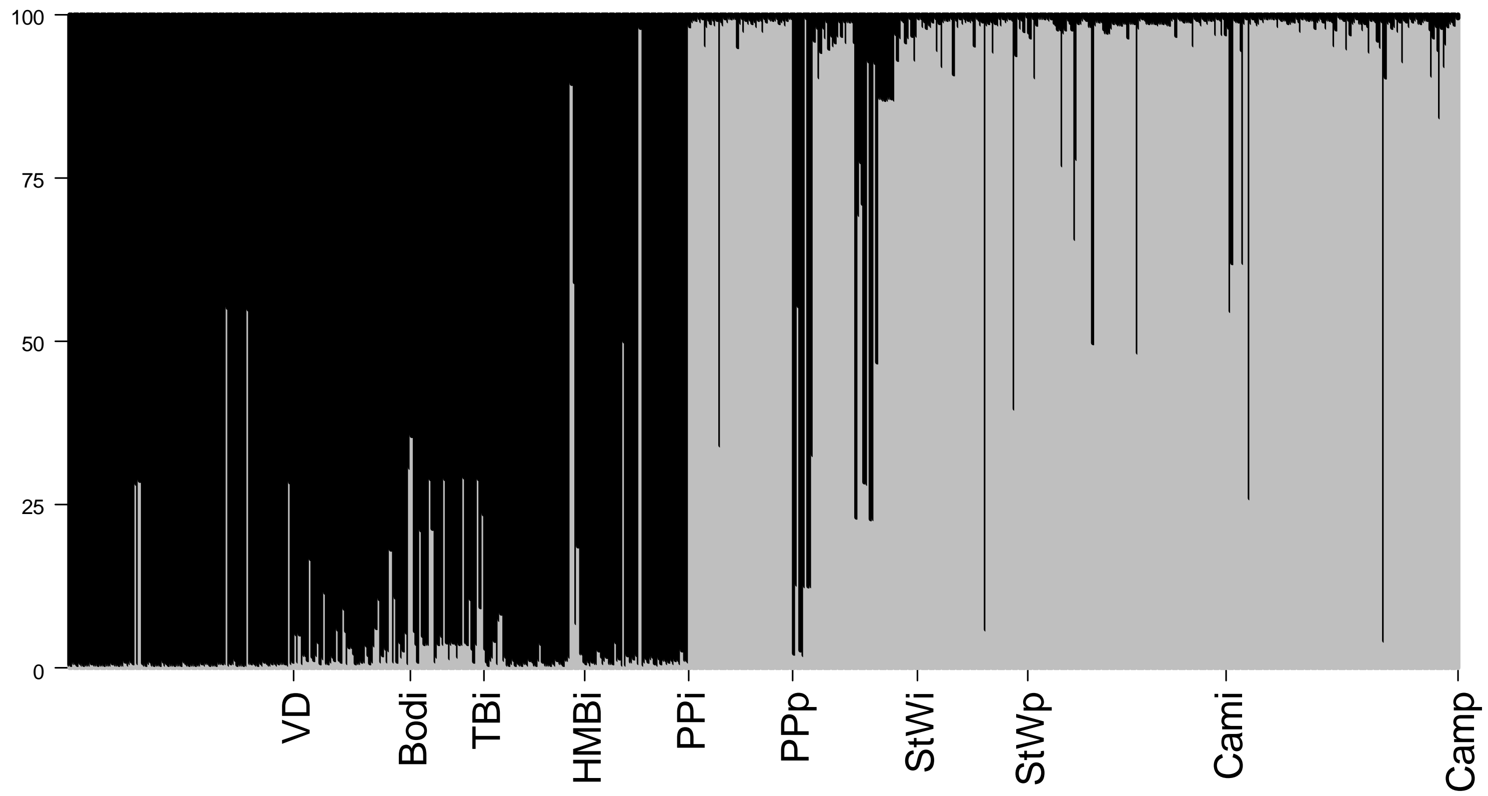

Figure 2.3. Structure results for the hierarchical analysis using the admixture ancestry model within the southern ancestral cluster. Support was similar for K=2 (A) and K=5 (B).

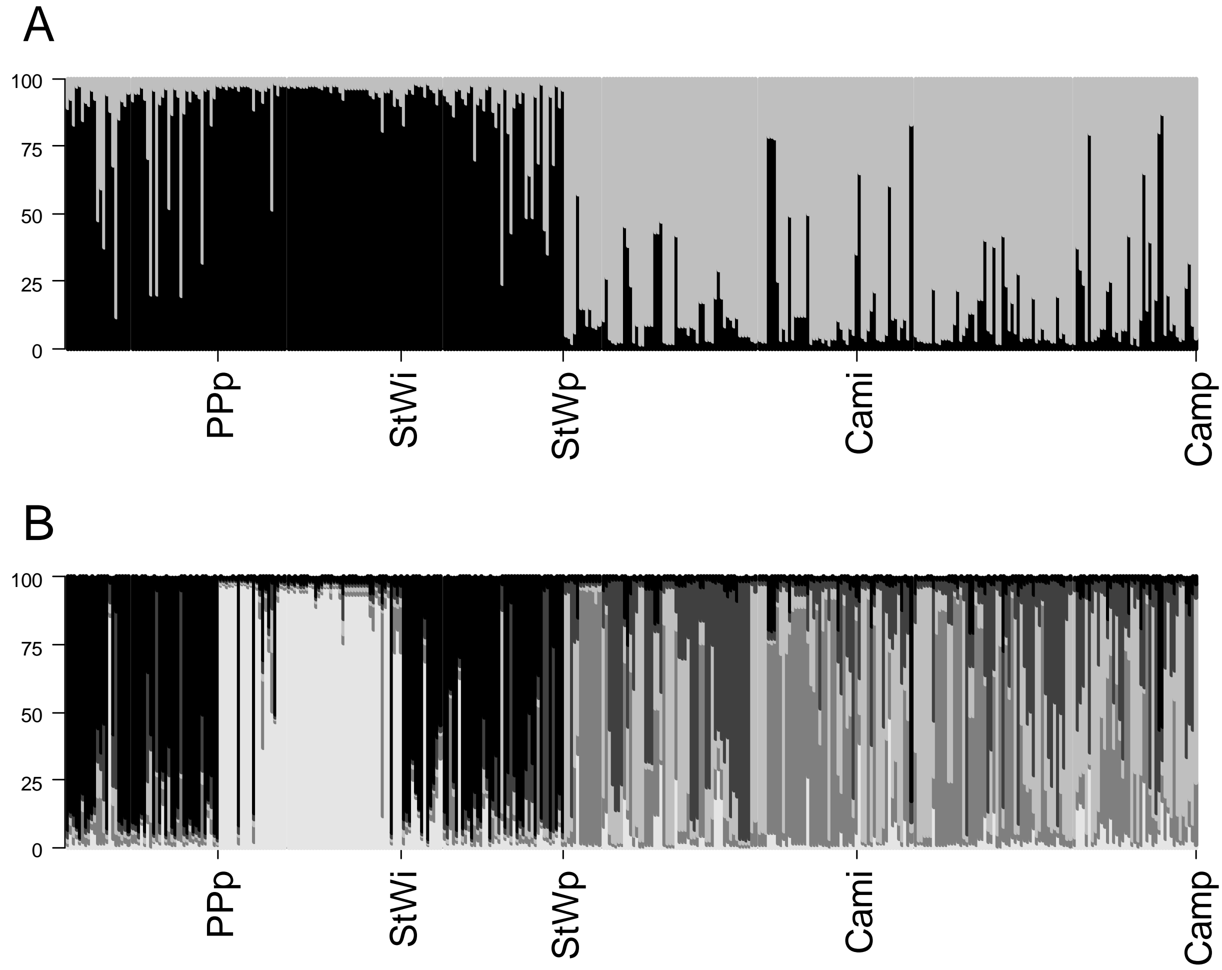

Figure 2.4. Structure results for the hierarchical analysis using the admixture ancestry model within the northern ancestral cluster. Support was similar for K=2 (A) and K=5 (B).

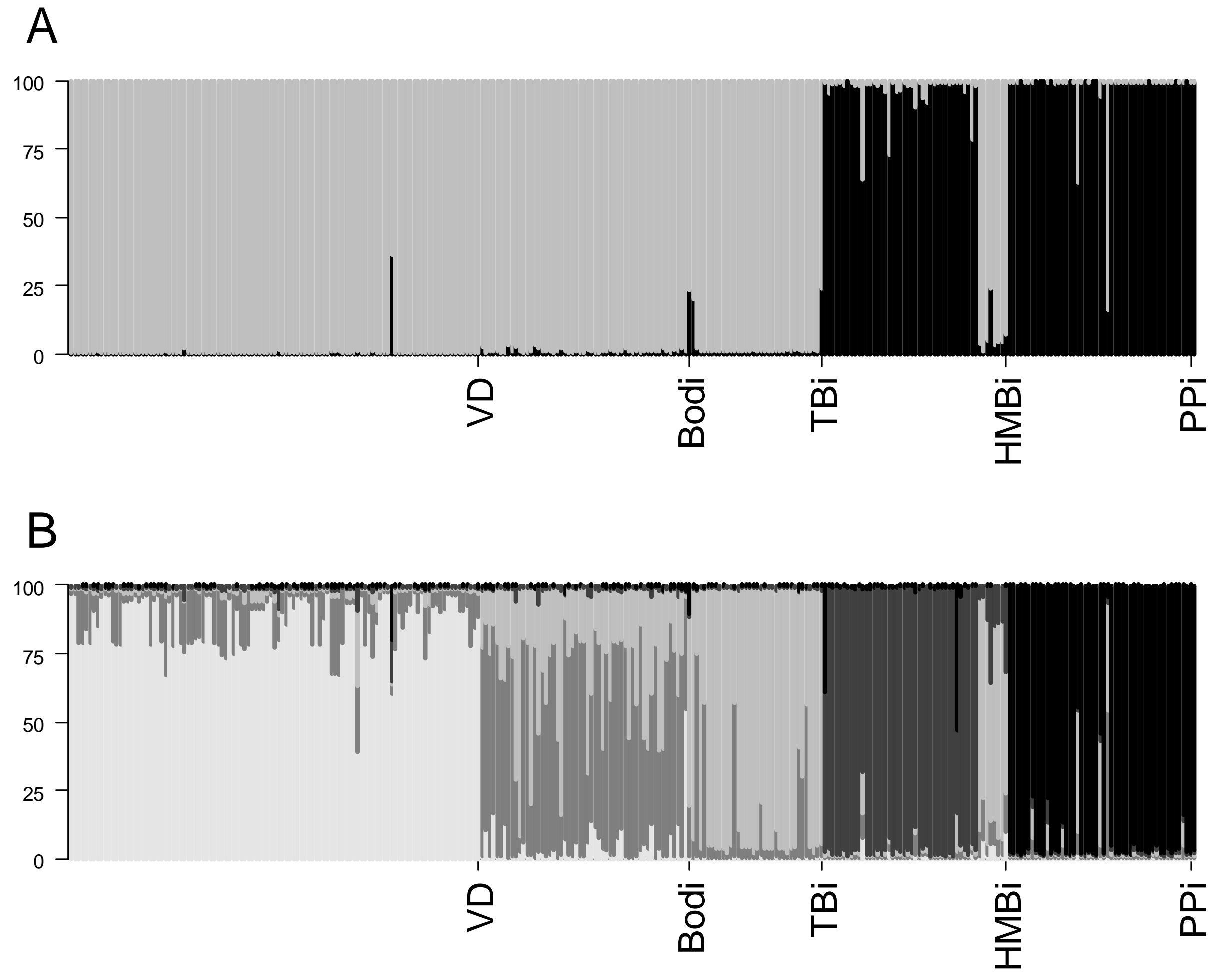

Figure 2.5. Structure results using the admixture setting within sample locations with both morphologies present for K=2. (A) Point Pinos, (B) Stillwater Cove, (C) Cambria.

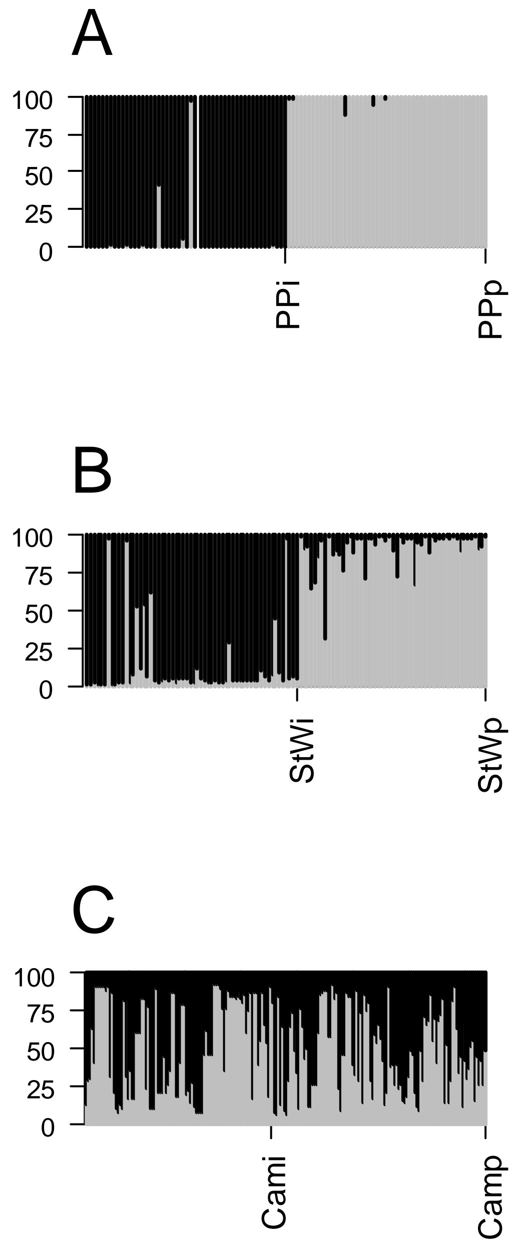

Figure 2.6. Assessment of first generation migrants for individuals at (A) Point Piños, (B) Stillwater Cove, and (C) Cambria. Individuals collected from the *M. pyrifera* patch appear as black points, and individuals collected from the *M. integrifolia* patch appear as red points. After likelihood analysis, individuals likely belonging to the *M. integrifolia* group are found to the right of the 1:1 line, whereas individuals likely belonging to the *M. pyrifera* patch are found to the left of the 1:1 line. A red point falling to the left of the 1:1 line represents an individual collected in an *M. integrifolia* patch that is likely a first generation migrant from the *M. pyrifera* patch and vice versa. Solid points indicate probability of assignment as a first generation migrant was P<0.05.

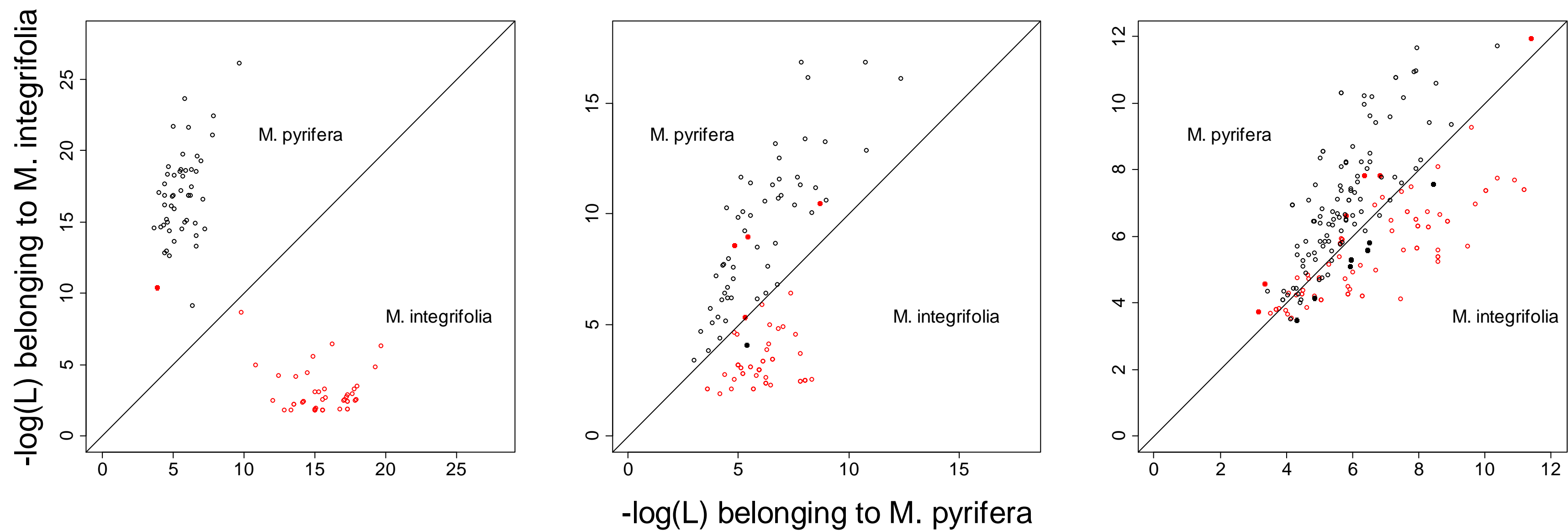

Figure 2.7: A-C Stillwater Cove, D-F Point Piños. Pairwise log mean distances regressed against Loiselle's kinship coefficients (black line). Comparisons are of all loci between individuals within patches the same morph. Dashed gray lines indicate the upper and lower 95% confidence intervals.

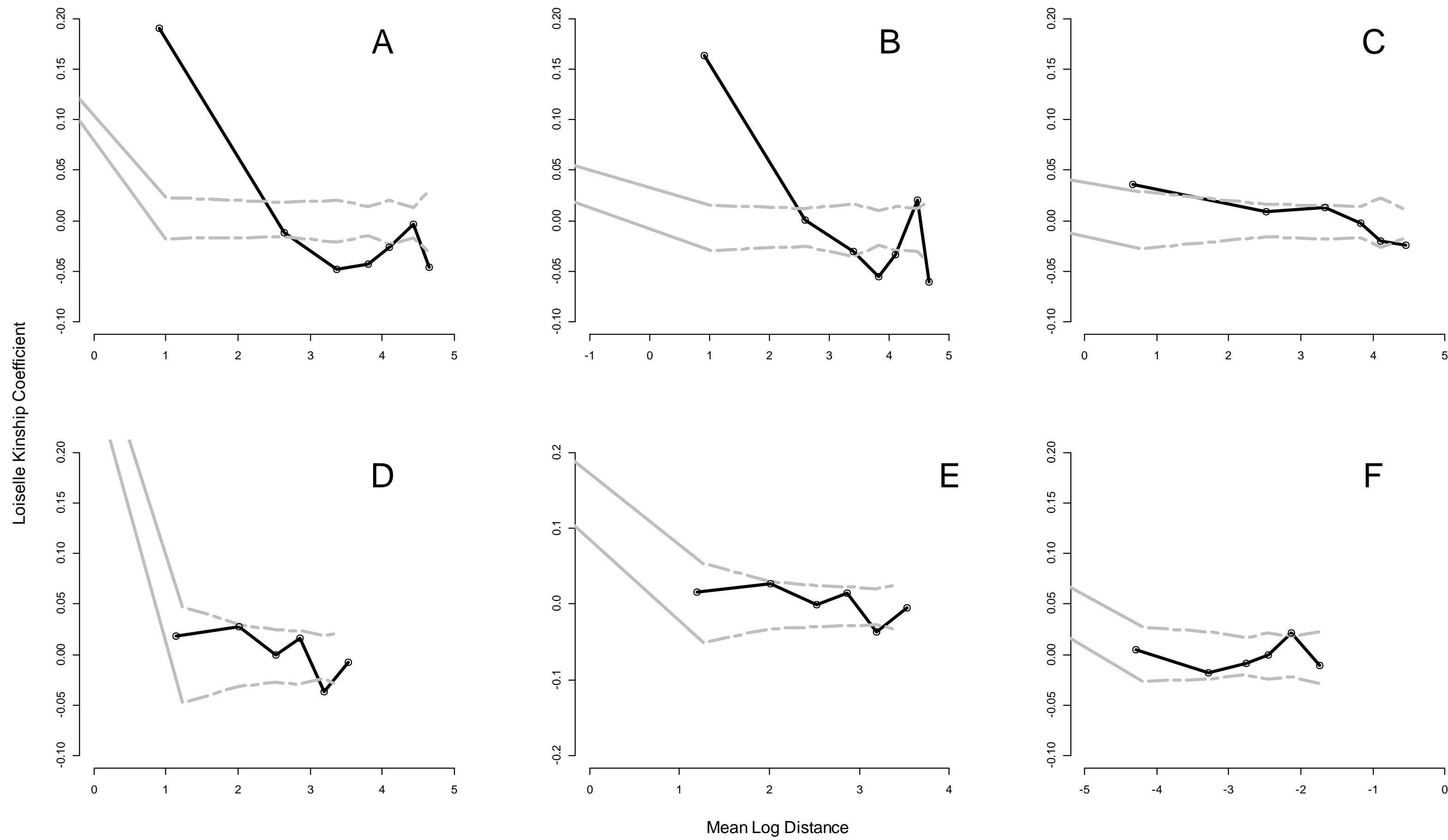

Figure 2.8: Spatial genetic variation of *Macrocystis spp.* in Stillwater Cove. Allele frequencies were adjusted, considering all individuals from both morphs to originate from the same population. 95% confidence intervals represent the expected relationship between distance and kinship when all individuals from both morphs are grouped together in a single population.

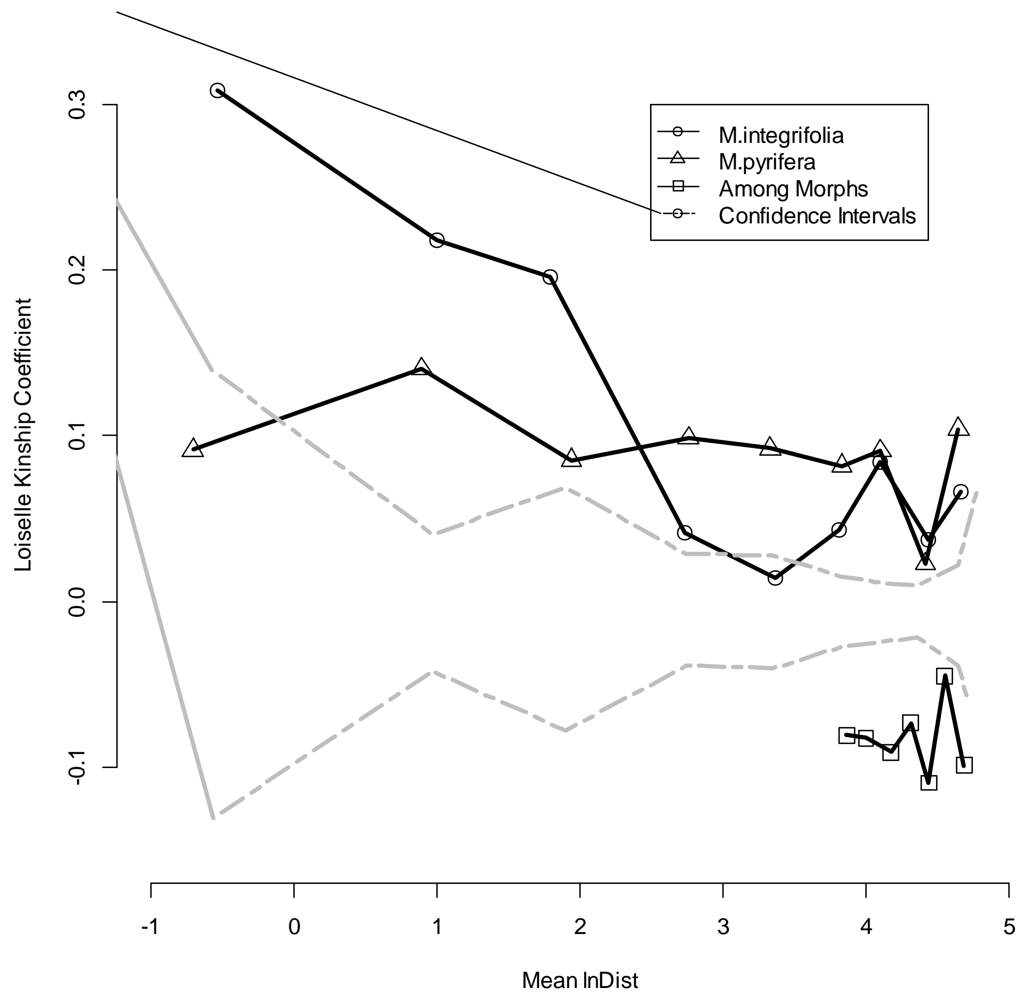

Figure 3.1. Observed counts from each developmental group and irradiance treatment. The first column (C) represents the control UV treatment. The second column (HPAR) are the developmental stages treated with elevated PAR for 30 minutes, the third column (UV) are the developmental stages treated with UVB for 30 minutes, and the fourth column (UV+HP) are developmental stages treated with both UVB and HPAR for 30 minutes. The rows represent the developmental stage that was treated with each irradiance treatment. Row one were unsettled spores, row two were the settled spores, row three were male and female differentiated gametophytes, and row four were one week old sporophytes. II indicates assignment to two *M. integrifolia* parents, PI to an *M. integrifolia* and an *M. pyrifera* parent, and PP to two *M. pyrifera*. Darker gray bars represent positive residual deviations from expected observed counts. Lighter gray bars indicate negative residual deviations. Positive residuals indicate higher than expected numbers of offspring assigned to a parent morph combination, and negative residuals indicate lower than expected counts. Strength of significance of deviation from expected frequency distributions; *=P<0.05, **=P<0.01, ***=P<0.001.

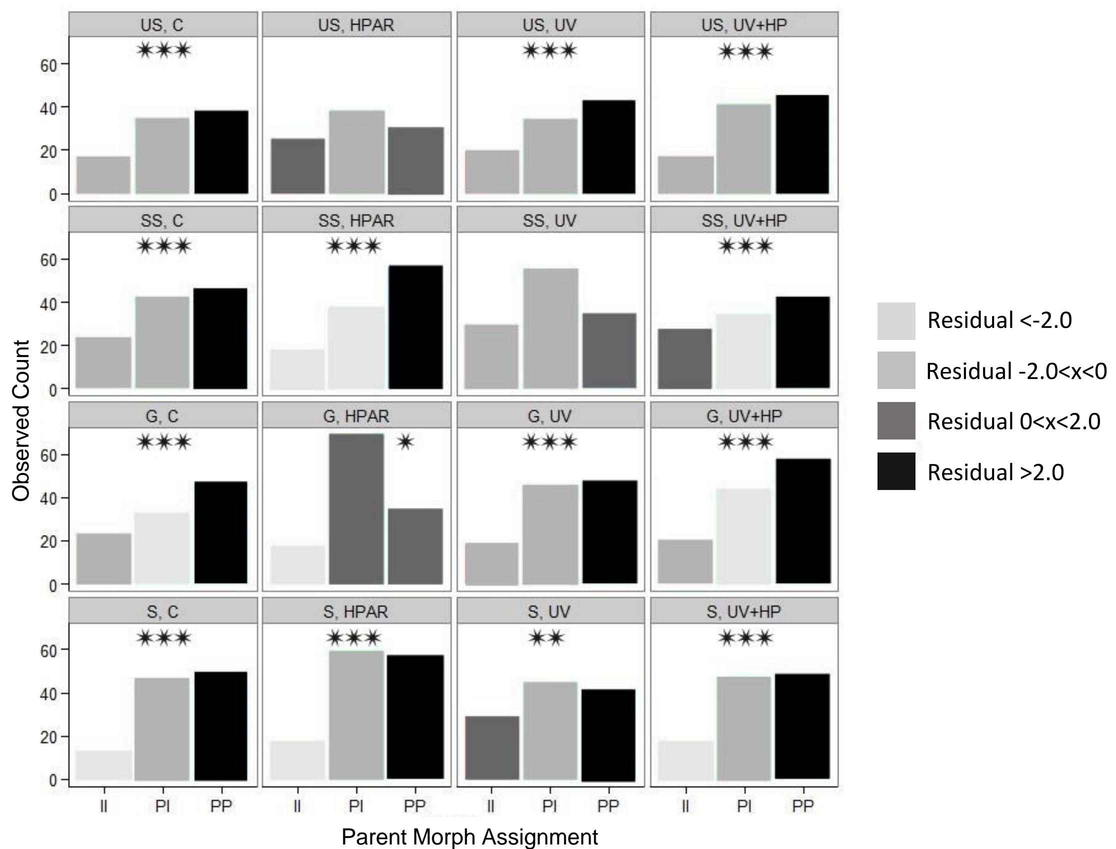

Table 1.1. Summary Statistics; Pc=*Pterygophora californica* data, Mp=*Macrocystis pyrifera* data, Pop = Population abbreviation, N = sample size, AR= allelic richness, He = Expected Heterozygosity, F_{IS} = Inbreeding Coefficient, *=0.01 significance level, **=0.001 significance level, ***=0.0001 significance level

Pop	Latitude	Longitude	Pc N	Mp N	Pc AR	Mp AR	Pc He	Mp He	Pc F_{is}	Mp Fis
Bul	34°27'31.98"N	120°20'0.36"W	52	52	7.43	11.42	0.611	0.767	0.016	0.183***
AH	34°28'18.72"N	120° 8'39.78"W	50	32	8.24	11.22	0.581	0.790	0.061	0.175***
AQ	34°28'7.62"N	120° 7'17.10"W	50	50	7.32	12.03	0.547	0.760	-0.018	0.121***
NP	34°25'20.40"N	119°57'10.56"W	50	49	6.06	12.93	0.567	0.777	-0.005	0.215***
IV	34°24'10.20"N	119°51'28.32"W	50	50	7.11	12.62	0.579	0.776	0.113*	0.215***
GB	34°24'49.62"N	119°49'20.64"W	50	47	7.17	12.11	0.585	0.768	0.101*	0.148***
AB	34°24'0.42"N	119°44'39.78"W	49	37	7.15	12.87	0.620	0.740	0.009	0.141***
Mk	34°23'39.60"N	119°43'48.00"W	50	44	8.35	13.46	0.616	0.795	0.228***	0.156***
Carp	34°23'32.70"N	119°32'37.68"W	52	50	5.95	11.78	0.513	0.780	0.041	0.134***

Table 1.2. Slope, intercept, and p-value of regression of single regression analyses for each of the three main factors and both measures of genetic differentiation (F_{ST} and D_{EST}) for *M. pyrifera* and *P. californica*. Bolded p-values indicate a significant correlation.

	$F_{ST}/1-F_{ST}$			D_{EST}		
Variable	Slope	Adjusted R^2	Regression P-value	Slope	Adjusted R^2	Regression P-value
Geographic Distance~						
Macrocystis	0.0003	0.1522	**0.0108**	0.0009	0.2148	**0.0026**
Pterygophora	0.0015	0.0857	**0.0462**	0.0010	0.0610	0.0792
Habitat Continuity~						
Macrocystis	-0.0023	0.2672	**0.0007**	-0.0061	0.3446	**0.0001**
Pterygophora	-0.0105	0.1465	**0.0122**	-0.0074	0.1371	**0.0150**
Oceanographic Transport~						
Macrocystis	0.0062	0.3445	**0.0031**	0.0150	0.3382	**0.0034**
Pterygophora	0.0316	0.2329	**0.0155**	0.0194	0.1701	**0.0359**

Table 1.3. ANCOVA results used to identify interactions between our predictions of genetic differentiation and the species used by examining the slopes of the regression lines. P-values indicate the significance of the interaction between the slopes of the two species regressions (see Figure 1.1) and the significance of the species effect using both measures of genetic differentiation.

	$F_{ST}/1\text{-}F_{ST}$	D_{EST}
Interaction	p-value	
Oceanographic Transport*Species	**0.0412**	0.6538
Habitat Continuity*Species	**0.0449**	0.6896
Geographic Distance*Species	0.1120	0.8797
Significance of Species Effect	p-value	
Oceanographic Transport	0.3508	0.9490
Habitat Continuity	**0.0021**	0.4295
Geographic Distance	0.3980	0.5571

Table 1.4. Results from multi-linear regression models incorporating geographic distance, habitat continuity, and minimum transport times during months of sporophyte production in *P. californica*. Significance of model was set at ($p<0.05$). Multi-linear regression models showing effects of transport time, along with habitat continuity and geographic distance independent from one another.

	Fst			Dest		
Variable	P-value	Adjusted R^2	AIC	P-value	Adjusted R^2	AIC
Single Regressions						
Geographic Distance	0.051	0.082	-202.10	0.174	0.047	-97.80
Habitat Continuity	0.011	0.151	-204.91	0.020	0.215	-101.88
January	0.011	0.260	-118.37	0.021	0.209	-101.71
February	0.349	-0.004	-111.96	0.511	-0.028	-96.20
March	0.025	0.198	-116.68	0.049	0.147	-100.11
April	0.017	0.225	-117.38	0.034	0.175	-100.82
May	**0.002**	**0.366**	**-121.60**	**0.007**	**0.291**	**-104.00**
November	0.007	0.288	-119.18	0.020	0.216	-101.89
December	0.035	0.171	-115.98	0.072	0.116	-99.38
Annual	0.002	0.366	-121.61	0.007	0.291	-103.99
Spring	0.005	0.318	-120.09	0.011	0.256	-103.00
Winter	0.060	0.131	-114.99	0.107	0.085	-98.65
Multiple Regressions						
~GeoDist+HabCont	0.016	0.173	-204.96	0.058	0.191	-100.36
~HabCont+Month						
January	0.012	0.322	-119.33	0.026	0.259	-102.20
February	0.041	0.220	-116.41	0.071	0.172	-99.89
March	0.014	0.309	-118.93	0.032	0.241	-101.72
April	0.009	0.345	-120.06	0.021	0.276	-102.69
May	**0.003**	**0.411**	**-122.31**	**0.011**	**0.327**	**-104.24**
November	0.006	0.370	-120.87	0.019	0.286	-103.00
December	0.019	0.285	-118.22	0.043	0.218	-101.07
~GeoDist+Mont						
January	0.016	0.296	-118.56	0.027	0.258	-102.17
February	0.166	0.090	-113.16	0.013	0.317	-103.91
March	0.043	0.216	-116.30	0.080	0.161	-99.60
April	0.015	0.304	-118.80	0.009	0.338	-104.58
May	**0.003**	**0.421**	**-122.67**	**0.006**	**0.368**	**-105.55**
November	0.022	0.274	-117.91	0.052	0.200	-100.61
December	0.091	0.149	-114.57	0.168	0.089	-97.87
Best Overall Multi-Regression						
~GeoDist+HabCont+April	**0.002**	**0.503**	**-125.04**	**0.002**	**0.501**	**-109.7**

Table 2.1. Distance classes manually defined for each pairwise kinship analysis in SPAGeDi. No. Dist Class=number of distance

Population	No. Dist Class	Distance classes (m)									
Point Piños											
M. integrifolia Pairs	6	5	10	15	20	30	45				
M. pyrifera Pairs	6	25	50	75	100	150	250				
Stillwater											
M. integrifolia Pairs	9	1	5	10	20	35	50	65	95	115	
M. pyrifera Pairs	9	1	5	10	20	35	50	65	95	115	
Stillwater (Adjusted)											
M. integrifolia Pairs	9	1	5	10	20	35	50	65	95	115	
M. pyrifera Pairs	9	1	5	10	20	35	50	65	95	115	
Among *M. pyrifera* and *M. integrifolia* Pairs	8	45	50	60	70	80	90	100	125		
All Pairs	10	1	5	10	20	35	50	65	95	115	125

Table 2.2. Delta K was calculated for each K to determine the highest support for each level of clustering. Both hierarchies are represented using the admixture setting. The number of genetic clusters receiving the most support (highest delta K) are bolded.

	Cluster Support								
	K=2 ΔK	K=3 ΔK	K=4 ΔK	K=5 ΔK	K=6 ΔK	K=7 ΔK	K=8 ΔK	K=9 ΔK	K=10 ΔK
Hierarchy 1									
All Populations	**2199.20**	1.97	2.54	167.64	5.53	0.87	64.32	0.91	0.00
Hierarchy 2									
Northern Populations	929.51	381.25	158.90	**948.86**	-	-	-	-	-
Southern populations	**682.47**	7.75	125.74	644.00	-	-	-	-	-

Table 2.3. Population data. Pop=Population; N=sample size, Morph=morphological classification, AR=allelic richness, meanA=mean number of alleles, He=expected heterozygosity, Ho=observed heterozygosity, Fis=inbreeding coefficient

Pop	N	Morph	Latitude	Longitude	AR	mean A	He	Ho	Fis
VD	109	Integrifolia	39.272677°	-123.792001°	2.14	4.29	0.2692	0.1846	0.3188
Bodi	56	Integrifolia	38.311278°	-123.053139°	2.614	2.57	0.3279	0.3309	0.0581
TBi	35	Integrifolia	38.196127°	-122.932873°	2.231	2.00	0.101	0.1025	0.1659
HMBi	49	Integrifolia	37.494461°	-122.497369°	3.054	2.86	0.3048	0.3081	0.4482
PPi	50	Integrifolia	36.633436°	-121.939125°	3.031	3.00	0.3362	0.3396	0.1922
PPp	50	Pyrifera	36.633275°	-121.945350°	4.731	4.86	0.5638	0.5695	0.0345
StWi	60	Integrifolia	36.565806°	-121.943861°	3.497	3.57	0.3996	0.4031	-0.0389
StWp	52	Pyrifera	36.565367°	-121.943650°	5.763	6.43	0.587	0.5928	0.0875
Cami	96	Integrifolia	35.453821°	-120.957521°	4.789	5.57	0.5419	0.5448	0.0776
Camp	111	Pyrifera	35.450393°	-120.960081°	5.446	6.00	0.5419	0.5444	0.052

Table 2.4. Pairwise genetic differentiation between population pairs. Pop *i* and Pop *j* represent populations being compared, GeoDist=geographic distance, Lin F_{ST}=Roussete's linearized measure of genetic differentiation $F_{ST}/(1-F_{ST})$, D_{EST}=Jost's estimate of genetic differentiation, lower CI and upper CI=95% confidence intervals following each measure of genetic differentiation

Pop *i*	Pop *j*	GeoDist	Lin F_{ST}	Lower CI	Upper CI	Jost's D_{EST}	Lower CI	Upper CI
Bodi	VD	135	0.3933	0.1401	0.8202	0.1061	0.0864	0.1285
TBi	VD	150	1.2114	0.3029	3.5558	0.1652	0.1426	0.1900
HMBi	VD	255	0.7470	0.2960	1.6399	0.1931	0.1644	0.2263
PPi	VD	365.5	1.1815	0.5124	2.3784	0.3594	0.3231	0.3972
PPp	VD	365	0.7138	0.2508	1.5176	0.3196	0.2901	0.3507
StWi	VD	375.1	0.7449	0.2256	1.4564	0.1800	0.1575	0.2047
StWp	VD	375	0.7062	0.2628	1.4284	0.3376	0.2922	0.3869
Cami	VD	530.5	0.7044	0.2329	1.7027	0.3125	0.2824	0.3401
Camp	VD	530	0.6287	0.2302	1.2321	0.3133	0.2861	0.3431
TBi	Bodi	15	0.2571	0.1618	0.3242	0.0420	0.0266	0.0592
HMBi	Bodi	115	0.4588	0.1443	1.2712	0.1163	0.0884	0.1484
PPi	Bodi	220.5	0.6728	0.2326	1.4231	0.2075	0.1736	0.2439
PPp	Bodi	220	0.3242	0.1998	0.5042	0.1859	0.1506	0.2265
StWi	Bodi	235.1	0.3247	0.0961	0.6529	0.0860	0.0687	0.1068
StWp	Bodi	235	0.3390	0.2034	0.5423	0.2308	0.1782	0.2867
Cami	Bodi	400.5	0.3561	0.1456	0.6773	0.1861	0.1552	0.2163
Camp	Bodi	400	0.3007	0.1040	0.5908	0.1589	0.1292	0.1914
HMBi	TBi	120	0.7957	0.1970	1.2999	0.1086	0.0848	0.1371
PPi	TBi	225.5	1.0606	0.5521	1.6035	0.2292	0.1966	0.2606
PPp	TBi	225	0.5067	0.1901	0.8832	0.1491	0.1208	0.1864
StWi	TBi	235.1	0.6929	0.1966	1.7647	0.1131	0.0959	0.1315
StWp	TBi	235	0.5323	0.2822	0.8450	0.2209	0.1759	0.2724
Cami	TBi	405.5	0.4399	0.2311	0.6372	0.1830	0.1616	0.2066
Camp	TBi	405	0.4251	0.1937	0.6915	0.1750	0.1546	0.1960
PPi	HMBi	110.5	0.4366	0.1500	0.7388	0.1179	0.0898	0.1499
PPp	HMBi	110	0.5726	0.3761	0.7483	0.3903	0.3359	0.4530
StWi	HMBi	125.1	0.6795	0.1779	1.6695	0.2215	0.1821	0.2644
StWp	HMBi	125	0.5518	0.3833	0.7361	0.4388	0.3795	0.5073
Cami	HMBi	275.5	0.4327	0.2601	0.6359	0.2774	0.2319	0.3203
Camp	HMBi	275	0.4347	0.2679	0.6215	0.3098	0.2643	0.3575
PPp	PPi	0.4	0.5228	0.2155	0.8818	0.2878	0.2498	0.3267
StWi	PPi	12	0.4972	0.1791	0.8100	0.1724	0.1538	0.1946
StWp	PPi	12.5	0.4890	0.2145	0.8212	0.2975	0.2486	0.3492
Cami	PPi	171	0.5420	0.2225	1.0396	0.3285	0.2880	0.3700
Camp	PPi	170.5	0.5168	0.2255	0.8727	0.3205	0.2833	0.3619
StWi	PPp	11.6	0.1982	0.0653	0.3702	0.0849	0.0600	0.1159
StWp	PPp	11.63	0.0080	0.0000	0.0166	0.0042	-0.0060	0.0217
Cami	PPp	170.5	0.1429	0.0663	0.2186	0.1192	0.0858	0.1573
Camp	PPp	170	0.1400	0.0708	0.2238	0.1079	0.0737	0.1450
StWp	StWi	0.1	0.1756	0.0753	0.3158	0.0880	0.0547	0.1357
Cami	StWi	160.6	0.3517	0.1677	0.5736	0.2267	0.1876	0.2647
Camp	StWi	160.1	0.3074	0.1659	0.4754	0.1960	0.1610	0.2296
Cami	StWp	160.5	0.1654	0.0909	0.2484	0.1573	0.1155	0.2034
Camp	StWp	160	0.1685	0.0784	0.3043	0.1366	0.1000	0.1743
Camp	Cami	0.5	0.0276	0.0133	0.0556	0.0247	0.0138	0.0366

Table 2.5. Clone statistics for the seven *M. integrifolia* patches. Pop=population; N*= the sample size at that population with individuals having no missing alleles, which were able to be used to calculate P_{gen} and P_{sex}; Distinct MLGs=number of unique multilocus genotypes in patch; Shared MLG groups=number of groups of units with members sharing the same multilocus genotype; Min MLG Dist=minimum distance between units sharing the same multilocus genotype (m); Max MLG Dist=maximum distance between units sharing the same multilocus genotype (m); Distinct Clonal Groups=number of groups with resampled multilocus genotypes that were unlikely to be resampled due to chance and given clone status; Min Clone Dist=minimum distance between clones (m); Max Clone Dist=maximum distance between clones (m) R=genotypic richness, calculated using (G-1)/(N-1), where G= the number of distinct MLGs; p E_e=the significance of the edge effect, $P<0.05$ indicates that genotypic richness may be overestimated due to spatial design, p A_C=the significance of aggregation, $P<0.05$ indicates that clones are more likely to be related to their neighbor than more distant individuals; AR=allelic richness; AR SD=standard deviation for allelic richness; NA=Distance values not applicable

Pop	N*	Distinct MLGs	Shared MLG groups	Mean # individuals within MLG Group	Min MLG Dist	Max MLG Dist	Distinct Clonal Groups	Min Clone Dist	Max Clone Dist	R	p E_e	p A_c	AR	AR SD
VD	72	62	7	2.75	0.10	1.56	6	0.10	1.56	0.86	0.017	0.000	2.59	0.13
Bod	51	50	1	2.00	0.10	0.10	1	0.10	0.10	0.98	0.000	0.000	2.84	0.07
TB	29	22	4	2.75	NA	32.94	0	NA	NA	0.75	0.428	0.297	2.14	0.12
HMB	35	32	3	2.00	0.76	21.07	1	0.76	0.76	0.91	0.006	0.019	2.28	0.04
PP	41	37	2	2.33	0.56	11.40	2	0.56	11.40	0.90	0.747	0.004	2.80	0.09
StW	43	25	7	3.71	0.22	5.15	7	0.22	5.15	0.57	0.509	0.000	3.49	0.32
Cam	46	38	7	2.14	NA	NA	7	NA	NA	0.82	0.453	0.000	5.03	0.17

Table 2.6. Parapatric populations with both morphs were tested at K=2, or two genetic clusters, using the admixture setting. The proportion of individuals of each morph were assigned to one of two clusters. The more even the proportions, the higher level of genetic admixture between the two patches. The more disparate the proportions, the more unique each genetic cluster is, and there for morphs are more genetically distinct.

Population	Inferred Cluster	
	1	2
Point Pinos		
M. integrifolia	0.034	0.966
M. pyrifera	0.991	0.009
Stillwater Cove		
M. integrifolia	0.120	0.880
M. pyrifera	0.910	0.090
Cambria		
M. integrifolia	0.533	0.468
M. pyrifera	0.472	0.528

Table 3.1. Parent sporophyte genetic diversity from 10 *M. integrifolia* individuals (Mi) and 10 *M. pyrifera* individuals collected in Stillwater Cove, CA. nA=number of alleles, pA=number of private alleles; HObs=observed heterozygosity, HExp= expected heterozygosity

Locus	nA	nA Mi	nA Mp	pA Mi	pA Mp	HObs	Hexp
BC-18	4	3	3	1	1	0.40	0.42
BC-19	2	2	2	0	0	0.35	0.45
BC-25	5	1	5	0	4	0.35	0.39
BC-4	6	4	6	0	2	0.80	0.77
Mpy-11	6	3	5	1	3	0.45	0.66
Mpy-8	5	2	5	0	3	0.75	0.65

Table 3.2. Offspring genetic diversity derived from 1727 individuals. Mi=*M. integrifolia* data, Mp=*M. pyrifera* data, nA=number of alleles, pA=number of private alleles; HObs=observed heterozygosity, HExp= expected heterozygosity

Locus	nA	HObs	Hexp
BC-18	5	0.413	0.431
BC-19	5	0.438	0.491
BC-25	6	0.476	0.538
BC-4	6	0.518	0.737
Mpy-11	8	0.410	0.705
Mpy-8	5	0.560	0.657

Table 3.3. Parentage assignments by each potential morph combination (Mi Mi, Mp Mi, Mp Mp) in each treatment group and developmental stage. Counts include both outcrossed and selfed assignments.

	Parent Morph Assignment		
Treatment Group	Mi Mi	Mi Mp	Mp Mp
USUV	19	34	42
USHPAR	25	38	30
USUV+HP	17	41	45
USC	16	34	38
SSUV	29	55	34
SSHPAR	17	37	56
SSUV+HP	27	34	42
SSC	23	42	45
GUV	18	45	47
GHPAR	17	69	34
GUV+HP	20	43	57
GC	23	32	46
SUV	29	44	41
SHPAR	17	59	56
SUVHP	17	47	48
SC	12	46	49

Table 3.4. Parentage assignments separated by the number outcrossed and the number selfed from each parent morph. Expected selfing rate is 0.05 based on 20 possible contributing parents.

Treatment Group	Parent Morph							
	M. integrifolia				*M. pyrifera*			
	Total	Outcrossed	Selfed	% Selfed	Total	Outcrossed	Selfed	% Selfed
USUV	19	7	12	0.632	42	20	22	0.524
USHPAR	25	14	11	0.440	30	12	18	0.600
USUV+HP	17	7	10	0.588	45	27	18	0.400
USC	16	11	5	0.313	38	19	19	0.500
SSUV	29	20	9	0.310	34	20	14	0.412
SSHPAR	17	9	8	0.471	56	26	30	0.536
SSUV+HP	27	16	11	0.407	42	18	24	0.571
SSC	23	15	8	0.348	45	23	22	0.489
GUV	18	7	11	0.611	47	26	21	0.447
GHPAR	17	13	4	0.235	34	20	14	0.412
GUV+HP	20	13	7	0.350	57	38	19	0.333
GC	23	12	11	0.478	46	22	24	0.522
SUV	29	13	16	0.552	41	28	13	0.317
SHPAR	17	8	8	0.471	56	34	22	0.393
SUVHP	17	7	10	0.588	48	29	19	0.396
SC	12	7	5	0.417	49	33	16	0.327

Table 3.5. Number of homozygous offspring who may have been derived via endomitosis. More than one parent genotype may be responsible for the same homozygous offspring multilocus genotype. Parent ID indicates the morph (I=*M. integrifolia;* P=*M. pyrifera*) and the individual identification number.

Parent ID	Offspring Count
I01	2
I02	27
I03	16
I04	14
I05	26
I06	14
I07	15
I08	17
I09	10
I10	10
P01	0
P02	0
P03	1
P04	0
P05	11
P06	0
P07	13
P08	10
P09	2
P10	0

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Appendices

Appendix A: *Pterygophora californica* microsatellite marker characterization

Genomic DNA was isolated using an initial nuclei isolation (Varela-Alvarez et al 2006) followed by standard cetylmethyl ammonium bromide (CTAB) (Doyle, 1987). DNA was digested with RsaI (Fermentas) and the total digested product was purified and ligated to annealed RsaI adaptors (RSA21: 5'-CTCTTGCTTACGCGTGGACTA-3' and RSA25: 5'-AGTCCACGCGTAAGCAAGAGCACA-3'). The enrichment procedure followed the protocol from Billote et al (1999) which used streptavidin-coated magnetic particles and biotinylated probes (Magnesphere, Promega, Madison, WI). We used a 5'-biotinylated $(CT)_{15}$ and $(GT)_{15}$ probes, with a 3'-dideoxyC end, to avoid the probe to work as a primer in the following PCR step (Koblizkova et al 1998). The enriched single stranded DNA was amplified by PCR using the RSA21 as a primer to recover double strand DNA. Previous PCR product was ligated into pGEM-T Easy vector (Promega, Madison, WI) and transformed into *Escherichia coli* competent cells (strain DH5α).

1140 positive clones were transferred from agar plates to 96 well microplates containing 150 µl of LB/Ampicilin solution, incubated (4 h, 37ºC), diluted 5x in ultrapure water (Sigma), and heated (10 minutes) to provide cell lysis. This solution was used as DNA template for PCR with standard SP6 and T7 primer amplification, and the products were transferred to Hybond N+ nylon membranes (Amersham) and hybridized with a 32P radiolabeled $(CT)_{15}$ and $(GT)_{15}$ probes. Insert sizes were estimated by agarose gel electrophoresis of the PCR product. A total of 81 clones were selected by the size and hybridization signal and the plasmids were extracted using the E.Z.N.A Plasmid Miniprep Kit (D6942-02, OMEGA Bio-Tek) and sequenced. Forty primer pairs were drawn with Primer 3 (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) from the ones that showed sufficiently large flanking regions and long microsatellite regions.

Microsatellite loci polymorphism was analyzed in one population, Arroyo Hondo, Santa Barbara Chanel, California, USA. Blade tissue from 48 individuals was collected from a 60 x 20 m area in the kelp bed and genomic DNA was extracted using the commercial kit Nucleospin 96 Plant Kit (Macherey-Nagel, Germany). PCR reactions were performed for 15 µl contained ±20 ng of DNA, 0.1 µM of each primer (Table I), 0.8 mM of dNTPs (Bioline), 2.0 or 2.5 mM of $MgCl_2$ (see Table I for locus optimization), 3.0 µl of 5x PCR Buffer and 0.4 U of GoTaq Polymerase (Promega, Madison, WI). Cycling conditions consisted of an initial denaturing step of 5 min at 95ºC, followed by 35 cycles of 30 s at 95ºC, 30 s at annealing temperature (see Table

I for locus optimization), 45 s at 72ºC, and a final elongation step at 72ºC for 20 minutes. All PCR reactions were performed on a GeneAmp 9700 thermocycler (PE Applied Biosystems).

ABI PRISM 3130xl DNA analyzer was used to analyze fragment length using the GeneScan Liz 500 size standard (Applied Biosystems). Raw allele sizes were scored with STRAND (http://www.vgl.ucdavis.edu/informatics/STRand), binned using the R package MsatAllele (http://www.ccmar.ualg.pt/maree/soft/msatalle.php), and manually reviewed for ambiguities. Genetix V. 4.02 (http://kimura.univ-montp2.fr/genetix/) was used to estimate linkage disequilibrium and conformity to the Hardy-Weinberg equilibrium.

A total of eight loci were selected as microsatellite markers after amplification and polymorphism screening (**Table S1**). The levels of genetic diversity were high; the number of alleles ranged from 2 to 14, and gene diversity from 0.2695 to 0.8746, mean = 0.6098 (Table I). Using MICROCHECKER software (http://www.microchecker.hull.ac.uk) we estimate that 2 loci (Pc-01 and Pc-14) were affected by the presence of null alleles.

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Appendix B - Characterization of eight microsatellite loci for *Pterygophora californica*

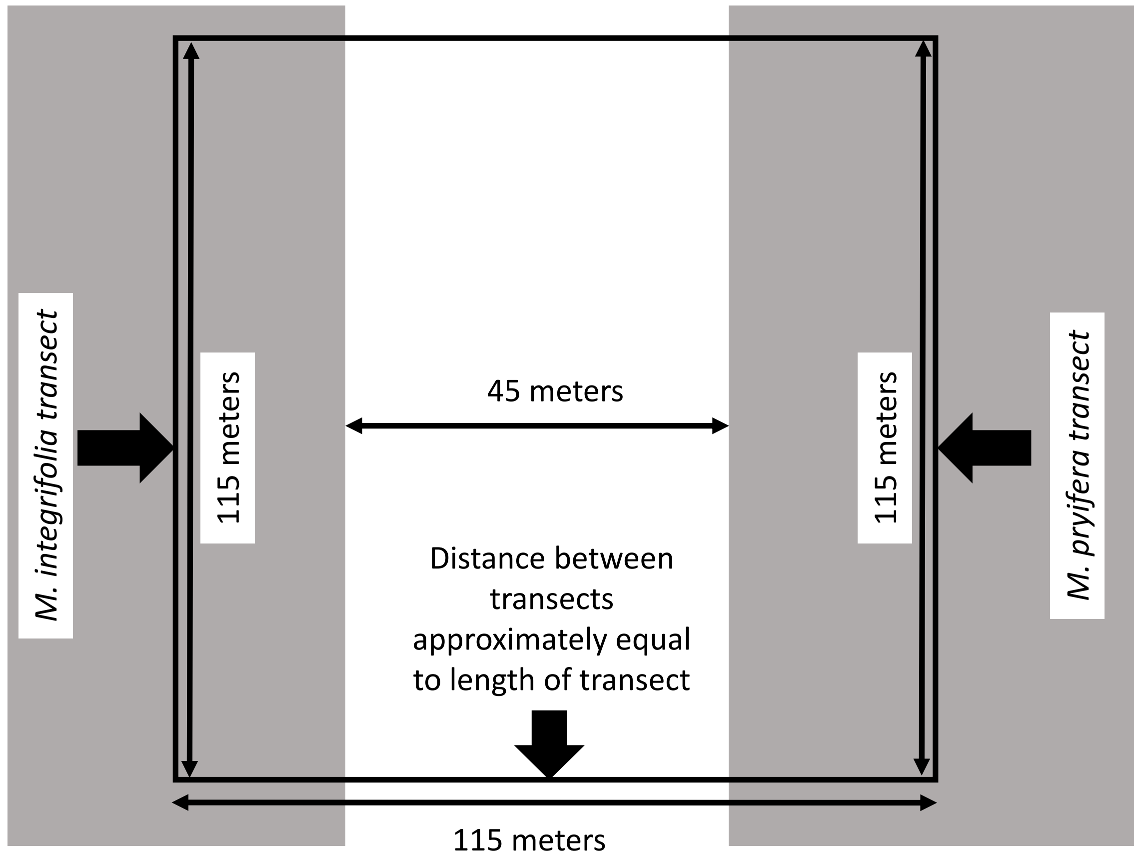
Locus name (Genebank no.)	Primer sequences 5' – 3'	Repeat motif	Clone size (bp)	Ta (ºC)	MgCl2 (mM)	Microchecker	*A*	Size range (bp)	*He*	*Ho*	F_{IS}
Pc-01 (KM392276)	F-CGTAGCTTGGCTTGGCTTG R-ACACACCACAACACGACAC	$(GTGTT)_{18}$	344	67	2.5	Null alleles	5	342-362	0.5840	0.3600	0.401
Pc-05 (KM392277)	F-AGATCGGGTTGGGGCATAC R-TGTAGTTGCGGGAGGTCAG	$(GA)_{16}$	204	60	2.0	nA	4	200-206	0.6233	0.7143	-0.136
Pc-06 (KM392278)	F-TTGACCACGGATCCCTTCC R-ACGCGCGCATATTGCAG	$(AG)_6AAG(GA)_{14}$	175	60	2.0	nA	4	174-193	0.3660	0.3617	0.022
Pc-10 (KM392279)	F-AGAGCAGTTAGGTGAAGCCC R-ACGCAGAGGGAGAAACAGG	$(CT)_{20}$	198	60	2.0	nA	5	146-209	0.2695	0.2245	0.177
Pc-14 (KM392280)	F-AGAAACGCAACCAGCCAAC R-GAAACTTGCGGAGAAGCGG	$(TC)_5G(CT)_{21}CG(CT)_6$	231	59	2.5	Null alleles	14	237-289	0.8746	0.5778	0.349
Pc-15 (KM392281)	F-ATAATTTTATACCAGGCAGACGG R-AATTGAAGCTCAGCGCACG	$(TC)_{13}C(CT)_2CC(CT)_7A(TC)_4$	157	60	2.0	nA	2	157-159	0.4981	0.4898	0.027
Pc-17 (KM392282)	F-ACCCTCTAGCACATTCTCGC R-AGAGAGGCGAAGCTAGCAC	$(CT)_6T(TC)_{25}$	232	59	2.5	nA	10	221-243	0.8540	0.8750	-0.009
Pc-19 (KM392283)	F-GGCACGAAACGGTGAGTTG R-GAGGCGGAGCACTGAGG	$(CT)_{11}G(TC)_{24}$	202	59	2.5	nA	12	163-226	0.8092	0.7755	0.052

Locus name and GeneBank accession number, primer sequence, motif repetition, clone size, PCR annealing temperature (Ta), presence of null alleles estimated with Microchecker, number of alleles found, fragment size range in base pairs (bp), gene diversity and inbreeding coefficient were estimated for the Arroyo Hondo bed.

Appendix C. Pairwise genetic differentiation between populations for both kelp species, and pairwise metrics used to determine drivers of genetic connectivity. GeoDist=Geographic Distance, HabCont=Habitat Continuity, TT=Oceanographic Distance

PopI	PopJ	Mp D_{EST}	Pc D_{EST}	Mp $F_{ST}/1\text{-}F_{ST}$	Pc $F_{ST}/1\text{-}F_{ST}$	GeoDist	HabCont	TT
Bul	AH	0.0454	0.0257	0.0233	0.0145	17.40	11.17	
Bul	AQ	0.0303	0.0495	0.0112	0.0535	19.48	11.06	4.626
Bul	NP	0.0444	0.0587	0.0187	0.0492	35.11	15.10	6.082
Bul	IV	0.0433	0.0386	0.0157	0.0311	44.11	11.98	6.309
Bul	GB	0.0622	0.0317	0.0214	0.0269	47.19	14.12	6.165
Bul	AB	0.0762	0.0305	0.0283	0.0210	54.50	9.96	
Bul	Mk	0.0771	0.0208	0.0353	0.0159	55.87	6.50	6.314
Bul	Carp	0.1348	0.1393	0.0581	0.1783	73.25	4.02	8.258
AH	AQ	0.0325	0.0381	0.0177	0.0653	2.13	11.41	
AH	NP	0.0727	0.0815	0.0332	0.0601	18.35	7.91	
AH	IV	0.0531	0.0292	0.0267	0.0166	27.40	10.85	
AH	GB	0.0733	0.0252	0.0306	0.0224	30.26	10.70	
AH	AB	0.1072	0.0267	0.0490	0.0194	37.60	7.32	
AH	Mk	0.0130	0.0544	0.0060	0.0285	39.01	11.04	
AH	Carp	0.0872	0.1703	0.0324	0.2160	56.21	8.76	
AQ	NP	0.0267	0.0953	0.0098	0.0977	16.25	7.28	4.202
AQ	IV	0.0287	0.0437	0.0107	0.0813	25.29	10.52	4.102
AQ	GB	0.0396	0.0643	0.0143	0.0645	28.14	10.52	4.815
AQ	AB	0.0512	0.0565	0.0231	0.0561	35.48	8.00	
AQ	Mk	0.0398	0.0707	0.0221	0.0521	36.89	10.83	5.567
AQ	Carp	0.1065	0.1816	0.0433	0.2759	54.09	8.62	7.506
NP	IV	0.0056	0.0654	0.0020	0.0563	9.06	18.15	4.117
NP	GB	0.0200	0.0399	0.0082	0.0281	12.09	16.62	3.608
NP	AB	0.0505	0.0539	0.0162	0.0401	19.39	7.25	
NP	Mk	0.0345	0.0671	0.0268	0.0467	20.78	14.46	4.101
NP	Carp	0.0849	0.2101	0.0305	0.2622	38.16	9.65	6.441
IV	GB	0.0251	0.0172	0.0062	0.0092	3.47	9.06	3.356
IV	AB	0.0236	0.0553	0.0128	0.0392	10.44	9.30	
IV	Mk	0.0781	0.0565	0.0308	0.0332	11.79	11.10	3.486
IV	Carp	0.0943	0.1670	0.0395	0.2132	17.48	6.86	5.923
GB	AB	0.0569	0.0260	0.0201	0.0233	7.34	9.54	
GB	Mk	0.0711	0.0358	0.0328	0.0234	8.75	12.56	3.165
GB	Carp	0.1157	0.1805	0.0372	0.2217	26.07	6.79	4.899
AB	Mk	0.0633	0.0139	0.0382	0.0095	1.45	9.83	
AB	Carp	0.1036	0.1806	0.0445	0.1837	18.80	8.52	
Mk	Carp	0.0711	0.1405	0.0260	0.1463	29.25	4.12	4.337

Appendix D. Layout of Stillwater Cove sampling. Grey areas represent the area around transect where samples were collected from. Spatial genetic structure was analyzed both within morph sampling areas, and between morph combinations.



Appendix E. High levels of gene flow between *M. pyrifera* patches drives high levels of genetic turnover and decreases generation time of patches. Infrequent migrants from *M. pyrifera* to *M. integrifolia* and between *M. integrifolia* patches coupled with asexual growth drives low levels of genetic turnover and increases the generation time of *M. integrifolia* patches.