

The mixed mating system of the sea palm kelp *Postelsia palmaeformis*: few costs to selfing

Allison K. Barner^{*,†}, Catherine A. Pfister and J. Timothy Wootton

Department of Ecology and Evolution, University of Chicago, 1101 East 57th Street, Chicago, IL 60637, USA

Naturally isolated populations have conflicting selection pressures for successful reproduction and inbreeding avoidance. These species with limited seasonal reproductive opportunities may use selfing as a means of reproductive assurance. We quantified the frequency of selfing and the fitness consequences for inbred versus outcrossed progeny of an annual kelp, the sea palm (*Postelsia palmaeformis*). Using experimentally established populations and microsatellite markers to assess the extent of selfing in progeny from six founding parents, we found the frequency of selfing was higher than expected in every population, and few fitness costs were detected in selfed offspring. Despite a decline in heterozygosity of 30 per cent in the first generation of selfing, self-fertilization did not affect individual size or reproduction, and correlated only with a marginally significant decline in survival. Our results suggest both that purging of deleterious recessive alleles may have already occurred and that selfing may be key to reproductive assurance in this species with limited dispersal. *Postelsia* has an alteration of a free-living diploid and haploid stage, where the haploid stage may provide increased efficiency for purging the genetic load. This life history is shared by many seaweeds and may thus be an important component of mating system evolution in the sea.

Keywords: self-fertilization; reproductive assurance; inbreeding; heterozygosity; microsatellites; parentage analysis

1. INTRODUCTION

Naturally isolated plant populations have conflicting selection pressures for successful reproduction versus avoidance of selfing. Selfing decreases heterozygosity in the population, which reveals higher frequencies of deleterious phenotypes generated by recessive alleles. Frequent self-fertilization decreases individual fitness, which causes population-level inbreeding depression and progeny discounting that then render the population more vulnerable to extinction [1–4]. Selfing thus adversely affects populations not only through decreases in individual fitness, but through the cumulative impact of these decreases, manifested in inbreeding depression. Nonetheless, species that have both restricted dispersal and limited reproductive opportunities (notably annuals) may be under strong selection to self, despite some costs via inbreeding depression.

Inbreeding depression can be a symptom of both population-level dynamics and individual traits. Selfing and associated inbreeding depression can dominate the mating system of small, fragmented populations that are characterized by patchy distributions and low numbers of actively reproducing individuals [5]. In the plant *Gentiana pneumonanthe*, inbreeding depression contributes to decreased reproductive success, decreased maximum population size, lower growth rate and increased extinction risk [6]. Inbreeding depression can also manifest in individual traits; for example, inbreeding

adversely affected larval survival, adult longevity and egg-hatching rate in a metapopulation of the butterfly *Melitaea cinxia* [2]. Using seed production, probability of flower formation, survival probability and reproduction to define fitness, Dudash [7] found that the fitness of outcrossed individuals was more than twice that of inbred individuals in experimental populations of self-fertile *Sabatia angularis* L. Whether by influencing individual- or population-level traits, the impact of inbreeding depression can lead to increased probability of population extinction [8].

However, individuals will sometimes self regardless of detrimental phenotypic effects [9–11]: an evolutionary trade-off described by the reproductive assurance hypothesis [12,13]. When species are limited in their opportunities to breed by seasonality or short lifespan, selfing can be a form of reproductive assurance [14,15]. Some plant and algae populations show little evidence of evolutionary or reproductive barriers to selfing [16–19]. Even highly outcrossing populations and those with specialized pollination systems can have mixed mating systems with moderate selfing rates, though inbreeding is high [20,21]. Although mixed mating systems have been documented in a number of species, relatively little is known about the frequency of selfing and the associated impact on fitness of selfed against outcrossed progeny in natural settings.

Seaweeds represent an especially interesting group of organisms to ask about the frequency and costs of inbreeding. Some abundant brown algae species (such as the rockweed species of temperate shores) show high

* Author for correspondence (barneral@science.oregonstate.edu).

† Present address: Zoology Department, Oregon State University, 3029 Cordley Hall, Corvallis OR 97331, USA.

levels of selfing [17,18], while other species such as the giant kelp (*Macrocystis pyrifera*) self while also showing conspicuous inbreeding depression [22]. Despite estimates of spore dispersal for kelp in excess of 1 km [23,24] and spore viability for several days [25], many kelp spores settle near to the parental source, a feature that may lead to selfing in nature. The alteration of generations in seaweed also provides a situation where purging deleterious recessives can be highly effective in the haploid stage.

To explore how the benefits or costs of selfing play out in nature, we asked whether a species with naturally patchy population structure across its geographical range exhibits barriers to selfing or shows deleterious fitness-related consequences of selfing. By establishing experimental populations in their natural habitat and using previously developed molecular markers [26], we describe the mating system of *Postelsia palmaeformis* (a species with limited dispersal), quantify its degree of selfing and determine the consequences of selfing to progeny fitness.

2. MATERIAL AND METHODS

(a) *Study system*

Postelsia palmaeformis (henceforth *Postelsia*) is a patchily distributed annual kelp that alternates between a haploid gametophyte stage and a diploid sporophyte stage. These kelp populations are restricted to the most wave-exposed sites in the rocky intertidal shores of the northeast Pacific [27–29]. *Postelsia* does not have resting stages, based on our field observations over 10 years, and thus persistence depends on reproductive success in each year. The diploid *Postelsia* sporophyte becomes conspicuous between late winter and early spring, and develops long, deeply ridged, leaf-like blades (fronds) from which the spores are released in late summer [27]. The sporophyte's fronds channel and concentrate haploid spores nearby, creating naturally dense patches fragmented from other clusters in the population [28]. Haploid spores are flagellated and develop into either male or female gametophytes at equal ratios in laboratory culture [30]. Motile sperm from male gametophytes travel to the sedentary female, a process mediated by pheromones [31]. Fertilization produces the recognizable diploid form of the sporophyte. Because the duration from spore production to conspicuous diploid extends from August to the following late winter and spring and is microscopic, the balance of time spent either as a gametophyte or as a sporophyte is poorly understood. *Postelsia* has restricted dispersal, typically 1–3 m from a parental source [28,32]. Most *Postelsia* individuals are highly related within clusters, with some having identical genotypes [33], and populations of *Postelsia* as little as 5 m apart are genetically differentiated [26]. From both observational and genetic data, we conclude that spores and sperm travel little from the parent plant. Establishment at new sites occurs when a dislodged individual or clump of individuals in reproductive condition is stranded in open space in the intertidal, where log damage or wave action has removed some of the mussel bed [32]. Only a single fertile plant can generate enough spores to establish a population [34], setting the stage for fitness consequences of self-fertilization. Thus, as an annual with a naturally fragmented population structure, *Postelsia* makes an excellent study system to examine questions about the prevalence of selfing and its fitness effects over time.

(b) *Experimental populations*

To examine the extent of selfing in natural conditions, seven experimental populations of *Postelsia* were analysed (abbreviated by locale; table 1). Between 1999 and 2006, a number of experimental patches were founded on Tatoosh Island at the northwest tip of the Olympic Peninsula, Washington (48°24' N, 124°44' W), as part of an ongoing experiment to test the effects of population size and genetic background on extinction risk. We used seven experimental populations that were founded with an individual from each of six different locations on Tatoosh Island and Cape Flattery known to have distinct genotypes (Finger, North Island, Rainbow Rock, West Rocks, East Cape Flattery, Slant Rock; see map in [26]). Because these experimental populations are being used to assess population size effects on extinction risk, they were thinned in their first year to population sizes of 20 and 50, resulting in 0–204 F₁ offspring removed in April or May of the first year only (table 1). Although we have established 18 such populations since 1999, we extensively genotyped only these seven because they provided sufficiently distinct genetic markers for the resolution necessary for parentage assignment, statistical analyses for inbreeding depression and tests for local adaptation.

Populations were established at south- and west-facing sites on Tatoosh known as the Finger and Strawberry Island ([34]; figure 1). Owing to both the constraints on the number of suitable sites for experimental populations and the fact that only approximately half of our attempted experimental populations established in any year, we initiated populations repeatedly from 1999 to 2006. Thus, although all experimental populations were not growing synchronously, they were all established with individuals from the same six founder populations that are known to differ genetically [26].

Using a technique adapted from Paine [34], the experimental populations were founded in the intertidal zone, using wire baskets to hold reproductive individuals for an extended period on sites that were cleared to establish a space for *Postelsia* progeny (gametophytes and sporophytes) to grow. Each experimental patch was founded with the fertile fronds (blades) and a section of the stipe from six individuals that were placed haphazardly into a chicken-wire packet (15 × 20 cm) in August. When in the packets, fronds of individuals overlapped and intertwined extensively. The packet was then attached to the rock in a cleared circle (1 m diameter) in the mussel bed at an approximate tidal height of 2 m above mean lower low water. The wire packet persists for at least four weeks, during which time the spores drop from the parent plants to the cleared substrate. The wire eventually disintegrates, leaving behind a population of developing microscopic gametophytes. Male gametophytes produce sperm that fertilize female gametophytes, although the extent of sperm movement in the field is unknown. The diploid sporophyte then grows into the easily recognizable kelp by the following March to June. We did not record the relative position of any of the six founders within a wire packet and assumed that, because an individual could be at most the basket dimension (15–20 cm away) from another, equivalent fertilization probabilities were maintained among all gametophytes. We also assumed that all founder individuals had approximately equivalent fecundity.



Figure 1. A map of the placement of the seven experimental populations of *Postelsia* at Tatoosh Island, Washington, USA.

Table 1. Details on experimental patches. Information about the seven patches used in the study: the year that they were founded; their initial population size and number removed to obtain it their population size in the first, second and third generations (F_1 , F_2 , F_3); the rate and frequency of selfing; and the mean H_t across individuals in F_1 . There was no correlation between F_2 or F_3 population size and frequency of selfing in the F_1 generation ($r = -0.567$, $t = 1.53$, d.f. = 5, $p = 0.185$; $r = -0.555$, $t = 1.49$, d.f. = 5, $p = 0.195$, Pearson's product-moment correlations, respectively). There was also no correlation between the F_2 or F_3 population size and mean H_t in the F_1 generation ($r = 0.481$, $t = 1.23$, d.f. = 5, $p = 0.274$; $r = 0.187$, $t = 0.426$, d.f. = 5, $p = 0.688$).

population	year established	initial count (removed)	F_1 count	F_2 count	F_3 count	frequency of selfing (F_1)	H_t in F_1
AG4	2006	20 (0)	15	23	10	0.33	0.311
G1	2006	50 (35)	45	125	45	0.44	0.294
G2	2003	50 (0)	46	54	4	0.63	0.217
G4	2002	50 (204)	12	1	0	1.00	0.046
G6	1999	50 (162)	47	75	79	0.58	0.198
GC3	2001	50 (71)	33	72	165	0.33	0.237
NF2	2006	20 (2)	16	0	0	0.73	0.222

The sporophyte progeny (F_1) were censused through spring and summer. These experimental populations mimic the natural fragmentation of *Postelsia* populations in the intertidal and allowed us to test for the dynamics of self-fertilization through analysis of parentage and quantifiable individual traits. Some individual traits could be measured throughout the summer (size, survivorship, density), while reproduction was assessed only near the end of the growing season, when the alga was mature. Some individuals may have become reproductive after this assessment but would have had a much reduced temporal window to release spores. Each month, we estimated alga size (as stipe diameter), assessed survivorship and mapped each plant using polar coordinates. Independent measures of individual plants show that stipe diameter explains 94 per cent of the variation in the total photosynthetic surface area as estimated from a LiCor area meter ($R^2 = 0.94$, $n = 60$, $p < 0.001$); thus, the diameter of the stipe at the base is a quick and accurate estimate of plant size. Individual polar coordinates were

quantified for each plant based on two stainless steel reference screws adjacent to each population, giving us spatial coordinates and thus density estimates over both individual-neighbourhood and population-level scales. The spatial coordinates also allowed us to track individual plants without using tags that might compromise survivorship. We used ARC GIS to map individuals within a patch and quantify Thiessen polygons [35,36], where the polygons are defined by lines drawn equidistant between each plant. Thus, large Thiessen polygons characterize isolated plants and polygon areas are a comparable metric of local density among populations. By collecting spatially explicit data, we estimated the degree of crowding among plants, a potentially important factor for growth and reproduction. Survivorship was assessed over each interval, and individual reproduction by the end of the growing season (August or September) was inferred based on the presence of darkened sporangia on the fronds. Although the presence of spores on the frond did not indicate progeny viability, it did indicate whether the alga was capable

of reproduction. In sum, we have estimates of individual size, growth, local density and whether an individual reproduced to link with genetic data.

(c) *Genetic data for Postelsia*

Tissue samples were collected from the basal meristem area of fronds for genetic analysis from all founding parents and progeny. While founder tissue was collected prior to patch establishment, progeny tissue was collected from individuals in July of each year and air-dried. Genetic analysis was performed on all individual tissue samples using nine microsatellite markers that had 3–13 alleles per locus and expected heterozygosity of 0.065–0.789 [37]. Some microsatellite loci deviated from Hardy–Weinberg equilibrium (HWE; low heterozygosity), a result expected in a species with inbreeding [37]. Among the source sites that we used, one to four of the nine loci were out of HWE, though the identity of the loci with heterozygote deficits differed among source populations [26]. PCR analysis was carried out at the DNA Core Sequencing Facility at the University of Chicago (Chicago, IL) and at the Field Museum Pritzker Laboratory for Molecular Systematics and Evolution (Chicago, IL). Genotypes were assigned to individuals using hand-scored GENE Mapper chromatograms.

(d) *Statistical analysis of parentage*

We estimated the frequency of self-fertilization by assigning parentage using the microsatellite information and a parentage assignment program, CERVUS [38]. CERVUS uses maximum likelihood to determine a statistical probability to any parental assignment by taking two parentage hypotheses and testing them against each other. A likelihood score is calculated for each parent at each locus, and then summed to find a cumulative likelihood score (LOD; logarithm of the likelihood ratio), where the candidate parent with the highest LOD score is the most likely parent [39]. The likelihood difference between the first and second most likely parent (Δ) is compared with a simulated probability distribution to create a confidence interval for which parentage is assigned with a level of statistical confidence. CERVUS assigns confidence intervals to parentage assignment based on these probability distributions. When CERVUS's parentage assignment was not within either of the two confidence ranges given by the probability density function (95 or 80%), that assignment was individually hand-checked to assure that it was not mismatching parents and progeny. Likelihood methods are an advantage here because some progeny were missing information at some loci owing to difficulties stemming from DNA extraction and amplification, especially from archived samples, and because not all parents differed at all loci.

(e) *Frequency of selfing and analysis of origin effects*

Given the parentage information, the observed frequency of selfing was calculated and compared with the expected rate. The expected selfing rate was assumed to be one in six (0.1667) if spores, assumed to be released equally from the six founders, interacted at random. Because all six founders were haphazardly placed into the wire packet and overlapped physically, and because we expect that spores can at least move several or tens of centimetres, random interaction was assumed. The expected selfing rate was tested for deviations with a *G*-test. A *G*-test was also used to examine whether any given locale had an increased probability of representation in the progeny, and whether these

genotypes were disproportionately the result of selfing or outcrossing. The raw *G*-values were calculated using data from our parentage analysis, where each offspring had two contributing parents and 0.01 was added to all values to allow analysis of zero values. Owing to the uncertainty in original source parental genotypes, we eliminated one population from locale analyses (G6).

(f) *Testing for fitness effects of selfing*

Given the data on the genetics, parentage, demography and spatial location of a plant, we asked what factors were significant predictors of individual plant fitness proxies with logistic and linear regression. Factors included Thiessen area, size and whether a plant was the result of self-fertilization, and these factors were analysed as predictors of fitness proxies: heterozygosity (H_t), and whether a plant reproduced in August or survived until August. The variables were both binary (reproduction, survival, self-fertilization) and continuous (stipe diameter, Thiessen polygon area, heterozygosity). Statistical relationships among these variables were estimated with logistic regressions for binary (reproduction, survival) dependent variables and linear regressions for continuous (stipe diameter, heterozygosity) dependent variables (R 2.8.1; [40]). Each regression was run using a May, July and August census. Because the mean range of stipe diameter values could vary across patches, the size measurement was standardized as stipe deviation. Stipe deviation estimates size as the deviation from the mean stipe diameter for that patch in that month, a metric that standardized size and minimized between-patch and -year differences. We tested whether H_t changed from the parents to offspring by estimating per-individual H_t as the summed binary heterozygosity at each locus for each individual. The reproduction regressions excluded one population (G6), as we did not collect reproductive information that year. Given binary survival data for each individual at each time step, we used the Cox proportional hazards model to test whether size, density or selfed/outcrossed status of an individual covaried significantly. In order to use the non-parametric Cox model, we assumed that all individuals were present in May, and thus the data are right-censored.

3. RESULTS

(a) *Parentage assignment*

Of the 211 progeny genotyped, five could not be assigned parentage with high confidence (one from G6, three from GC3 and one from NF2) and were thus excluded from our analysis. In two other cases (both in G6), we were unable to assign parentage because the parents were genetically identical but for a single allele at a single locus, and difficulties in DNA extraction and genotyping made this problem impossible to resolve. In spite of this difficulty, we knew that one individual was the result of outcrossing. Whether we assigned the other individual as selfed or outcrossed made no significant difference in our regression results. For the remaining 206 progeny, results were based on an average of 8.56 loci per individual, and we were always able to recognize whether they were selfed versus outcrossed, even when there was some uncertainty in the source population assignment. However, there was no systematic bias in which source population parentage assignments lacked confidence.

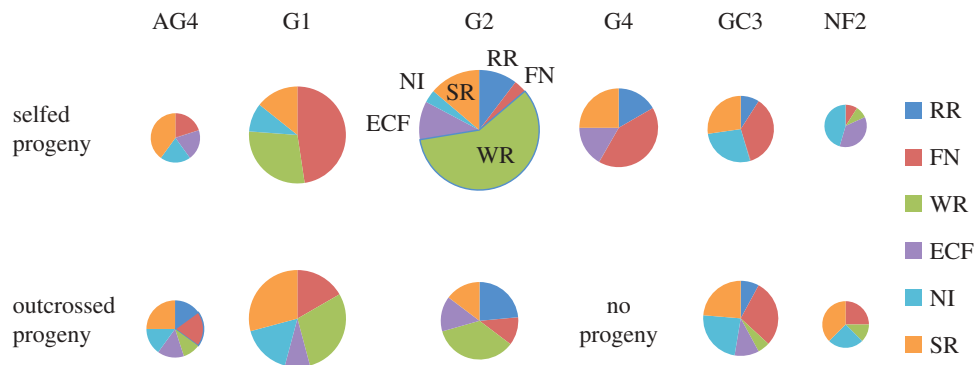


Figure 2. Originating locales of offspring genotypes for selfed and outcrossed progeny in the F_1 generation among six experimental populations. The size of the circle is scaled to the number of progeny that resulted (from 0 to 58). Although the distribution of parental genotype in the progeny generation deviates from a random distribution ($G = 201$, d.f. = 6, $p < 0.001$), there is no single origin genotype in the parental generation that contributes significantly more to the genotype array of the progeny generation (Kruskal–Wallis $\chi^2 = 5$, d.f. = 5, $p = 0.416$). Given an expected frequency of selfing (0.167), there is a significant deviation from random mating, with every patch showing a higher-than-expected frequency of selfing ($\chi^2 = 216$, $p < 0.001$).

(b) Frequency of selfing

We found no evidence for barriers to self-fertilization in any of the seven experimental populations. Rates of selfing ranged from 0.333 to 1 (all progeny were selfed in G4); all were greatly in excess of the 1 in 6 (0.167) rate expected by chance (table 1; $\chi^2 = 216$, $p < 0.001$). When we quantified selfing or outcrossing in each patch, there were only three founding individuals across all patches whose genotype was not represented in progeny; the remaining 33 individuals allowed us to assess whether a parent genotype contributed more progeny via outcrossing or selfing. Selfing was as great as or greater than outcrossing in 13 of those 33 founders. Thus, selfing was prevalent among all source genotypes and all experimental populations.

Although the representation of genotypes from each of the six sites differed among the offspring of six of the experimental populations ($p < 0.001$; figure 2; G6 excluded; see §2), this contribution of genotypes did not systematically deviate by site (Kruskal–Wallis on raw G values, $p = 0.555$). Thus, while individuals from different source populations might be more likely to survive and reproduce in different patches, no one population was more likely to contribute and succeed across all patches (figure 2).

Similarly, when we asked whether there was differential source contribution to only the selfed progeny, differences were found among experimental populations ($p < 0.001$), but again these representations did not differ systematically (Kruskal–Wallis: $p = 0.416$ for selfing; $p = 0.351$ for outcrossing). Note that even when there was a high incidence of selfing, multiple founders were responsible. Thus, even though all F_1 from patch G4 were selfed, all six founders contributed to this selfing rate. In sum, a source contribution could show deviations from expected in any given experimental population, but these deviations were never consistent across populations, regardless of whether the progeny were selfed or outcrossed (figure 2). However, because we were unable to quantify individual spore production at the time populations were founded, we cannot rule out the possibility that *a priori* differences in individual fecundity were important.

Although we controlled for location origin, the experimental design did include one instance where a parent population (NF2) was out-planted near its original environment (FN). However, there was no evidence

that these parents contributed disproportionately to progeny and our results were unchanged when we excluded the NF2 experimental population.

Parthenogenesis has been induced in the laboratory for other kelps [41–43], and it could affect our interpretations of selfing if *Postelsia* is parthenogenic to any extent in nature. To check for this, we quantified the incidence of identical founder genotypes in the progeny to assess whether parthenogenesis might contribute to self-fertilization rates by examining only founders that were not homozygous at all loci (25 individuals over the seven populations). When we estimated the fraction of selfed individuals that could have been parthenogens (e.g. those that were identical to a founder), we found that 22 out of 85 selfed progeny had identical genotypes and thus could be potential parthenogens (25.9%), while 63 were the result of self-fertilization events of gametes from the same individual. Based on the parental haplotypes for these selfed individuals, we would have expected 36 individuals (42.5%) to be homozygous and thus potential parthenogens.

(c) Fitness effects of selfing

Although a number of possible factors could affect the size of individual *Postelsia*, size was not significantly predicted by any of these factors for any of the three time points we used (table 2). Variation in stipe diameter was not significantly explained by *Postelsia* density (May, $p = 0.707$; July, $p = 0.637$; August, $p = 0.725$) nor by whether the plant was selfed or outcrossed (May, $p = 0.213$; July, $p = 0.111$; August, $p = 0.247$). Because size was not statistically correlated to *Postelsia* density or genetics, we then used these three factors as independent variables to explain changes in survival and reproduction.

Survivorship was modelled as a function of plant size, local density and whether it was a result of self-fertilization or outcrossing (table 2). Survivorship declined over the course of the summer, but the rate of decline was not significantly explained by density, individual size or whether an individual was selfed (Cox proportional hazard test, $r^2 = 0.013$; likelihood ratio test = 2.73, $p = 0.436$). Survival of an individual to August showed some weak relationships with the degree of selfing through the

Table 2. Regression results. Coefficients of regressions asking whether size (stipe diameter deviation in cm), neighbourhood density (Thiessen polygon area) and whether the individual was selfed (0 = outcrossed, 1 = selfed) significantly explained variation in reproduction, survival to August or size. Regressions used pooled data over all patches and all years.

		dependent variables		
		reproduction	survival	size
May	size	0.035	0.008	—
	Thiessen	221.07 [†]	77.22	-42.61
	selfed	0.351	-0.637 [†]	-0.716
	intercept	0.239	0.842*	0.496**
July	size	0.177**	0.017	—
	Thiessen	253.28*	-3.56	81.41
	selfed	0.771	-0.548 [†]	-1.421
	intercept	-0.297	0.864**	0.531**
August	size	0.193**	—	—
	Thiessen	352.62*	—	58.82
	selfed	0.707	—	-1.114
	intercept	-0.452**	—	0.341**

*0.001 < p < 0.05.

** p < 0.001.

[†]0.05 < p < 0.10.

first generation when analysed with a multiple regression, with a marginally significant negative relationship between selfing and survival in May and July ($p = 0.058$ and 0.066 , respectively; table 2). Survival to August was not significantly correlated with either individual size (May, $p = 0.867$; July, $p = 0.949$; table 2) or *Postelsia* local density (May, $p = 0.287$; July, $p = 0.464$; table 2).

We asked if variation in whether *Postelsia* reproduced was explained by the same independent variables used for survival: plant size, local density and self-fertilization. Individual reproduction was related positively to plant size (July, $p < 0.001$; August, $p < 0.001$) and to Thiessen area (May, $p = 0.0752$; July, $p = 0.022$; August, $p = 0.018$), but reproduction was not correlated with selfing (May, $p = 0.495$; July, $p = 0.148$; August, $p = 0.182$). Thus, reproduction was more likely for larger, more isolated plants, but it was not significantly affected by selfing.

Our power to detect significant effects of selfing was greater for the size of the individual than for survivorship or reproduction. When we used only paired contrasts between selfed and outcrossed individuals, our power to detect a 10 per cent difference in size was near 80 per cent, with an approximate power of 99 per cent to detect the difference we observed. We had approximately 85 per cent power to detect the 24.6 per cent difference we estimated in reproduction and an approximately 65 per cent power for the observed survivorship difference, but very low power (less than 50%) to detect a difference as small as 10 per cent. We note also that the difference in the incidence of reproduction was always opposite to the cost of selfing hypothesis.

(d) *The relationship between selfing and heterozygosity*

Heterozygosity was significantly lowered by selfing (figure 3), with heterozygosity 30 per cent lower for

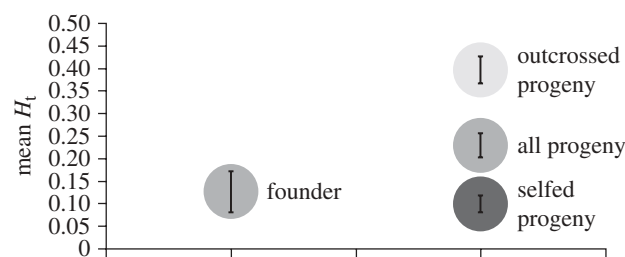


Figure 3. The mean heterozygosity ($H_t \pm$ s.e.) for founding *Postelsia* and their progeny. Heterozygosity declined by 30 per cent within a single generation for selfed progeny compared with an increase in heterozygosity for outcrossed progeny. The coefficients of selfing on heterozygosity was statistically significant ($H_{\text{JULY}} = -0.297\text{selfed} + 0.397$, $p < 0.001$).

selfed offspring compared with outcrossed offspring ($p < 0.001$). Although selfing was associated with a significant 30 per cent decline in H_t in the first year, fitness costs to this high rate of selfing were relatively few, only manifesting itself in a marginal survival cost. The overall heterozygosity of the progeny was higher than the heterozygosity of the parents ($t = 3.879$, d.f. = 72, $p < 0.001$), because of the formation of new genotypes in F_1 via outcrossing among different source populations. Thus, even though there was a range of heterozygosity available in the progeny to explore the effects of selfing and outcrossing, we nevertheless found no strong effect of parental mating on the progeny.

(e) *Long-term population trends*

Individuals were not genotyped past the first generation because we would no longer have had distinct parental genotypes to continue to track parentage. However, population fates were followed into the second and third generation (F_2 and F_3) by counting individuals in August. When we tested for a correlation between selfing rate or heterozygosity in F_1 and population size in F_2 or F_3 , we found no significant relationship (table 1). We also found no relationship between the frequency of selfing in F_1 and the probability of extinction in F_2 or F_3 (table 1).

4. DISCUSSION

(a) *Evolution and the genetics of inbreeding depression*

Purging of the genetic load may explain why we found few detectable fitness effects of self-fertilization in *Postelsia*. Inbreeding depression may not have been observed because of the possible purging of deleterious alleles costly to plant fitness, a finding that has empirical support in other plant studies [44,45]. If inbreeding depression is due mostly to mutations of large effect, then previous inbreeding should have purged these mutations [1,45,46]. Lower costs to selfing have been shown experimentally by Willis [47], who found that after 335 lines of *Mimulus guttatus* were formed, maintained by self-fertilization over five generations and then crossed to create an outcrossed population, the average fitness of the experimentally crossed progeny doubled compared with the fitness of the original population. Inbreeding depression was found for only one of 11 traits in an experimental self-compatible population of *Leavenworthia*

alabamica, supporting the hypothesis that self-fertilization purges the genome of deleterious, recessive or partially recessive alleles [48]. The persistence of *Postelsia* as a naturally highly selfing organism and the few genetic costs to selfing that we found suggest that purging may have occurred in the distant past, leaving few detectable deleterious phenotypic effects of inbreeding.

Highly inbred populations have been shown to have low inbreeding depression, and in that case increased self-fertilization is strongly selected for [49]. Thus, populations that have evolved high selfing rates will probably remain at that high rate and not be susceptible to invasion by genotypes favouring outcrossing. With few negative fitness effects, selfing can therefore be evolutionary advantageous.

(b) *Determinants of Postelsia fitness in nature*

Because survival was measured as the persistence of the sporophyte through the late summer, we do not know whether survival differed during the gametophyte or microscopic sporophyte stage (from approximately September to April). If deleterious effects of selfing occur at the haploid gametophyte stage, where all alleles are exposed to selection, the gametophyte may serve as a stage with strong selection. Detrimental outcomes of selfing would then be only a result of those genes that act selectively on the sporophyte stage. For this reason, it follows that inbreeding depression would generally be less prevalent in taxa with a free-living gametophyte stage (such as some seaweeds), in contrast to more well-studied groups that lack one (e.g. vascular plants and animals).

Further, if there is differential mortality at young life stages, then the demography of the population could be altered prior to our census [50,51]. And if deleterious genotypes are produced by increasing inbreeding through self-fertilization but are then purged, purging should preferentially occur for traits expressed early in life, as suggested for pollen and ovule production by Carr & Dudash [52] and Husband & Schemske [53]. Variation in survival over an individual's lifetime makes it difficult to track the genetic factors contributing to this variation. We found no statistically significant predictors for individual size, indicating a possibly strong effect of microhabitat in shaping fitness outcomes, strong enough to override genetics. However, the duration of the haploid gametophyte stage and the selective pressures associated with it remain little understood in marine algae.

Finally, persistence may have more to do with factors related to stochastic demography than to genetic factors [54]. Local persistence of *Postelsia* populations is dependent upon patch size; a population of 1–30 individuals has a probability of persistence for a single year of only 0.36, while those populations with 50 individuals have a persistence probability of 0.50 [34]. Although Paine [34] did not specifically consider genetic factors, stochastic demography alone could dominate persistence patterns. Similar analysis of *Postelsia* populations to date found that demographic effects of small size generate significant effects on time to local extinction (J. T. Wootton & C. A. Pfister 1999–2010, unpublished data), suggesting that persistence may also be affected by variability in demography that is unrelated to selfing.

While persistence in the intertidal may not be governed by genetic factors in the long term, in the short term self-fertilization provides a means of persistence for colonizing species. The few fitness consequences of self-fertilization in a single generation in this kelp mean that *Postelsia* can successfully begin a population with only one individual and can increase that population by selfing.

(c) *Implications for Postelsia mating system*

Another possible mechanism of persistence for *Postelsia* is parthenogenesis, a form of selfing. *Postelsia* offspring that have identical offspring to parental genotypes could be the result of selfing or cloning via parthenogenesis. Parthenogenesis has been shown in the laboratory in other kelps [41,42], including the persistence via parthenogenesis alone in *Laminaria japonica* for a dozen generations [43]. Although we do not know the incidence of parthenogenesis in nature for *Postelsia*, studies of other kelp from this region show that many species are capable of parthenogenesis in the laboratory (J. D. Collens & C. A. Pfister 2007, unpublished data). In our populations, we had a total of 30 progeny in the F₁ that were the result of selfing, but were identical (or nearly so) to the parent and thus might have been the outcome of parthenogenesis. If we remove these putative parthenogens from our analyses, we find no differences in any of the results presented. Furthermore, the expected rates at which completely homozygous individuals were generated from known parental haplotypes were more than adequate to explain the presence of these individuals. In sum, although we cannot rule out the occurrence of parthenogenesis in our data, there was no evidence that parthenogenesis was increasing the selfing rate. Additionally, whether progeny were parthenogens or the result of selfing among parental haplotypes, there was little evidence for short-term costs of selfing.

The ecology of *Postelsia* is that of a naturally isolated and patchily distributed kelp. Few costs to selfing in the first generation, as we have demonstrated here, mean that *Postelsia* can successfully begin a population with only one individual and increase that population by selfing. Selfing has been recognized as adaptive when fitness costs for not reproducing are high [9,11–13], as would be expected for the annual *Postelsia*. Short-lived species could have greater selective pressure to ensure persistence, via selfing for reproductive assurance. For algal species that occupy areas of currents and turbulent flow, mechanisms of reproductive assurance might include synchronous spore release [55], spore release at times that minimize transport [56] and few barriers to selfing ([18] and this study). In *Postelsia*, we found few short-term costs for selfing, and selfing may provide a means of reproductive assurance in this relatively short-lived species.

Funding was provided by the NSF (OCE no. 01-17801 to J.T.W. and C.A.P.), the Faculty Research Fund of the University of Chicago (to C.A.P.) and University of Chicago Biological Sciences Collegiate Division Summer Fellowship (to A.K.B.). Many people have contributed to this project over the years, notably H. Kusumo, K. Barnes and Y. Seligman. Comments from G. Dwyer, R. Hudson, S. Pruett-Jones and two anonymous reviewers improved the manuscript. We extend our continued gratitude to the Makah tribe for allowing access to Tatoosh Island.

REFERENCES

- 1 Charlesworth, D. & Charlesworth, B. 1987 Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.* **18**, 237–268. (doi:10.1146/annurev.es.18.110187.001321)
- 2 Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998 Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494. (doi:10.1038/33136)
- 3 Herlihy, C. R. & Eckert, C. G. 2002 Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* **416**, 320–323. (doi:10.1038/416320a)
- 4 da Silva, A. *et al.* 2008 Heterozygosity–fitness correlations revealed by neutral and candidate gene markers in roe deer from a long-term study. *Evolution* **63**, 403–417. (doi:10.1111/j.1558-5646.2008.00542.x)
- 5 Dudash, M. R. & Fenster, C. R. 2000 Inbreeding and outbreeding depression in fragmented populations. In *Genetics, demography and viability of fragmented populations* (eds A. G. Young & G. M. Clarke), pp. 55–74. Cambridge, UK: Cambridge University Press.
- 6 Oostermeijer, J. G. B. 2000 Population viability analysis of the rare *Gentiana pneumonanthe*: the importance of genetics, demography and reproductive biology. In *Genetics, demography and viability of fragmented populations* (eds A. G. Young & G. M. Clarke), pp. 313–334. Cambridge, UK: Cambridge University Press.
- 7 Dudash, M. R. 1990 Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* **44**, 1129–1139. (doi:10.2307/2409277)
- 8 Nieminen, M., Singer, M. C., Fortelius, W., Schops, K. & Hanski, I. 2001 Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *Am. Nat.* **157**, 237–244. (doi:10.1086/318630)
- 9 Darwin, C. 1876 *The effects of cross and self fertilization in the vegetable kingdom*. London, UK: John Murray.
- 10 Müller, H. 1883 *The fertilization of flowers*. London, UK: Macmillan and Co.
- 11 Baker, H. G. 1955 Self-compatibility and establishment after ‘long-distance’ dispersal. *Evolution* **9**, 347–349. (doi:10.2307/2405656)
- 12 Lloyd II, D. G. 1992 The selection of self-fertilization. *Int. J. Plant Sci.* **153**, 370–380. (doi:10.1086/297041)
- 13 Kalisz, S., Vogler, D. W. & Hanley, K. M. 2004 Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* **430**, 884–887. (doi:10.1038/nature02776)
- 14 Aarssen, L. W. 2000 Why are most selfers annuals? A new hypothesis for the fitness benefit of selfing. *Oikos* **89**, 606–612. (doi:10.1034/j.1600-0706.2000.890321.x)
- 15 Rathcke, B. J. 2003 Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *Am. J. Bot.* **90**, 1328–1332. (doi:10.3732/ajb.90.9.1328)
- 16 McCall, C., Waller, D. M. & Mitchell-Olds, T. 1994 Effects of serial inbreeding on fitness components in *Impatiens capensis*. *Evolution* **48**, 818–827. (doi:10.2307/2410489)
- 17 Engel, C. R., Daguin, C. & Serrao, E. A. 2005 Genetic entities and mating system in hermaphroditic *Fucus spiralis* and its close dioecious relative *F. vesiculosus* (Fucales, Phaeophyceae). *Mol. Ecol.* **14**, 2033–2046. (doi:10.1111/j.1365-294X.2005.02558.x)
- 18 Perrin, C., Daguin, C., Van de Vliet, M., Engel, C. R., Pearson, G. A. & Serrao, E. A. 2007 Implications of mating system for genetic diversity of sister algal species: *Fucus spiralis* and *Fucus vesiculosus* (Heterokontophyta, Phaeophyceae). *Eur. J. Phycol.* **42**, 219–230. (doi:10.1080/09670260701336554)
- 19 Busch, J. W. 2005 Inbreeding depression in self-incompatible and self-compatible populations of *Leavenworthia alabamica*. *Heredity* **94**, 159–164. (doi:10.1038/sj.hdy.6800584)
- 20 Fenster, C. B. & Martén-Rodríguez, S. 2007 Reproductive assurance and the evolution of pollination specialization. *Int. J. Plant Sci.* **168**, 215–228. (doi:10.1086/509647)
- 21 Kephart, S. R., Brown, E. & Hall, J. 1999 Inbreeding depression and partial selfing: evolutionary implications of mixed-mating in a coastal endemic, *Silene douglasii* var. *oraria* (Caryophyllaceae). *Heredity* **82**, 543–554. (doi:10.1038/sj.hdy.6885250)
- 22 Raimondi, P. T., Reed, D. C., Gaylord, B. & Washburn, L. 2004 Effects of self-fertilization in the giant kelp, *Macrocystis pyrifera*. *Ecology* **85**, 3267–3276. (doi:10.1890/03-0559)
- 23 Kinlan, B. & Gaines, S. 2003 Propagule dispersal in marine and terrestrial environments: a community perspective. *Ecology* **84**, 2007–2020. (doi:10.1890/01-0622)
- 24 Alberto, F., Raimondi, P. T., Reed, D. C., Coelho, N. C., Leblois, R., Whitmer, A. & Serrão, E. A. 2010 Habitat continuity and geographic distance predict population genetic differentiation in giant kelp. *Ecology* **91**, 49–56. (doi:10.1890/09-0050.1)
- 25 Reed, D. C., Amsler, C. D. & Ebeling, A. W. 1992 Dispersal in kelps: factors affecting spore swimming and competency. *Ecology* **73**, 1577–1585. (doi:10.2307/1940011)
- 26 Kusumo, H. T., Pfister, C. A. & Wootton, J. T. 2006 Small-scale genetic structure in the sea palm *Postelsia palmaeformis* Ruprecht (Phaeophyceae). *Mar. Biol.* **149**, 731–742. (doi:10.1007/s00227-006-0254-z)
- 27 Abbott, I. A., Hollenberg, G. J. & Johansen, H. W. 1976 *Marine algae of California*. Stanford, CA: Stanford University Press.
- 28 Paine, R. T. 1979 Disaster, catastrophe, and local persistence of the sea palm *Postelsia palmaeformis*. *Science* **205**, 685–687. (doi:10.1126/science.205.4407.685)
- 29 Blanchette, C. A. 1996 Seasonal patterns of disturbance influence recruitment of the sea palm, *Postelsia palmaeformis*. *J. Exp. Mar. Biol. Ecol.* **197**, 1–14. (doi:10.1016/0022-0981(95)00141-7)
- 30 Druehl, L. D., Robinson, B. R. & Button, D. K. 1989 Characterizing and sexing laminarialean meiospores by flow cytometry. *Mar. Biol.* **101**, 451–456. (doi:10.1007/BF00541646)
- 31 Müller, D. G., Maier, I. & Gassman, G. 1985 Survey on the sexual pheromone specificity in Laminariales (Phaeophyceae). *Phycologia* **24**, 475–477.
- 32 Dayton, P. K. 1973 Dispersion, dispersal and persistence of the annual intertidal alga *Postelsia palmaeformis* Ruprecht. *Ecology* **54**, 433–438. (doi:10.2307/1934353)
- 33 Coyer, J. A., Olsen, J. L. & Stam, W. T. 1997 Genetic variability and spatial separation in the sea palm kelp *Postelsia palmaeformis* (Phaeophyceae) as assessed with M13 fingerprints and RAPDs. *J. Phycol.* **33**, 561–568. (doi:10.1111/j.0022-3646.1997.00561.x)
- 34 Paine, R. T. 1988 Habitat suitability and local population persistence of the sea palm *Postelsia palmaeformis*. *Ecology* **69**, 1787–1794. (doi:10.2307/1941157)
- 35 Mithen, R., Harper, J. L. & Weiner, J. 1984 Growth and mortality of individual plants as a function of ‘available area’. *Oecologia* **62**, 57–60. (doi:10.1007/BF00377373)
- 36 Karagatzides, J. D., Manson, H. R. & Tsuji, L. J. S. 2003 Spatial distribution and performance of *Scirpus americanus* ramets across a temperate intertidal marsh resource

- gradient. *Plant Ecol.* **169**, 215–226. (doi:10.1023/A:1026015218282)
- 37 Kusumo, H. T., Pfister, C. A. & Wootton, J. T. 2004 Dominant (AFLP) and co-dominant (microsatellite) markers for the kelp *Postelsia palmaeformis* (Laminariales). *Mol. Ecol. Not.* **4**, 372–375. (doi:10.1111/j.1471-8286.2004.00657.x)
- 38 Marshall, T. C., Slate, J., Kruuk, L. E. B. & Pemberton, J. M. 1998 Statistical confidence for likelihood based paternity inference in natural populations. *Mol. Ecol.* **7**, 639–655. (doi:10.1046/j.1365-294x.1998.00374.x)
- 39 Meagher, T. R. 1986 Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most likely male parents. *Am. Nat.* **128**, 199–215. (doi:10.1086/284554)
- 40 R Development Core Team 2008 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- 41 Nakahara, H. & Nakamura, Y. 1973 Parthenogenesis, apogamy and apospory in *Alaria crassifolia* (Laminariales). *Mar. Biol.* **18**, 327–332.
- 42 Nakahara, H. 1984 Alternation of generations of some brown algae in unialgal and axenic cultures. *Sci. Pap. Inst. Algol. Res. Hokkaido Univ.* **7**, 77–194.
- 43 Lewis, R. J., Jiang, B. Y., Neushul, M. & Fei, X. G. 1993 Haploid parthenogenetic sporophytes of *Laminaria japonica* (Phaeophyceae). *J. Phycol.* **29**, 363–369. (doi:10.1111/j.0022-3646.1993.00363.x)
- 44 Crnokrak, P. & Barrett, S. C. H. 2002 Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**, 2347–2358.
- 45 Lande, R. & Schemske, D. W. 1985 The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40. (doi:10.2307/2408514)
- 46 Barrett, S. C. H. & Charlesworth, D. 1991 Effects of a change in the level of inbreeding on genetic load. *Nature* **352**, 522–524. (doi:10.1038/352522a0)
- 47 Willis, J. H. 1999 The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* **53**, 1678–1691. (doi:10.2307/2640431)
- 48 Busch, J. W. 2004 Inbreeding depression in self-incompatible and self-compatible populations of *Leavenworthia alabamica*. *Heredity* **94**, 159–164. (doi:10.1038/sj.hdy.6800584)
- 49 Charlesworth, D., Morgan, M. T. & Charlesworth, B. 1990 Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* **44**, 1469–1489. (doi:10.2307/2409330)
- 50 Santelices, B. 1990 Patterns of reproduction, dispersal and recruitment in seaweeds. *Oceanogr. Mar. Biol. Ann. Rev.* **28**, 177–276.
- 51 Martinez, E. A. & Santelices, B. 1998 Selective mortality on haploid and diploid microscopic stages of *Lessonia nigrescens* Bory (Phaeophyta, Laminariales). *J. Exp. Mar. Biol. Ecol.* **229**, 219–239. (doi:10.1016/S0022-0981(98)00063-X)
- 52 Carr, D. E. & Dudash, M. R. 1996 Inbreeding depression in two species of *Mimulus* (Scrophulariaceae). *Am. J. Bot.* **83**, 586–593. (doi:10.2307/2445917)
- 53 Husband, B. C. & Schemske, D. W. 1996 Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**, 54–70. (doi:10.2307/2410780)
- 54 Lande, R. 1988 Genetics and demography in biological conservation. *Science* **241**, 1455–1460. (doi:10.1126/science.3420403)
- 55 Pearson, G. A. & Serrão, E. A. 2006 Revisiting synchronous gamete release by furoid algae in the intertidal zone: fertilization success and beyond? *Int. Comp. Biol.* **46**, 587–597. (doi:10.1093/icb/icl030)
- 56 Pearson, G. A. & Brawley, S. H. 1996 Reproductive ecology of *Fucus distichus* (Phaeopyceae): an intertidal alga with successful external fertilization. *Mar. Ecol. Prog. Ser.* **143**, 211–223. (doi:10.3354/meps143211)