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Looking into the black box: simulating the role of self-fertilization and mortality in the genetic structure of *Macrocystis pyrifera*

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Abstract

Patterns of spatial genetic structure (SGS), typically estimated by genotyping adults, integrate migration over multiple generations and measure the effective gene flow of populations. SGS results can be compared with direct ecological studies of dispersal or mating system to gain additional insights. When mismatches occur, simulations can be used to illuminate the causes of these mismatches. Here, we report a SGS and simulation-based study of self-fertilization in *Macrocystis pyrifera*, the giant kelp. We found that SGS is weaker than expected in *M. pyrifera* and used computer simulations to identify selfing and early mortality rates for which the individual heterozygosity distribution fits that of the observed data. Only one (of three) population showed both elevated kinship in the smallest distance class and a significant negative slope between kinship and geographical distance. All simulations had poor fit to the observed data unless mortality due to inbreeding depression was imposed. This mortality could only be imposed for selfing, as these were the only simulations to show an excess of homozygous individuals relative to the observed data. Thus, the expected data consistently achieved nonsignificant differences from the observed data only under models of selfing with mortality, with best fits between 32% and 42% selfing. Inbreeding depression ranged from 0.70 to 0.73. The results suggest that density-dependent mortality of early life stages is a significant force in structuring *Macrocystis* populations, with few highly homozygous individuals surviving. The success of these results should help to validate simulation approaches even in data-poor systems, as a means to estimate otherwise difficult-to-measure life cycle parameters.

Keywords: giant kelp, inbreeding depression, mating system, microsatellites, self-fertilization, spatial genetic structure

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Introduction

Inbreeding may decrease the survival and fecundity of the offspring of related individuals (or the same individual, in the case of selfing); such a decrease in the fitness of inbred offspring relative to outcrossed is termed inbreeding depression (Charlesworth & Willis 2009). The decrease in fitness may take the form of defective phenotypes that do not survive beyond early life stages, but is more often apparent through decreased growth, survival and fecundity (Charlesworth & Willis 2009). In spite of the potential for significant negative impacts of inbreeding, self-fertilization (the fertilization of an egg by a sperm from the same hermaphroditic individual) is relatively common in both plants and animals (Jarne & Charlesworth 1993). Between 35% and 84% of hermaphroditic angiosperms

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are at least partially self-compatible and over 50% of flowering plants self-fertilize at least some of the time (Jarne & Charlesworth 1993; Barrett *et al.* 1996). Although research on rates of self-fertilization is less well developed in other taxonomic groups, self-compatible hermaphroditism and indirect selfing (i.e. fertilization between male and female gametophytes produced by the same sporophyte) are common among algae and nonvascular plants (e.g. Perrin *et al.* 2007; Barner *et al.* 2011).

Inbreeding depression (ID) is generally assessed through controlled crosses, either by comparing the fitness of outcrossed vs. inbred progeny from within a single population for within-population ID, or by comparing crosses within a subpopulation to crosses between members of different subpopulations to identify among-population ID (Keller & Waller 2002). For many species, it may be difficult or impossible to perform controlled crosses. In addition, such crosses may significantly overestimate the impacts of ID because the level of inbreeding studied often exceeds that which is naturally present (Keller 1998; Raimondi *et al.* 2004). ID can also be estimated in natural populations using known or reconstructed pedigrees (Pemberton 2004) or with population genetic tools by (i) correlating measures of an individual's fitness (such as growth rate, size or reproductive output) with their heterozygosity (Mitton 2000); (ii) assessing changes in the inbreeding coefficient (*F*) of a population over time (Ritland 1990); or (iii) correlating measures of relatedness between parents with offspring performance (Bensch *et al.* 1994). For species, such as algae, fungi and ferns that produce large numbers of small (or microscopic) progeny, it may be impossible to collect genetic data or track mortality for early life stages. Thus, results from the assessments of adults are indicative of ID in survivors that have become large enough to track and will significantly underestimate the contribution of early-lethal ID.

Simulation studies offer an alternative method to estimate population genetic parameters, such as ID, which may otherwise be difficult or impossible to measure accurately. Computer simulations are used to generate *in silico* data sets, using models of population genetic structure and evolution parameterized with known or hypothesized scenarios (Hoban *et al.* 2012). These data sets can then be used to assess the interactions between evolutionary forces or, when compared with real data, to reconstruct historical population genetic processes (Arenas 2012; Hoban *et al.* 2012).

The advent of hypervariable markers has fostered detailed studies of the spatial genetic structure (SGS) of populations. Often, the objective of SGS studies is to indirectly estimate gene flow from the change in pairwise kinship, or other genetic relatedness statistics, as a function of spatial distance between individuals. An SGS pattern results from the restricted movement of individuals or other dispersing agents such as gametes, seeds or spores over multiple generations. Related individuals will tend to aggregate in genetic neighbourhoods, with neighbourhood size closely related to the species' dispersal potential (Rousset 1997). Spatial genetic autocorrelation, the decline in mean pairwise genetic kinship with spatial distance, is the pivotal analytical tool in different SGS regression models (Rousset 2000; Vekemans & Hardy 2004). The slope of the regression line (hereafter termed SGS*b*) is an indicator of SGS strength and geneflow limitation.

One important factor influencing the strength of the SGS pattern is the mating system, because it defines the genotypic composition of the dispersal vectors that are produced by sexual reproduction and influences kinship and heterozygosity in the progeny (Vekemans & Hardy 2004). Generally, higher selfing rates are likely to lead to higher mean kinship in the immediate neighbourhood and steeper SGS*b* than found for species that rely more on outcrossing (Vekemans & Hardy 2004).

Population density is also tightly related to selfing rate due to the density dependence of mate encounters. Self-compatible species are expected to self-fertilize less at higher densities (Karron *et al.* 1995; Eppley & Pannell 2007). At low densities, the larger distances between self-compatible hermaphrodites may become limiting, reducing the probability of finding non-self-mating partners or gametes, and thus reducing the probability of outcrossing.

Spatial genetic structure patterns, as estimated by genotyping adult populations, integrate migration over multiple generations and are a measure of the 'effective' past gene flow. These can be compared with direct measurements or theoretical predictions of dispersal or with other mating system characteristics to gain additional insights into the processes that determine effective gene flow. Mismatches in such comparisons are opportunities to test null hypotheses using computer simulations to illuminate the underlying processes (Epperson *et al.* 2010; Hoban *et al.* 2012). Mismatches between simulated and observed patterns can be minimized by running simulations that use realistic parameter values and fitting simulated to observed data (e.g. selection and larvae retention parameters in Galindo *et al.* 2010).

A mismatch between mating system expectations, derived from direct or theoretical studies, and SGS patterns is expected when inbreeding mortality removes pre-adult individuals from the population before sampling. In this case, a lower-than-expected SGS*b* and mean kinship in the observed data would be estimated. The population selfing rate (*S*) has been typically estimated from molecular marker data by using inbreeding coefficient (F_{IS}) estimates and the expectation $S = 2F_{IS}/(1 + F_{IS})$ (Li 1955). If mortality of the most inbred individuals occurs before sampling, then this relationship is likely to underestimate the actual S, or the proportion of gametes produced through selfing, while correctly estimating the effective S, the proportion of adults produced as a result of selfing. Thus, inbreeding, its impact (lethal vs. nonlethal) and the life stages it affects are key elements to consider when investigating mating systems.

Research on spore dispersal in macroalgae has suggested that dispersal kernels may be highly skewed, with the majority of spores settling within metres to hundreds of metres from the parent individual, but with significant numbers of spores dispersing as far as kilometres (Reed *et al.* 1988; Gaylord *et al.* 2002; Alberto *et al.* 2010). Using hydrodynamic transport models, Gaylord *et al.* (2006) predicted that selfing probabilities in the giant kelp *Macrocystis pyrifera* should range from ~20% to >40% for a typical spacing between individuals of 1–10 m, with selfing rates increasing as the spacing between individuals increases. Selfing should be most prominent in kelp forests with widely spaced individuals or in isolated patches (Gaylord *et al.* 2012). The impact of this level of inbreeding, particularly in isolated kelp forests, is not well understood. However, the costs of inbreeding appear to be significant in giant kelp and may have an impact at multiple life stages. In laboratory and outplanting experiments, Raimondi *et al.* (2004) found that inbreeding resulted in significantly lower survivorship of juveniles and adults, significantly decreased spore production and significantly fewer zygotes produced, when compared with outcrossed treatments. Their results also suggested that the impacts of ID were significantly increased by intraspecific competition. Many of the impacts Raimondi *et al.* (2004) observed occurred in the 'black box' of the microscopic stages of the *Macrocystis* life cycle, where *in situ* studies are significantly hampered by the small sizes of the organisms, sediment and organic particles, morphological similarity across taxa and otherwise challenging conditions (Schiel & Foster 2006).

In this study, we demonstrate how SGS is surprisingly weak in three stands of giant kelp, given the theorized expectations of substantial inbreeding (Gaylord *et al.* 2006). To unravel the underlying causes of this result, we employ a simulation-based search for the level of selfing and early mortality rates (assumed to be ID related) that would fit the individual heterozygosity (IH) distribution in the observed data. By doing so, we estimate the impact of lethal ID on early microscopic life stages of the haplo-diploid life cycle of kelp (e.g. in the 'black box'). As a standard for comparison, our best-fitting selfing rates can be contrasted with those derived from hydrodynamic particle transport models (Gaylord *et al.* 2006), while our ID rates can be compared to those found in a controlled cross-experiment by Raimondi *et al.* (2004). We also compare our selfing results with the results of other possible mating scenarios: full-siblings, half-siblings and first cousins. The specific goals of our study were to (i) test the hypothesis that high rates of local spore settlement (within a few metres of the parent) lead to increased inbreeding; and (ii) use simulations to determine the selfing and mortality rates (presumed lethal ID) needed to produce the levels of IH and spatial genetic structure that we observed in the field.

Materials and methods

Macrocystis life history

Macrocystis pyrifera, the giant kelp, is the largest alga in the world and forms extensive forests in the northeast and south Pacific (Wormersley 1954). Like all kelps, *M. pyrifera* has a biphasic life history, alternating between a large diploid sporophyte stage that releases equal numbers of male and female spores and a microscopic haploid gametophyte stage with separate sexes. Individual kelp can produce extremely high numbers of spores (exceeding 10^8 spores released per individual per day; Gaylord *et al.* 2002), and spores are the primary dispersive stage for giant kelp. In contrast to marine organisms with planktonic larval stages, fertilization occurs after rather than prior to dispersal (Reed *et al.* 1992). Fertilization, and the formation of the next generation of sporophytes, occurs when male and female spores settle in sufficient proximity for sperm from the male gametophyte to be able to detect pheromones released by the female gametophyte and thereby locate the egg (Boland *et al.* 1983). Because this pheromone is thought to be effective only over distances of <1 mm (Boland *et al.* 1983), successful sporophyte recruitment generally requires a spore settlement density of >1 spore/mm^2 (Reed 1990; Reed *et al.* 1991).

Sample collections

Samples for genetic analysis were collected from *M. pyrifera* at three sites, hereafter referred to as populations, along the mainland coast of the Santa Barbara Channel: Carpinteria (34º22.508N, 119º32.309W), Mohawk (34º23.672N, 119º43.779W) and Goleta (34º24.855N, 119º49.299W), all monitored by the Santa Barbara Coastal Long Term Ecological Research (SBC-LTER) project since 2001 (http://sbc.lternet.edu/). Sampling within each population was spatially explicit, with x and y coordinates recorded for every specimen sampled. For this purpose, eight parallel, sixteen metre long, east–west oriented transects were set along shore, spaced 2 m apart from each other. Given our expectation of significantly elevated selfing (and pairwise kinship) within a few metres of the parent sporophyte (Gaylord *et al.* 2006), we believe that this scale is adequate to capture any SGS in the population. Samples were collected from up to 20 individuals (at least 1 m tall, identified as having a single holdfast) per transect, distributed evenly along the transect length. If less than 20 individuals were present on a transect, then all individuals were sampled. To avoid duplicating samples between transects, all sampling took place south of each transect line.

Genotyping

Genomic DNA was extracted with the NucleoSpin 96 Plant Kit (Macherey-Nagel, Duren, Germany). All individuals were genotyped for eleven microsatellite loci (Mpy7, Mpy8, Mpy9, Mpy11, Mpy14, Mpy19, BC4, BC8, BC18, BC19 and BC25; Alberto *et al.* 2009) using forward fluorochrome-labelled primers on a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA, USA); PCR conditions are described in Alberto *et al.* (2009). Fragment length was analysed on an ABI PRISM 3130 DNA analyzer (Applied Biosystems) using the GeneScan-500 LIZ standard. Raw allele sizes were scored with STRand v.2.4.59 (Locke *et al.* 2007) and binned into allele classes using the R package MsatAllele (Alberto 2009).

Analysis

Summary statistics. We calculated summary statistics (allelic richness, observed and expected heterozygosity, F_{IS}; Table 1) using custom scripts in R (R Development Core Team 2012). To assess the impact of null alleles and scoring errors on our heterozygosity results, we used Micro-Checker v.2.2.3 (Van Oosterhout *et al.* 2004). One locus (Mpy9) was identified as having null allele problems and was not used in analysis or simulations. To test for departures from Hardy–Weinberg equilibrium in our three populations, we used a Hardy–Weinberg probability test as implemented in the software GENEPOP v.4.1.1 (Rousset 2008). A significant departure from Hardy–Weinberg equilibrium would be consistent with our expectation that selfing occurs in our three populations of *M. pyrifera*.

Table 1 Summary statistics

	A	SD (A)	H_E	H_O	F_{IS}	P
Carpinteria	12.057	0.127	0.718	0.642	0.105	<0.0001
Goleta	11.701	0.097	0.732	0.671	0.083	<0.0001
Mohawk	12.473	0.234	0.708	0.665	0.061	<0.0001

Allelic richness (A), standard deviation of the allelic richness, expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), probability of the observed F_{IS} under the null hypothesis of Hardy–Weinberg equilibrium (P).

Spatial genetic structure analysis. To test the hypothesis that high local settlement leads to increased inbreeding and increased kinship at short distances, we regressed average pairwise Loiselle's kinship (Loiselle *et al.* 1995) on log-transformed average pairwise spatial distance, between all possible pairs of individuals collected for each of the three populations. Averages were estimated based on arbitrarily defined distance classes with maximum distances of 1, 2, 4, 6, 8, 10, 15 and 30 m. A significant negative slope and significant elevation of kinship in the smallest distance class would indicate SGS. For each population, spatial locations were randomly permuted among individuals 10 000 times in order to test, for each spatial distance class, whether the observed mean kinship values were different from those expected under a random distribution of genotypes. To test the significance of the observed SGS pattern, a distribution of regression slopes was also constructed using a permutation test, and P values for the observed regression were estimated as the fraction of this distribution greater than the observed slope. All spatial autocorrelation analyses were performed using the software SPAGeDi v.1.3 (Hardy & Vekemans 2002).

Simulations. To investigate the processes that might lead to the observed heterozygosity, we simulated a variety of mating conditions and contrasted the results with the observed data. Using the complete set of observed data for a given population as a starting pool of genotypes, we simulated different data sets of complete inbreeding (selfing), full-siblings, half-siblings and first cousins at levels ranging from 0% to 100% for a single generation. We also simulated more complex situations, with various combinations of our four mating conditions. These failed to improve the results of the single-condition simulations and are not shown. For all simulations except the 100% levels, the remaining proportion of simulated individuals not meeting the simulation condition was fully outcrossed. In all simulations, we generated 10 000 simulated individuals and then calculated individual heterozygosity (the individual proportion of heterozygous loci) as a summary statistic to contrast with observed data.

In each simulation, we randomly sampled all the observed data from a single population to construct parent matrices and then randomly sampled the genotypes in these matrices to construct our offspring. Outcrossed offspring were created by sampling one allele per locus from each of two, randomly selected parental genotypes. For selfed offspring, two alleles per locus were randomly sampled with replacement from a single parent to construct a single selfed genotype. Full-siblings were created by sampling one allele per locus from each of two parents twice, to make two offspring genotypes. Half-siblings resulted from sampling single alleles from one parent into two separate offspring genotypes and sampling the remaining alleles for each offspring genotype from different parental genotypes. First cousins required two rounds of sampling. In the initial sampling, a matrix of full-siblings and a matrix of outcrossed offspring were constructed according to the rules above. In the second sampling round, one allele per locus was taken from each individual in the full-sib matrix and combined with an allele from the corresponding individual in the outcrossed matrix to create a single offspring genotype.

To fit different mating system simulations with observed data, we calculated a total absolute difference between observed and simulated proportions (summed over the eleven classes, from zero to ten heterozygous loci) for each mating system, increasing the outcrossing level by 1% steps. We also calculated the difference in means of IH between observed and simulated data for each 1% increase in outcrossing. Finally, we used a chi-square goodness-of-fit test to identify the range of simulated outcrossing percentages yielding distributions that could not be statistically differentiated from the observed values. Mean and standard error for the single best-fitting percentage of selfing with mortality was calculated from 10 000 bootstrap replicate simulations.

For all simulations, we also included a mortality function that removed highly homozygous individuals from the simulation, if those individuals were in excess relative to the left tail of the observed IH distribution. Because simulated and observed data sets differ in the mortality that potentially removes observations in the latter (affecting life stages smaller than the sampled individual sizes), we considered the excess of the most homozygous individuals in simulations as the proportion that dies due to lethal ID during the early life stages (Raimondi *et al.* 2004). Thus, the proportion of individuals removed was considered as a mortality probability that was fitted with a logistic regression using IH as the independent variable and location as categorical predictor to understand whether there were differences between populations. We also derived a measure of fitness for each IH class, based on the proportion of individuals from each class that survived. We estimated ID for the best-fitting selfing rate for each population as the difference between outcrossed and inbred fitness values (in this case, their survival probabilities) divided by the outcrossed fitness value (Lande & Schemske 1985). All statistical analyses and simulations were conducted in R (R Development Core Team 2012).

Results

Summary statistics

The 10 microsatellite loci used were able to discriminate all sampled sporophytes, yielding a total of 163, 161 and 181 individual genotypes for Carpinteria, Goleta and Mohawk, respectively. One locus (Mpy9) was identified as having significant null allele problems and was dropped from further analysis and was not used in simulations. All three populations displayed a significant positive departure from Hardy–Weinberg equilibrium ($P < 0.001$; Table 1). In all three cases, this departure was due to a deficit of heterozygotes relative to the random mating expectation, consistent with the expectation of selfed offspring in our data set.

Spatial genetic structure analysis

Carpinteria showed no significant relationship between kinship and interpair distance ($b = 0.000672$, $P = 0.6042$; Fig. 1, Table S1, Supporting information). Goleta had a significant negative slope ($b = -0.00298$, $P = 0.0125$) and a significantly higher kinship coefficient than expected by chance for the first (0–1 m) spatial class ($P < 0.0001$; Fig. 1, Table S1, Supporting information). Mohawk had a significant negative slope ($b = -0.0019$, $P = 0.0445$) and a significantly higher kinship than expected by chance in the 1- to 2-m distance class ($P = 0.05$), but not in the 0- to 1-m distance class ($P = 0.8$; Fig. 1, Table S1, Supporting information).

Simulations

Density plots of our observed data showed a single normal or near-normal curve centred between six and seven heterozygous loci (Fig. 2). As the level of selfing increased from zero (Fig. 2, first column), a second mode appeared to the left of the initial one, centred at around three heterozygous loci. This second mode grew, until the initial mode disappeared (between 60% and 100% selfing). Increasing levels of full-siblings (Fig. 2, second column) in the simulated population from 0% to 100% slightly shifted the mean value of heterozygosity in the simulated population to the right of the observed data, that is, towards higher numbers of heterozygous loci. Increasing the level of half-siblings (Fig. 2, third column) shifted the mean value of

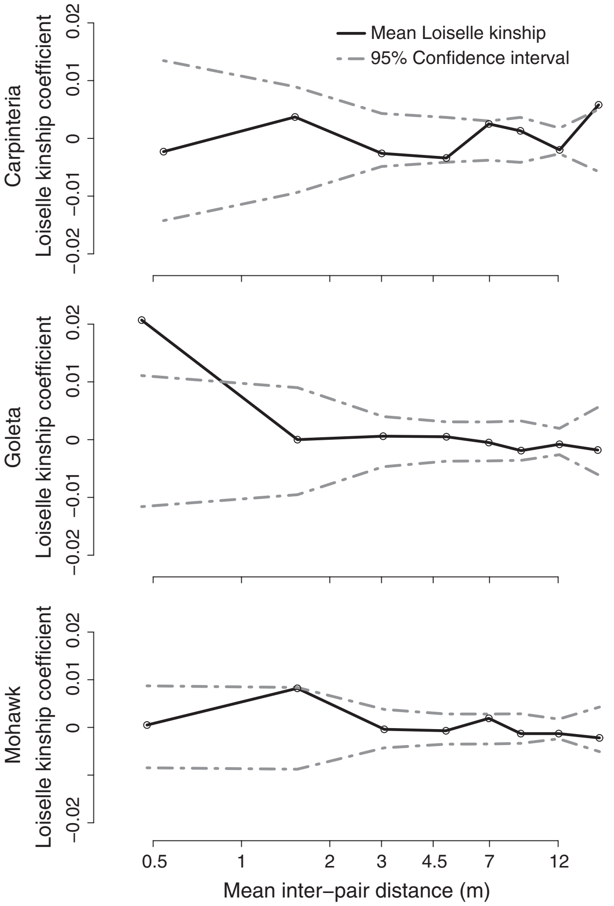

Fig. 1 The relationship between pairwise Loiselle kinship and pairwise geographical distance between individuals for the three giant kelp populations. Spatial distance class limits are defined as 1, 2, 4, 6, 8, 10, 15 and 30 m. Mean pairwise distances are shown on a log scale.

heterozygosity of the simulated population to the right of the observed and to a greater extent than seen in the full-sibling simulations. Changing the level of first cousins (Fig. 2, fourth column) seemed to have little effect on the distribution of heterozygous loci in the simulated population.

The difference between observed and simulated IH means (Fig. 3) illustrated the important difference between selfing and the other three mating conditions. In all three populations, only selfing lead to an excess of homozygous individuals relative to the observed population as the percentage of nonoutcrossed offspring increased. All three other mating conditions maintained a deficit of homozygous individuals in the left tail of the IH distribution across the full range of simulated percentages. Thus, only selfing produced offspring that could justifiably be culled by lethal ID to produce a better fit between simulated and observed distributions.

The total absolute difference in proportions between the simulated and observed populations (Fig. 4, Table 2) showed the relationship between goodness of fit and the percentage of individuals meeting the mating condition. Only selfing with mortality showed an improved fit at intermediate percentages of nonoutcrossed offspring. For selfing with mortality, the best fit was achieved at a mean of 37.61% selfing for Carpinteria (SE: 0.020), 43.93% for Goleta (SE: 0.003) and 32.21% for Mohawk (SE: 0.008). The fit for full- and half-siblings became progressively worse at higher percentages of simulated siblings in the data set, while the fit for first cousins was essentially unchanged across all the simulated percentages. Goodness-of-fit test results indicated that only the selfing-with-mortality simulation was consistently similar to the observed data (chi-square not significantly different from zero; Table 2). For Carpinteria, levels of selfing with mortality between 16% and 58% were not significantly different from the observed population. For Goleta, values of selfing with mortality between 18% and 62% did not differ significantly from the observed population. Mohawk showed the widest spread of nonsignificant values of selfing with mortality, with values between 2% and 65% not significantly different from the observed population. The fits of all but one of the other simulations (Mohawk selfing, no mortality) were significantly different from the observed distribution for all simulated proportions. For Mohawk selfing simulations without mortality, selfing levels between 0% and 12% were not significantly different from the observed population distribution.

In our simulations, the most homozygous classes (approximately 0–4 heterozygous loci*individual^{-1}) were overwhelmingly composed of selfed offspring. Thus, mortality due to high level of homozygosity impacted selfed offspring disproportionately. For Carpinteria, the mortality rate for selfed offspring was 69% vs. <0.1% for outcrossed; for Goleta, 73% of selfed offspring were removed vs. 0.3% of outcrossed offspring; for Mohawk, the comparison was 72% vs. 0.1%. As a result, the effective selfing rate (i.e. the rate measured among surviving adults) was much lower than the actual selfing rate; the effective selfing rates for Carpinteria, Goleta and Mohawk were 16%, 18% and 12%, respectively.

Logistic regression indicated a significant negative relationship between heterozygosity and the odds of dying ($P < 0.0001$, Fig. 5, Table S2, Supporting information). There was no significant interaction between population and heterozygosity ($P = 0.32$), and therefore, the decay in the probability of dying was assumed to be similar across populations. For each additional heterozygous locus added (because we used a sample of ten loci, this corresponds to an estimated increase of 10% in

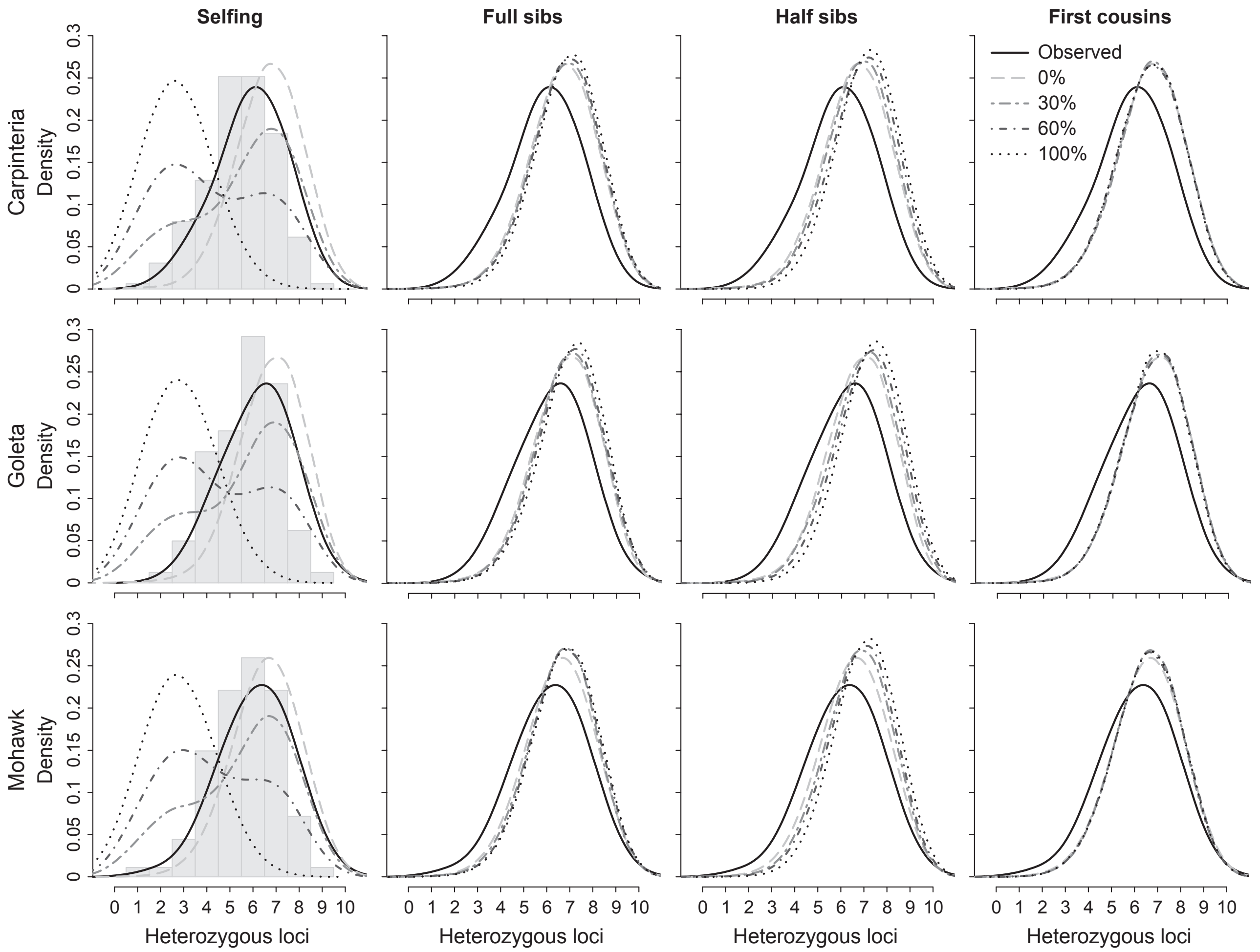

Fig. 2 Individual heterozygosity distributions for simulated and observed data from three giant kelp populations. Data are discrete (proportion of individuals in each heterozygosity class), but are presented as smoothed density lines for ease of visualization. Histograms of observed samples are shown in the first column. Different lines are labelled with the proportion of offspring simulated under that mating system (e.g. column one selfing); the remaining individuals are outcrossed.

whole-genome heterozygosity), the odds of dying were reduced by 91.6%. However, differences between populations were observed, and Carpinteria had significantly lower mortality probability, for the same level of heterozygosity, than either Goleta (odds of dying 4.5 times greater, $P = 0.00335$) or Mohawk (odds of dying three times greater, $P = 0.02596$), while the odds of dying for Goleta and Mohawk were not significantly different from one another ($P = 0.432$). The difference in intercept is visible as a leftward shift in the Carpinteria line, when data are plotted as probability of dying at a given level of heterozygosity (Fig. 5). This also seems to be reflected in our calculations of ID, where Carpinteria showed lower ID (0.6932) than either Goleta (0.7275) or Mohawk (0.7203). For all three populations, the fitness (survivorship) of individuals with 0 or 1 heterozygous loci was 0%, and for individuals with ≥5 heterozygous loci was 100%. For 2, 3 and 4 heterozygous loci per individual, fitness varied across populations. For Carpinteria, fitness was 7%, 28% and 74%; for Goleta, 0%, 8% and 37%, and for Mohawk, 14%, 10% and 40% for 2, 3 and 4 heterozygous loci, respectively.

Discussion

The links between life history, dispersal, self-fertilization and fitness, while potentially of immense importance, have rarely been addressed, as noted in Raimondi *et al.* (2004). One of the rare studies that attempted to connect life history, selfing and fitness found high rates of selfing but little or no apparent decrease in fitness in the sea palm kelp, *Postelsia palmaeformis* (Barner *et al.* 2011). Unusual among marine species, *P. palmaeformis* is characterized by significant genetic differentiation at the metre scale, due to a very low capacity for dispersal (limited to 1–3 m or less;

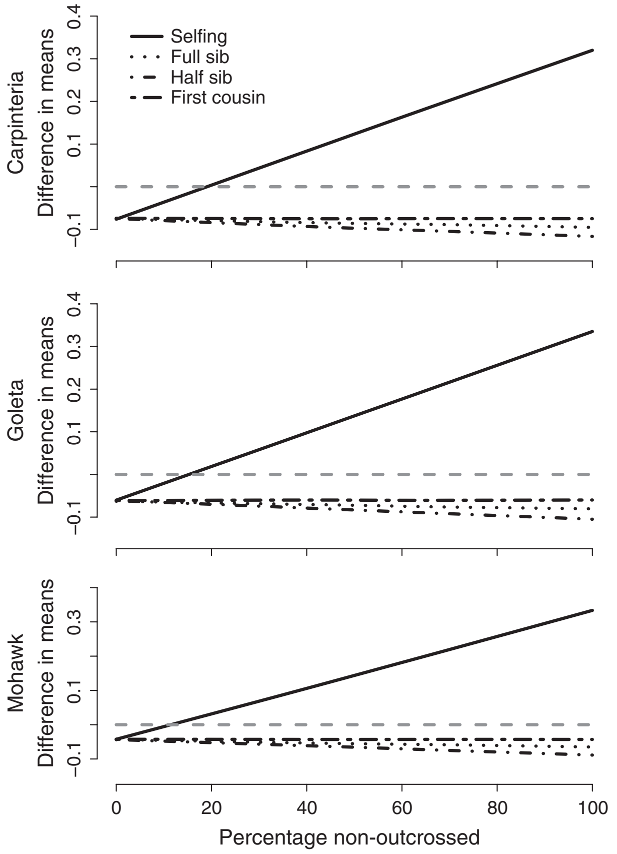

Fig. 3 Difference in means between simulated and observed individual heterozygosity distributions as a function of increasing proportion of nonoutcrossed offspring. Mating systems (e.g. selfing) are indicated by different line styles. Zero difference in means is indicated by the grey dashed line.

Kusumo *et al.* 2006). Although *Macrocystis pyrifera* has a significantly greater capacity for dispersal than *P. palmaeformis*, empirical and modelling studies have suggested that ~50% of *Macrocystis* spores disperse <100 m in flow conditions typical of kelp forests in southern California (Gaylord *et al.* 2002). Raimondi *et al.* (2004) and Gaylord *et al.* (2006) reflected on the relatively short-range dispersal of *Macrocystis* spores, along with the perceived short lifespan of most resultant gametophytes in the field (Deysher & Dean 1986; Reed *et al.* 1994, 1997) and hypothesized that self-fertilization at levels of 10% or more was a likely consequence.

In laboratory and field experiments, Raimondi *et al.* (2004) found that there were severe fitness consequences to self-fertilization in *M. pyrifera,* and their results suggested three important attributes of these consequences: (i) there was no self-incompatibility at the point of syngamy; (ii) costs were manifest very early in the life history (during fertilization or very early survival) and very late in the life history (reproduction); and (iii) fitness consequences appeared to be amplified

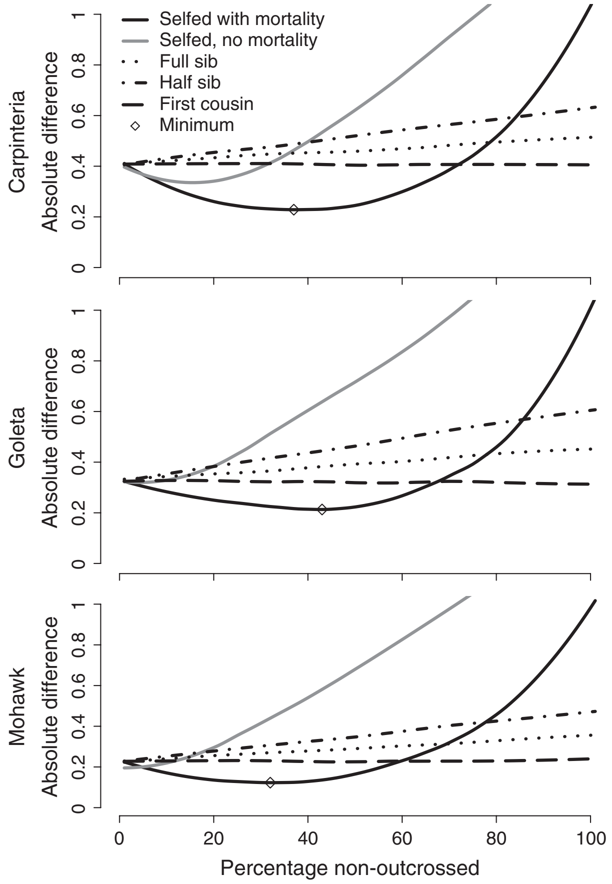

Fig. 4 Absolute proportion difference between simulated and observed individual heterozygosity distributions as a function of increasing percentage of nonoutcrossed offspring. Mating systems are indicated by different line styles. Best fit is indicated by the diamond on the Selfed w. Mort. line for each population.

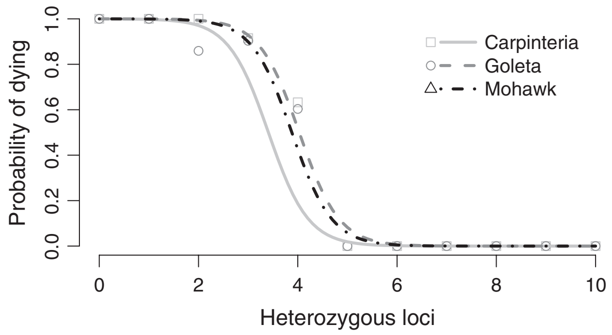

Fig. 5 Probability of dying as a function of the number of heterozygous loci per individual giant kelp, as modelled using a logistic regression. The decrease in the probability of dying with increasing heterozygosity was not significantly different among populations. Individuals from Carpinteria had significantly lower odds of dying than Mohawk and Goleta for the same level of individual heterozygosity.

under competition with outcrossed individuals. Gaylord *et al.* (2006, 2012) followed this work and developed a hydrodynamic model to predict that levels of self-fertilization in the field could reach 20–40% with increasing rates of selfing associated with decreasing density of plants. Such assumptions (or predictions) concerning levels of selfing could not be tested, as high resolution genetic markers were unavailable.

There are three key and essentially opposing hypotheses that follow from the previous work described above, which we tested in the current study: (i) self-fertilization is rare in nature, and selfed sporophytes do not exist in the field; (ii) self-fertilization is relatively common and consistent with modelled estimates, and selfed sporophytes are common in the field; and (iii) self-fertilization is relatively common and consistent with modelled estimates, but because of costs incurred at the time of fertilization or very early increased mortality in the presence of outcrossed individuals, very few if any selfed sporophytes (sampled as adults) occur in the field. Of these hypotheses, only #2 is relatively easy to test using conventional genetic approaches. In giant kelp, given the expected correlation between selfing and aggregated uniparental spore settlement, we would expect to measure a significantly elevated kinship in the smallest spatial distance classes (as described in the Methods), and estimates of selfing calculated from the $S = 2F_{IS}/(1 + F_{IS})$ (Li 1955) relationship should match expectations from hydrodynamic models. In our findings, while both Goleta and Mohawk had significantly negative slopes for modelled kinship vs. interpair distance, only Goleta showed a significant increase in kinship in the first spatial distance class; in all three populations, the effective selfing rate underestimated model predictions. Thus, we found no evidence to support the notion that selfed *Macrocystis* sporophytes are common in the field (i.e. hypothesis #2).

Hypotheses #1 and #3 are very difficult to differentiate using conventional approaches. Hypothesis #1 seems less likely to be true, given that no barriers to self-fertilization are known in *Macrocystis* (Raimondi *et al.* 2004) and the expectations from hydrodynamic modelling of 20–40% selfing (Gaylord *et al.* 2006). Although our consistently significant heterozygote deficiency in 1+ metre-sized sporophyte populations provide a hint that some type of inbreeding may be occurring, which is still detectable in adult sporophytes, the key differences between the two hypotheses result from unknown processes occurring during the microscopic stages of the *Macrocystis* life cycle. Simulations using realistic mating parameters represent a way to estimate these unknown processes. Distributions of IH without selfing (i.e. full-sibs, half-sibs, cousins) at all three study populations developed very differently than simulations with selfing as the level of outcrossing decreased. Only in simulations with selfing and mortality did mean heterozygosity shift towards that of the observed population. In other simulations, the mean shifted to the right of the observed or was unchanged. Similarly, goodness of fit only improved significantly in simulations with selfing and mortality. In other cases, the fit either remained the same (first cousins) or became worse as the level of outcrossing decreased (full- and half-sibs). These results show that the genetic signature of adults in a population with no selfing was clearly distinguishable from that for adults in a population with considerable selfing. They also suggest that there is considerable purging of selfed individuals at very early life stages. These simulation results are thus most consistent with hypothesis #3 that selfing does occur at rates similar to those predicted by hydrodynamic models, but that many of these selfed individuals are lost to the population prior to reaching adulthood.

Table 2 Simulation results

	Carpinteria	Goleta	Mohawk
Selfing plus mortality			
Best fit (%)	37.61	43.93	32.21
Standard error	0.020	0.003	0.008
Absolute difference	0.227*	0.209*	0.123*
NS range (%)	16–58	18–62	2–65
Selfing no mortality			
Best fit (%)	14	3	0
Absolute difference	0.335	0.32	0.195*
NS range (%)	N/A	N/A	0–12
Full-sibs			
Best fit (%)	0	0	0
Absolute difference	0.411	0.333	0.224
NS range (%)	N/A	N/A	N/A
Half-sibs			
Best fit (%)	0	0	0
Absolute difference	0.406	0.326	0.229
NS range (%)	N/A	N/A	N/A
First cousins			
Best fit (%)	51	100	45
Absolute difference	0.404	0.313	0.225
NS range (%)	N/A	N/A	N/A

Best fit indicates the percentage with the lowest absolute difference between simulated and observed distributions. For Selfing plus mortality, this value is the mean lowest value from 10 000 simulations per population. Standard error of the best fit calculated from 10 000 simulations. Absolute difference is the total difference between observed and simulated distributions at the best-fitting percentage. NS range (%) indicates the range of percentages for which the chi-square values were statistically indistinguishable from zero (N/A indicates that all values were significant).

*Nonsignificant chi-square difference between simulated and observed distributions.

Spatial genetic structure analysis

If selfed individuals are formed near the parental spore release site and a significant proportion of them survive to become sporophytes, then we would expect to see a significant increase in kinship at the smaller spatial distance classes. This was not generally the case, suggesting that adult sporophyte populations are characterized mostly by outcrossed individuals (Vekemans & Hardy 2004), although two populations showed significant negative slopes in kinship vs. distance models (Goleta and Mohawk), and one showed elevated kinship in the first distance class (Goleta). Predictions based on hydrodynamic transport models suggest that we should find a 20–40% selfing rate, with most selfed individuals settling within a few metres of the parent plant (Gaylord *et al.* 2006). Thus, our results imply a significant mismatch between expectations based on previous studies and the observed pattern.

Simulations

In the absence of mortality, all simulations were a poor fit to the observed data (all chi-square comparisons significant). Of the simulated mating schemes, only selfing produced an excess of highly homozygous individuals relative to the observed data; these individuals would be susceptible to mortality due to inbreeding depression. Thus, only selfing with mortality resulted in distributions of heterozygosity that were not significantly different from the distributions of the sampled population. Best fits were seen when selfing was simulated at levels between 32% and 44%, which is remarkably similar to 30–40% selfing rate predicted by Gaylord *et al.* (2012) for the densities of sporophytes that we observed. The need for a mortality factor to achieve meaningful improvements in fit as levels of selfing changed also fits well with expectations based on prior work. Using empirical data from Raimondi *et al.* (2004) for comparing sporophyte densities resulting from outcrossed and selfed treatments and assuming that the number of sporophytes observed in their outcrossed treatment is the null expectation for selfing treatments (i.e. the number that would survive if not for ID-related mortality), we estimated that selfed sporophytes had a mortality of 68% after 18 days of growth. Our study matches these results from Raimondi *et al.* (2004) nearly perfectly; for the three populations studied in our simulations, early mortality (occurring before sampling size was reached) among selfed individuals ranged from 69% to 73% relative to outcrossed individuals.

Density-dependent mortality of early life stages is undoubtedly a significant force in structuring adult populations of *Macrocystis*. With spores needing to settle at densities of >1 spore/mm^2 for fertilization to take place (Reed 1990) and final adult sporophyte densities estimated to average 2–4 per 10 m^2 (Dayton *et al.* 1984, 1992; Graham *et al.* 1997), survivorship to adulthood is a vanishingly small 0.00003 per cent of potentially available zygotes. Strong competition for light and space regulates the carrying capacity of kelp forests (Graham *et al.* 2007), and the reproductive excess produced by *Macrocystis* is considerable.

As previously suggested by Raimondi *et al.* (2004), we estimated high levels of ID for all three of our study populations, suggesting little purging of deleterious alleles by *Macrocystis*. The maximum effect of purging is expected with highly recessive alleles and hard selection (Whitlock 2002). In the case of *Macrocystis*, density-dependent mortality due to intense competition for space removes >99% (as calculated above) of all individuals, regardless of the presence of selfed offspring. The higher mortality of selfed individuals, relative to their outcrossed competitors, should fall within this range of normal mortality, leading to soft, not hard, selection. Furthermore, given the intense competitive atmosphere of the kelp forest, there is no reason to assume that the competitive disadvantage of homozygous individuals requires highly deleterious alleles. Instead, inbreeding theory would lead us to predict that ID in *Macrocystis* is the result of numerous rare, mildly deleterious alleles that are very difficult to purge (Charlesworth & Willis 2009). The large effective population sizes of *Macrocystis*, which may exceed 2500 individuals under realistic conditions (Alberto *et al.* 2010), may also impede the effectiveness of purging by constantly inputting new mildly deleterious mutations into the population (Charlesworth & Willis 2009).

Conclusions

Results of our simulations suggest that the lack of a significant strong relationship between interindividual kinship and distance that we observed is best explained by strong effects of ID acting on early life stages rather than a low selfing rate. Our findings of approximately 32–44% selfing, depending on the kelp forest, fit well with model predictions of a significant fraction (20–40%, Gaylord *et al.* 2006) of new sporophytes resulting from self-fertilization. However, our models suggest that few of the most inbred, highly homozygous individuals survive to adulthood in any of the three kelp forests. This is supported by estimates of the densities of microscopic gametophytes in the forest (typically 10–100/mm^2; Reed *et al.* 1997) vastly exceeding the densities of macroscopic sporophytes, such that overall survivorship is around 0.00003 per cent. Under these conditions of intense competition, the high costs of selfing identified in *Macrocystis* (Raimondi *et al.* 2004) are likely to render selfed offspring incapable of competing against outcrossed competitors. Our model results suggest that relative survivorship among selfed *Macrocystis* sporophytes in the 'black box' is very low (27–31%) and that for highly homozygous individuals, survivorship approaches zero.

It must be noted, however, that simulations that achieved a good fit to the observed data dealt exclusively with selfing, and not lesser degrees of inbreeding. While costs appear to be clear and immediately catastrophic for selfed individuals, biparental inbreeding could (and likely, would) cause a temporal propagation of ID that could eventually yield population consequences. Selfing would be expected to occur commonly in populations regardless of their age structure, with rates varying due to individual densities (with the caveat that selfing might occur at higher rates in older populations, due to lowered density). Because density-dependent competition removes huge numbers of individuals between zygotic and visible sporophyte stages regardless of inbreeding status, the mortality of selfed individuals is likely to fall within the range of normal background mortality, resulting in soft selection (Ridley 2004), and no particular population consequences. Inbreeding that arises from consanguineous mating or due to population isolation and drift (Wright 1984), however, may be inevitable in *Macrocystis pyrifera*, as local spore production and settlement is likely to be vastly higher than spores arriving from external populations, particularly in isolated populations. It is also likely that purging will not effectively remove deleterious alleles from *Macrocystis* populations, as the effect of most deleterious alleles is likely to be relatively weak, and populations continue to receive input of deleterious alleles through mutation and migration (Keller & Waller 2002). Under such conditions, the effects of inbreeding can be expected to increase with population age and eventually negatively impact population persistence.

Simulation studies are a powerful alternative approach to estimate otherwise difficult-to-measure life history parameters. Our study used a combination of empirical and simulation analyses of microsatellite data to characterize a cryptic mating system and early mortality rates in *M. pyrifera*. We found that the observed pattern of individual heterozygosity was likely driven by selfing at rates of 32–44%, with little or no survivorship of the most homozygous individuals. These estimates were highly consistent with those from earlier theoretical and empirical studies (Raimondi *et al.* 2004; Gaylord *et al.* 2006). Although such detailed contextual information may not be available for many study systems, our results should help to validate simulation approaches in other systems, even in the absence of data, as a means to unravel otherwise challenging parameters of many life cycles.

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M.L.J. assisted with study design, designed R code, ran models, performed statistical analyses and drafted the manuscript. P.T.R. and D.C.R. designed the study and provided the samples. N.C.C. carried out microsatellite PCR amplifications and preliminary analysis. E.A.S. assisted with study design and project coordination. F.A.A. designed the study, provided samples, assisted with analysis and R coding, coordinated the project and helped to draft the manuscript. All authors edited and approved the final manuscript.

Data accessibility

R scripts: available on DRYAD (doi:10.5061/dryad.s1b07). Sample locations and microsatellite data: available on DRYAD (doi: 10.5061/dryad.s1b07) and through the data portal on the Santa Barbara Coastal Long Term Ecological Research website: (http://sbc.lternet.edu//data/index.html).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Spatial genetic structure results.

Table S2 Logistic regression input data.