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EFFECTS OF SELF-FERTILIZATION IN THE GIANT KELP, *MACROCYSTIS PYRIFERA*

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Abstract. The costs of self-fertilization were evaluated for the giant kelp, *Macrocystis pyrifera*. *Macrocystis*, like other kelps, has a biphasic life history in which the dispersal stage is the 1N spore generation. Once spores settle, they grow into sedentary and microscopic male or female gametophytes, which produce sperm and eggs. Fertilization ensues, and the resultant 2N sporophyte, the spore-producing form, grows rapidly to a height of up to 25 m. Based on previous empirical studies and modeling field surveys, the potential for self-fertilization is expected to be high, largely due to the shape and extent of spore dispersal distributions. The costs of self-fertilization were assessed with laboratory and field experiments over the entire life history of the sporophyte stage. There was no evidence for self-incompatibility, and fitness was dramatically lower in selfed compared to outcrossed individuals. In addition, there was evidence that costs were more severe under intraspecific competition with outcrossed individuals. The demonstrated severity of the consequences of self-fertilization suggests a species that typically outcrosses (at least under the assumption that costs result from rare recessive deleterious alleles). However, costs were more pronounced in later life history phases, suggesting routine self-fertilization. One implication of significant levels of self-fertilization in *Macrocystis* is a sort of inbreeding-mediated localized population senescence, leading to striking oscillations in populations, a pattern seen in many Californian kelp beds.

Key words: California, USA; competition; dispersal; inbreeding; kelp; Macrocystis pyrifera; *outcrossing; self-fertilization; spores.*

INTRODUCTION

Self-fertilization, the fusion of male and female gametes from a single genetic hermaphroditic (2N) entity, occurs in many plants and animals (Jarne and Charlesworth 1993). Over 50% of all flowering plant species, for instance, self-fertilize at least occasionally (Barrett et al. 1996). Among hermaphroditic angiosperms, 35–84% show some level of self-compatibility (Jarne and Charlesworth 1993). Hermaphroditism also occurs commonly in algae, lower plants, and in a variety of animals, although group-wide estimates of rates of self-fertilization of these group have not been produced (but see Soltis and Soltis 1988, Soltis et al. 1988, and Korpelainen and Kolkkala 1996 for estimates of self-fertilization in *Equisetum* spp.).

Self-fertilization has a number of widely recognized potential consequences, all of which result from effects of inbreeding depression. These effects include decreased survivorship, stunted growth, and lower fecundity. Two hypotheses have been proposed for the genetic cause of inbreeding depression (see Charlesworth and Charlesworth 1987). First, recessive deleterious alleles that are maintained at (generally) low frequencies in populations may be expressed in inbred (including selfed) individuals that become homozygous at critical loci. Second, reduced heterozygosity, even in the absence of identifiable deleterious recessives, may itself result in a loss of fitness.

While costs related to self-fertilization are well acknowledged, there are also at least three potential benefits to self-fertilization (reviewed in Jarne and Charlesworth 1993). First, selfing may elevate reproductive assurance in cases where gametes would otherwise go unfertilized (this is particularly important when adults are rare and not clustered or when gametes possess a poor capacity for dispersal). Second, self-fertilization may work to maintain ''good'' genotypes. Third, self-fertilization may allay the genetic costs of outcrossing, which derive from either the cost of male function or meiosis (Lively and Lloyd 1990).

Although there are clearly trade-offs among the above costs and benefits, net inbreeding depression almost always has been detected, when investigated, in flowering plants where self-fertilization occurs (Charlesworth and Charlesworth 1987, Jarne and Charlesworth 1993). Many measurements of inbreeding de-

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pression are also likely underestimates due to difficulties associated with field assessments and accounting for all stages in the life cycle (Charlesworth and Charlesworth 1987, Dudash 1990). The few documented exceptions where inbreeding depression has not been found are in species that rely almost entirely on inbreeding, where deleterious recessive alleles tend to be purged quickly from populations (Stebbins 1950, Wyatt 1990, Barrett and Charlesworth 1991, Jarne and Charlesworth 1993). These findings have provided empirical support for theories suggesting that: (1) outcrossing is favored when fitness costs resulting from inbreeding depression exceed 50% (relative to outcrossed individuals) and, (2) there may be advantages accrued by self-fertilization when the costs of inbreeding depression are <50% (Lande and Schemske 1985, Schemske and Lande 1985, Charlesworth and Charlesworth 1987, Jarne and Charlesworth 1993).

Some organisms (e.g., kelps, large brown macroalgae of the order Laminariales), have biphasic life histories that may strongly influence both the predominance of self-fertilization and its consequences for fitness. The conspicuous life phase in kelps is the sporophyte, a large diploid individual that releases equal numbers of haploid male and female spores. These spores, following dispersal and settlement, develop into sessile, free-living, microscopic male and female gametophytes (gamete-producing, 1N individuals). The next generation's sporophyte (2N) then arises from a zygote produced by fusion of sperm from the male gametophyte and egg from the female gametophyte (Luning and Muller 1978, Muller et al. 1985). An important feature of this algal biphasic life history is that unlike most marine animals, fertilization occurs after planktonic dispersal and settlement and will only occur if male and female spores settle close to one another to ensure fertilization at the gametophyte phase (Dayton 1985, Reed 1990). However, the odds that spores will settle close to one another diminish with dispersal distance due to dilution effects. Therefore, factors that promote limited spore dispersal also increase the probability of self-fertilization (i.e., fertilization between male and female gametophytes produced by the same sporophyte). The extent to which self-fertilization occurs in the field is completely unknown; there are no published studies of the spatial patterns of genetic structure of *Macrocystis*. However, Coyer et al. (1997) examined a related kelp species, *Postelsia palmaeformis*, and found that populations separated by as little as 25 m could be distinguished genetically.

The extent to which dispersal affects the rate of self-fertilization can be influenced by the size of the spore source. Increasing the size of the spore source (either by increasing the number of plants releasing spores or the number of spores released per plant) increases the probability that some spores will travel long distances before they dilute to a density that is insufficient for fertilization (Reed et al. 1997). Although the conditions believed to promote self-fertilization in kelps (i.e., low sporophyte density, limited dispersal, and a small spore source) are common, the frequency of self-fertilization and its consequences to individual fitness have never been explored. In other systems, particularly for flowering plants, there have been numerous studies exploring mechanisms that act to decrease incidence of self-fertilization (Bertin 1993), but to our knowledge such mechanisms have not been found for macroalgae. Those investigations that consider dispersal from *Macrocystis* individuals have indicated that dispersal leading to gametophyte densities sufficient for reproduction can be very limited, on the order of meters to tens of meters (Anderson and North 1966, Reed et al. 1988, 2004*b*). Modeling efforts largely agree with the empirical results. Gaylord et al. (2002) found that, under flow conditions common in kelp beds, ~50% of *Macrocystis* spores dispersed <100 m. In that study spore settlement density was not estimated, but it is likely that it decreases dramatically as a function of dispersal distance, decreasing the likelihood of successful sexual reproduction. Field investigations of the density of *Macrocystis* recruits as a function of distance from the source plant or population have produced a negative exponential relationship (Reed et al. 1988, 2004*a*, *b*). Such a distribution, which appears to be the norm, would promote aggregations of uniparental gametophytes and as a result, self-fertilization. Recently Reed et al. (2004*b*) showed empirically that maximum estimated dispersal from individual plants was variable but that in >50% of the trials dispersal did not exceed 30 m. An investigation of the effect of sporophyte density, synchrony in spore release, and dispersal distance on the distribution of settled spores and the likelihood of self-fertilization in *Macrocystis* can be found in P. T. Raimondi, D. C. Reed, B. Gaylord, and L. Washburn (*unpublished manuscript*).

A kelp's biphasic life history may also affect the ensuing costs of self-fertilization because rare deleterious alleles are potentially expressed in every gametophytic (1N) generation. This relies on the assumption that loci expressed in the gametophyte generation are also expressed in the sporophyte stage. To our knowledge this idea has never been investigated for algae. However, there is evidence for such cross-generational expression in vascular plants (Honys and Twell 2003, Walsh and Charlesworth 1992) and there is no a priori reason to think it would not occur in algae. If expression of rare deleterious alleles does occur in every gametophytic (1N) generation, there may be the capacity for purging of harmful alleles through rapid selection, a phenomenon that has been observed in a number of haplo-diploid systems (Saito et al. 2000).

The link between life history, dispersal, self-fertilization, and fitness, while potentially of immense importance, has rarely been addressed and has never been investigated in a marine species. Here we present the results of a study that explores such self-fertilization

PLATE. 1. Diver sampling a giant kelp plant. The plant being sampled has about 40 fronds and is in a medium-density kelp forest (~6 plants per 100 m^2). Note the sporophyll bundle at the base of the plant. Photo credit: Richard Herman.

issues in a dominant player in many subtidal, coastal habitats. Specifically, in the present study we document the lifetime fitness costs of self-fertilization for the sporophyte stage of the giant kelp, *Macrocystis pyrifera* (see Plate 1). We found that fitness was dramatically lower in selfed compared to outcrossed individuals and that these costs were more severe under intraspecific competition with outcrossed individuals. We also found that these costs were more pronounced in later life history phases, which is typical of species that routinely self-fertilize. Finally we discuss kelp bed dynamics in the context of demography, dispersal, and frequency dependent self-fertilization rates.

METHODS

Experimental populations of *Macrocystis* with different ratios of selfed vs. nonselfed sporophytes were used to evaluate the individual costs and population consequences of self-fertilization. Because the effects of self-fertilization can be manifested at any life stage, we tested the performance of selfed vs. outcrossed sporophytes at multiple stages, from zygote to reproductive maturity (approximately one year postfertilization). Laboratory populations were employed to evaluate rates of successful zygote production, while age-specific survivorship and adult fecundity were assessed via experimental individuals outplanted to the field.

Zygote production

Laboratory kelp populations were established by inoculating 5-mL plastic culture dishes with one of three different spore mixtures, each leading to a distinct level of selfing. These three mixtures represented combinations of selected ratios of sib/nonsib spores extracted from 20 different sporophyte individuals (collected haphazardly in the field, with minimum distance separating plants >10 m). The first mixture incorporated spores from a single sporophyte, yielding suspensions with 100% selfed zygotes after fertilization. The second mixture combined spores from a single sporophyte (70% of the suspension) with an equal-part concoction of spores from the other 19 individuals (the remaining 30%). This yielded a suspension containing zygotes with expected selfing rates of ~50% (see Expression 1). The third mixture was created with equal numbers of spores from each of the 20 sporophytes, yielding selfing rates of 5%. All three spore mixtures were then replicated 20 times; for the 100% and 50% selfing mixtures, a different ''primary'' sporophyte was used for each replicate. Because it was not possible to produce a 0% selfing (i.e., 100% outcrossed) treatment using this approach, the 5% selfing rate was used as a logistically attainable approximation to full outcrossing in our experiments (as a consequence, our estimates of the costs of self-fertilization are slight underestimates). One consequence of this protocol is that in the 100% and 50% selfing treatments there was a single focal individual for each replicate, whereas in the 5% treatment all 20 replicates had the same mixture of spores (from all 20 parents). We consider this a feature of the design. By using the same adults for all treatments we were able to: (1) not introduce novel genotypes to just one of the treatments and, (2) mimic the fertilization scenario for a kelp forest. There are two possible concerns with this design. The first concern is independence among replicates. Millions of spores are in the spore slurry, and the odds that the resulting sporophytes are genetically identical among the replicates is so low as to be essentially zero. Hence, we view the replication in the outcrossed treatment to represent independent estimates of ourcrossed progeny that resulted from a population of 20 individuals (the same population as was used for the other treatments). Second, the estimate of variance among replicates might be lower in the 5% treatment than in the other two treatments, which would cause heteroscedascity. This did not occur (see *Results*).

Five trials of this experiment were run from January to May 1998 (approximately monthly) to allow for potential temporal variability in response. All three self-

ing ratios were used in trials 2–5. In the first trial we only used the 5% and 100% selfing mixtures.

The concentrations and volumes of all suspensions used to seed the above cultures were controlled to achieve a settlement density of ~100 spores/mm^2 for all levels of selfing, near the high end of the range of densities we have observed in nature (Reed et al. 1997). Once inoculated, the dishes were maintained under mild agitation in nutrient-enriched (Provosoli 1968) seawater (changed weekly), in an environmentally controlled room at 15°C, with an irradiance of 40–50 μmol·m^{-2}·s^{-1}, using a 14:10 h L:D photoperiod. At 17 d postsettlement, the production of zygotes (defined here as individuals consisting of two to four cells) in laboratory populations was then determined using an inverted compound microscope at a magnification of 400×. The ratio of zygotes/number of female gametophytes was used as the quantity of interest rather than the number of zygotes to account for any differences in initial density among replicate trials conducted on different dates using different batches of spores.

In developing the above selfing combinations, selfing rates (which we define as the rate of self-fertilizations relative to all fertilizations) were calculated as:

$$\sum_{i=1}^{n} (p_i)^2 \qquad (1)$$

where p_i is the proportion of spores from parent i and n is the number of parent sporophytes. The assumption here is that the level of self-fertilization between gametophytes in experimental populations is directly proportional to the ratio of sib/nonsib spores that were used to establish the populations. Such an assumption is only valid if the fertilization rate is similar in sibling and nonsibling matings. We know from earlier experiments (Lewis and Neushul 1994) and from data reported below that complete self-incompatibility (i.e., the inability of an ova and sperm produced by sibling male and female gametophytes to fuse) does not occur in *Macrocystis*. The extent to which partial self-incompatibility (defined here as a partial barrier to the fusion of sibling gametes whose effect is to increase the time required for sperm to penetrate the ovum) occurs in *Macrocystis* is unknown. A potential consequence of partial self-incompatibility in *Macrocystis* is a reduced rate of self-fertilization due to asymmetrical competition between sib and nonsib sperm. The 50% selfing treatment allowed us to assess whether partial self-incompatibility occurs in *Macrocystis*. No self-incompatibility would be implied if the 50% selfing treatment produced a response (e.g., higher zygote production than the 100% selfing treatment) approximately halfway between the 100% and 5% treatments. On the other hand, a response by the 50% selfing treatment that was identical to the 5% treatment would suggest partial self-incompatibility. As is discussed in the *Discussion*, the 50% selfing treatment also allowed us to detect those costs of self-fertilization that are only expressed under competition with outbred individuals (Charlesworth and Charlesworth 1987).

Age-specific survivorship and fecundity

Experimental field populations were produced by artificially seeding natural rocks with the same spore mixtures of *Macrocystis* used in the laboratory experiments. These rocks were then outplanted to a local reef (Naples Reef located 1.6 km offshore and 209 km west of Santa Barbara, California, USA; 34°25.353′ N, 119°57.182′ W) within 18 h as per the methods of Reed (1990). Because recruitment success (i.e., production of sporophytes via the intervening gametophyte stage) in *Macrocystis* varies unpredictably in time and space (Deysher and Dean 1986, Reed 1990), we repeated the outplants on five different occasions (each time using a different batch of spores obtained from 20 new sporophytes) to increase opportunities for successful recruitment of sporophytes derived from the gametophytes growing on our experimental rocks. Indeed, there was only one outplant for which there was successful recruitment in the field. Age-specific survivorship in experimental field populations was determined by periodically counting the number of sporophytes present on each of the 60 outplanted rocks. Reproductive status and fecundity were estimated 365 d postsettlement by measuring the number and size of sori (the structures in kelps where spores are produced) on each sporophyte using the methods employed by Reed et al. (1996).

Results

Zygote production

We examined the effects of self-fertilization on zygote production using an ANOVA procedure where level of selfing was considered a fixed effect and trial a random effect (Appendix Table A1; Fig. 1). Both trial ($n = 5$, $P < 0.001$) and level of selfing ($n = 3$, $P = 0.003$) were significant. A series of a priori hypotheses were then tested. First we compared zygote production from the 100% selfed treatment with the outcrossed treatment (5% selfing) to test the general idea that there is a potential cost to self-fertilization. Outcrossed (5%) treatments produced 40% more zygotes than did the 100% selfing treatment. To determine the likelihood that this was the result of self-incompatibility we made two other comparisons: (1) 50% vs. 5% and, (2) 50% vs. expected if no self-incompatibility. If there is no self-incompatibility, then there should be a linear relationship between level of selfing and zygote production that can be defined by two endpoints, 5% and 100% selfing. A significant positive deviation from the expected level of zygote production in the 50% selfing treatment would have been evidence for partial self-incompatibility; a positive deviation indicates that eggs not fertilized by sibling sperm (due to self-incompat-

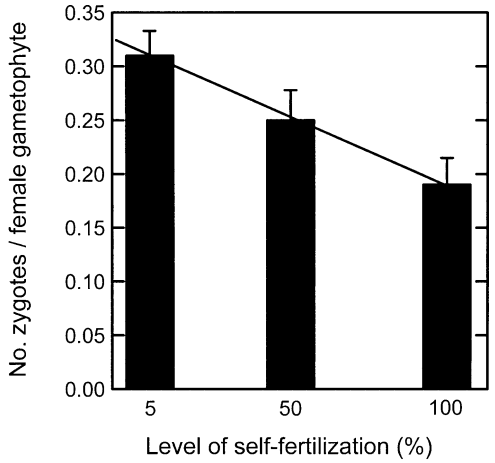

FIG. 1. Zygote production (number of zygotes/number of female gametophytes) as a function of degree of self-fertilization. The line indicates the prediction for the 50% treatment under the assumption of no self-incompatibility.

ibility) were fertilized by nonsibling sperm. There was no evidence of deviation from expected under the assumption of no self-incompatibility: the 50% treatment was significantly lower than the 5% treatment ($F = 4.13$, df = 1, 263, $P < 0.043$) and nearly identical to the expectation ($F = 0.001$, df = 1, 263, $P < 0.985$).

Juvenile and adult survivorship in experimental field populations

One of the five field outplants resulted in sporophyte recruitment sufficient to provide adequate replication for field trials. Densities of sporophytes produced from this outplant were sampled 135, 169, and 365 d after the start of the experiment to evaluate whether the cost of selfing identified at the zygote stage changed over time. The results of the experiment were striking. Over the lifetime of a selfed individual there was a nearly complete loss of fitness. Initially we found that the abundance of sporophytes decreased linearly with level of self-fertilization soon after fertilization. We were interested in determining if this relationship continued over the lifetime of an individual; hence differences in density attributable to level of self-fertilization were evaluated using a repeated-measures ANCOVA approach. In this model the level of self-fertilization was treated as a covariate because our earlier results suggested that the 50% treatment had costs predicted by a linear equation (costs as a function of level of self-fertilization). The results of this analysis indicate that sporophyte abundance varied inversely with level of self-fertilization (Appendix Table A2, $P = 0.04$), that sporophyte density generally decreased over time irrespective of level of self-fertilization ($P < 0.0001$), and most importantly, that the relationship between level of self-fertilization and sporophyte density did not vary over time (days × LSF interaction, $P = 0.90$). Differences in sporophyte density among the levels of self-fertilization in the field mirrored those observed for zygote production in the laboratory (Fig. 2). The density of juvenile sporophytes (~2–5 cm tall) sampled 135 d after outplanting was ~40% lower in the 100% selfing treatment relative to the 5% treatment; the density of juveniles in the 50% treatment was intermediate between the 5% and 100% treatments. Although sporophyte density declined in all treatments over time, the relative differences in density among treatments remained relatively constant. Thus, after a year in the field when most of the plants were >10 m tall, sporophyte density remained ~40% and 20% lower in the 100% and 50% selfing treatments, respectively, relative to the 5% selfing treatment.

Spore production in experimental field populations

The effect of self-fertilization on adult spore production 365 d after outplanting was evaluated in two ways. First we compared the frequency of sporophytes that had fertile (i.e., darkened) sori using chi square analysis (Fig. 3a). The frequency of reproductive sporophytes was similar for the 5% and 50% selfing treatments (~50%) and approximately five times higher than in the 100% treatment (11%). While this gave an estimate of the per capita effect it could be argued that the replicate of statistical interest is the replicate rock, not the individual (e.g., results might be biased by nonindependence of replicate sporophytes if sporophytes were not evenly distributed across rocks). Hence, we also compared the frequency of replicate rocks having reproductive sporophytes (Fig. 3b). The results were the same as in our evaluation of the per capita effect:

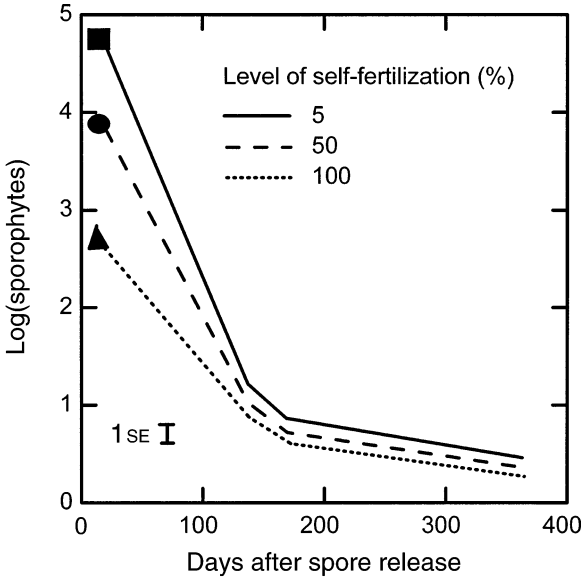

FIG. 2. Log sporophyte density (measured as no./100 cm^2) in field outplants over time as a function of level of self-fertilization. The common standard error (SE) is indicated in the lower left corner. Solid symbols represent data from the laboratory. Lines are from field results.

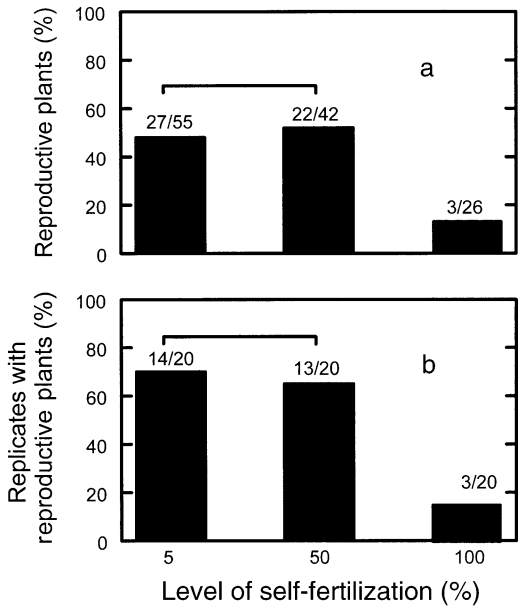

FIG. 3. (a) Proportion of sporophytes that became reproductive for each of the three levels of degree of self-fertilization. Chi-square tests were used to compare distributions. The first comparison includes all levels of self-fertilization (chi-square = 12.61, df = 2, $P = 0.0018$); the second comparison includes only the 5% and 50% levels to evaluate the effect of the 100% treatment to overall level of significance (chi-square = 0.169, df = 1, $P = 0.68$). (b) Proportion of replicate rocks having reproductive sporophytes for each of the three levels of self-fertilization. Again the first comparison includes all levels of self-fertilization (chi-square = 14.8, df = 2, $P = 0.0006$), while the second comparison includes only the 5% and 50% levels to evaluate the effect of the 100% treatment to overall level of significance (chi-square = 0.114, df = 1, $P = 0.74$).

frequency of rocks having reproductive sporophytes was similar for the 5% and 50% selfing treatments (~75%) and about four and a half times higher than in the 100% treatment (15%). Note also that this result is not due to simple differences in the number of rocks that had sporophytes (reproductive or nonreproductive). Both at the beginning and end of the field surveys most rocks in all selfing treatments had sporophytes (18, 20, and 18 rocks in beginning and 12, 14, and 15 rocks out of 20 rocks at the end in the 100%, 50%, and 5% selfing treatments, respectively).

We also compared sorus area (a measure of fecundity) among the three selfing treatments in sporophytes that survived to reproduce. Since the number of reproductive sporophytes differed dramatically among treatments (see Fig. 3), we opted not to use inferential statistics. Instead we simply present the data in Fig. 4 and note that the pattern is similar to that shown in Fig. 3, except that the differences among selfing treatments are even more striking. Per capita sorus area was similar for the 5% and 50% selfing treatments (~1200 cm^2) and nearly 10 times greater than that of the 100% treatment (150 cm^2).

The accumulated costs of self-fertilization on fitness (estimated as the product of costs incurred on zygote production, survival to adulthood, development of reproductive structures, and fecundity) in *Macrocystis* are remarkable (Table 1). Selfed individuals had, on average, <2% of the fitness of outcrossed individuals. Furthermore, this value is probably an underestimate of the lifetime cost of self-fertilization in *Macrocystis* due to our inability to: (1) produce a 0% selfing treatment (we approximated it with a 5% selfing rate), and (2) evaluate the costs of selfing in the gametophyte generation.

DISCUSSION

The two major results of this work are that for *Macrocystis pyrifera*: (1) there is no evidence for any level of self-incompatibility and, (2) the costs of self-fertilization are extremely high. Given that limited dispersal is common in *Macrocystis* (Anderson and North 1966, Reed et al. 1988, 2004*b*), the opportunity for self-fertilization should be great. These results are surprising in view of the common belief that the cost of self-fertilization is purged through the removal of deleterious alleles as incidence of selfing increases (Davenport 1908, Jones 1917). Moreover, it has been suggested that if the potential for self-fertilization is high and costs are chronically very severe some sort of self-incompatibility will evolve. However, while studies on flowering plants show that the costs of selfing frequently decrease with incidence of self-fertilization (Barrett and Charlesworth 1991, Parker et al. 1995, Husband and Schemske 1996), they rarely vanish. Furthermore, costs of selfing may remain substantial even in populations that have significant levels of selfing (Charlesworth and Charlesworth 1987, Jarne and Charlesworth 1993, Eckert and Barrett 1994, Husband and Schemske 1996).

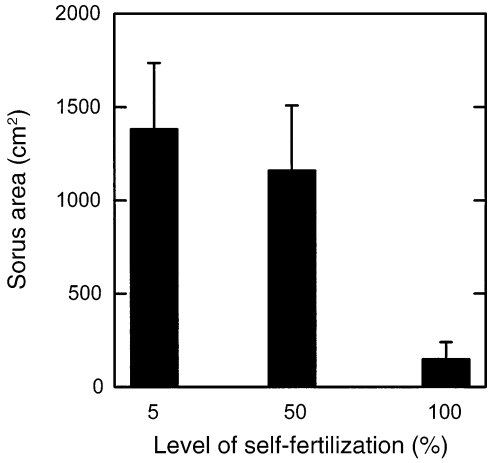

FIG. 4. Sorus area per sporophyte for the three levels of self-fertilization (mean + 1 SE). Only sporophytes that produced sori were included in calculations of the means.

TABLE 1. Estimates of the cost of self-fertilization on stage-specific and accumulated fitness.

Fitness component	Proportional difference between selfed (100% treatment) and outcrossed (5% treatment) individuals	Accumulated fitness of selfed individuals relative to outcrossed individual (proportion)
1) Zygote production	0.61	0.61
2) Survival to adulthood	1.01	0.62
3) Development of reproductive structures	0.24	0.15
4) Fecundity	0.11	0.0165

Notes: Proportional difference for each fitness component was calculated as the estimate for outcrossed individuals divided by that for selfed individuals. Accumulated fitness was calculated as the product of proportional differences. Estimates of fitness components were derived as follows: (1) zygote production—comparison of values for 100% and 5% treatments in Fig. 1; (2) survival to adulthood—comparison of mortality rates for 100% and 5% treatments in Fig. 2; (3) development of reproductive structures—comparison of proportion of reproductive individuals for 100% and 5% treatments in Fig. 3a; and (4) fecundity—comparison of sorus areas for 100% and 5% treatments in Fig. 4.

Theory suggests that the costs of self-fertilization tend to be manifested in later life stages for species that usually self-fertilize (Husband and Schemske 1996). This is based on the idea that selection is weakest against mutations that are expressed late in life as the relative contribution to reproduction decreases with age (Williams 1957, Hamilton 1966, Charlesworth 1980, Rose 1991). Plant species that primarily self-fertilize generally express inbreeding depression in later life stages, especially during growth and reproduction, whereas species that typically outcross generally express inbreeding depression across all life stages (e.g., seed production, germination, survival, growth, and reproduction; Husband and Schemske 1996). For *Macrocystis*, we found the effects of inbreeding depression were most pronounced during reproduction (Table 1), consistent with results for species that routinely self-fertilize.

Our results also indicated that intraspecific competition increased the severity of inbreeding depression. Our estimate of inbreeding depression in the sporophyte phase (Table 1) did not take into account results of the 50% selfing treatment because following fertilization there was no way to determine which individuals were the result of self- or cross-fertilization. Production of reproductive structures in the 50% treatment did not differ from those in the 5% treatment and both were much greater than in the 100% treatment (Figs. 3 and 4). These results strongly suggest that all of the adults produced in the 50% treatment were outcrossed individuals, indicating that there was decreased juvenile survivorship for selfed individuals in this treatment. If this is true, then this cost was only expressed under competition with outcrossed individuals, as juvenile survivorship was similar for selfed and outcrossed individuals when there was no competition (compare curves for 5% and 100% treatments in Fig. 2). This is consistent with studies that indicate that inbreeding depression is more severe in competitive environments (Carr and Dudash 1995). One important implication of this result is that selfed macroscopic individuals will decrease disproportionately as a function of outcrossing probability because of increased competition with outcrossed individuals.

It is possible that the high level of inbreeding depression seen in this study may be (in part) attributable to differences between our study and most other studies examining the effects of self-fertilization. First, nearly all of this study was carried out in the field, and the greatest costs incurred from inbreeding were found in the field. The severity of the effects is consistent with findings that levels of inbreeding depression are generally higher when measured under natural conditions in the field compared to artificially controlled conditions in the laboratory (Charlesworth and Charlesworth 1987, Dudash 1990, Barrett and Harder 1996). It is worth noting that some studies looking for inbreeding depression in the field through comparison of individuals in populations that varied in level of inbreeding have found little evidence for inbreeding costs (Keller and Waller 2002). This sort of evaluation is problematic because there is no direct comparison of performance of inbred to outcrossed individuals (in competition with each other). When such comparisons have been made, as in our study, outcrossed individuals typically outperform inbred ones (Keller and Waller 2002). Second, we examined the cost of self-fertilization throughout the entire sporophyte phase (2N) of *Macrocystis*. Fitness consequences due to self-fertilization can be thought of as the product of costs manifested at separate life stages (see Table 1 for an example). Hence, there is the potential for underestimating the fitness consequence in any study addressing only part of a species' life history.

In the *Introduction* we suggested that investigations of self-fertilization for species with biphasic life histories like that of kelps may be particularly interesting because the expression of rare deleterious alleles potentially occurs in every gametophytic (1N) generation, which could lessen the effects of inbreeding depression

in the sporophyte generation. Although we did not explicitly test this idea, the high level of inbreeding depression in *Macrocystis* does not suggest a general purging of deleterious genes in the gametophytic phase. We evaluated the costs of self-fertilization in the 2N sporophyte phase only, and we found the costs of selfing were most severe during the production of spores (23 times greater than for all other stages). Recall, however, that microscopic gametophytes of *Macrocystis* are the gamete-producing phase in the kelp life history. The structures (e.g., sori) associated with the production of spores do not occur in gametophytes, and it seems unlikely that deleterious alleles associated with spore production would be purged in the gametophyte stage. The severity of inbreeding depression found for traits specific to the sporophyte phase (as opposed to those potentially expressed in both gametophytic and sporophytic phases) points to a tantalizing possibility that purging of deleterious alleles occurs only primarily for those processes expressed in both generations of *Macrocystis*.

In some ways the alternation of haploid and diploid phases in a biphasic life history like that of kelps is similar to that of haplo-diploid systems in which males are 1N and females are 2N (e.g., social insects). A major difference between the two life histories is in the timing of the haploid phase. In biphasic systems the free-living haploid phase occurs in alternating generations, whereas in haplo-diploid systems the haploid phase occurs in each generation in males. In haplo-diploid species it has been found that deleterious alleles are selected out through the 1N males (Smith and Shaw 1980, Matsuda 1987, Atmer 1991), which causes the equilibrium frequency of deleterious alleles to be lower than in diplo-diploidy systems (Atmer 1991). The frequency of deleterious alleles in haplo-diploid species may be related to whether both males and females express the traits; if genes are limited to expression in females, then there may be no significant purging of those genes through haploidy (Crozier 1985, Saito 1994). Recent work by Saito et al. (2000) has confirmed one of the predictions of this hypothesis: that there are deleterious genes governing only the traits of adult females in wild populations of haplo-diploid organisms that are not removed through haploidy. These investigators found a sharp reduction in fecundity but no evidence for inbreeding effects on early survival for inbred lineages of the mite *Schizotetranychus miscanthi* compared to outcrossed lineages. This result is strikingly similar to our findings. In both mites and giant kelp the manifestation of inbreeding depression was most prevalent in reproductive traits in the diploid phase.

Finally, our results suggest that the incidence of self-fertilization may be quite high particularly when spore dispersal is limited. Although highly motile, the small size of kelp spores (~5–10 μ in diameter) causes their dispersal to be determined largely by hydrodynamic conditions; current velocity is thought to increase dispersal distance and elevated turbulence to decrease it (Gaylord et al. 2002). Available information suggests that kelp beds alter flow by reducing current velocities and perhaps by increasing turbulence (Jackson and Winant 1983, Jackson 1984, Eckman et al. 1989, Jackson 1998; but see Gaylord et al. [*in press*] for a discussion of turbulence in kelp beds). Such phenomena would cause spore dispersal to be much more limited within populations (i.e., inside of kelp beds) than that among populations (i.e., outside of kelp beds; Graham 2003). If this is true, then one might expect kelp populations on isolated reefs or in areas of slow currents to display higher and more variable levels of self-fertilization than populations on continuous reefs or in areas exposed to faster currents. Given the results presented herein, periodic local extinctions of *Macrocystis* is a potential outcome resulting from high levels of inbreeding. In fact, kelp populations worldwide undergo frequent episodes of decline and recolonization, much of which may involve dispersal over scales of hundreds to thousands of meters (Ebeling et al. 1985, Dayton et al. 1992, Reed et al. 2000). In California, populations of giant kelp fluctuate on cycles of 3–5 years (Dayton et al. 1984, Nisbet and Bence 1989, Burgman and Gerard 1990, Dayton et al. 1992, Graham et al. 1997). The extent to which inbreeding depression resulting from self-fertilization affects the dynamics of kelp populations has never been considered, yet may be significant.

The ecology and life history of *Macrocystis pyrifera* provides a fascinating and nearly completely unexplored system in which to test evolutionary theory. Beyond this system there awaits the intriguingly complicated suite of life histories in other marine algal systems, particularly the red algae. Coupled with recent advances in tools to study population structure, nearshore oceanography, and small-scale hydrodynamics, we are entering a phase of investigation of the evolutionary ecology of marine populations and communities. Such studies will complement the long history of ecological studies in these systems to provide a richer understanding of the structure and organization of communities in time and space.

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Literature Cited

Anderson, E. K., and W. J. North. 1966. In situ studies of spore production and dispersal in the giant kelp, *Macrocystis pyrifera*. Proceedings of the International Seaweed Symposium **5**:73–86.

Atmer, W. 1991. On the role of males. Animal Behavior **41**: 195–205.

Barrett, S. C. H., and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature **352**:522–524.

Barrett, S. C. H., and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Ecology and Evolution **11**:A73–A79.

Barrett, S. C. H., L. D. Harder, and A. C. Worley. 1996. The comparative biology of pollination and mating in flowering plants. Philosophical Transactions of the Royal Society of London B Biological Sciences **351**:1271–1280.

Bertin, R. I. 1993. Incidence of monoecy and dichogamy in relation to self-fertilization in angiosperms. American Journal of Botany **80**:557–560.

Burgman, M. A., and V. A. Gerard. 1990. A stage-structured, stochastic population model for the giant kelp, *Macrocystis pyrifera*. Marine Biology **105**:15–23.

Carr, D. E., and M. R. Dudash. 1995. Inbreeding under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. Heredity **75**:437–445.

Charlesworth, B. 1980. Evolution in age-structured populations. Cambridge University Press, Cambridge, UK.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics **18**:237–268.

Coyer, J. A., J. L. Olsen, and W. T. Stam. 1997. Genetic variability and spatial separation in the sea palm kelp *Postelsia palmaeformis* (Phaeophyceae) as assessed with M13 fingerprints and RAPDs. Journal of Phycology **33**:561–568.

Crozier, R. H. 1985. Adaptive consequences of male-haploidy. Pages 201–202 *in* W. Helle and M. W. Sabelis, editors. Spider mites, their biology, natural enemies and control. Volume 1A. Elsevier, Amsterdam, The Netherlands.

Davenport, C. B. 1908. Degeneration, albinism and inbreeding. Science **28**:454–455.

Dayton, P. K. 1985. The ecology of kelp communities. Annual Review of Ecology and Systematics **16**:215–245.

Dayton, P. K., V. Curie, T. Gerrodette, B. D. Keller, R. Rosenthal, and D. Ven Tresca. 1984. Patch dynamics and stability of some California kelp beds. Ecological Monographs **54**:253–289.

Dayton, P. K., M. J. Tegner, P. E. Parnell, and P. B. Edwards. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp forest community. Ecological Monographs **62**:421–445.

Deysher, L. E., and T. A. Dean. 1986. In situ recruitment of sporophytes of the giant kelp *Macrocystis pyrifera* (L) Agardh, C.A.: effects of physical factors. Journal of Experimental Marine Biology and Ecology **103**:41–63.

Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L (Gentianaceae)—a comparison in 3 environments. Evolution **44**:1129–1139.

Ebeling, A. W., D. R. Laur, and R. J. Rowley. 1985. Severe storm disturbances and reversal of community structure in a southern California kelp forest. Marine Biology **84**:287–294.

Eckert, C. G., and S. C. H. Barrett. 1994. Inbreeding depression in partially self-fertilizing decodon *Verticillatus* (Lythraceae)—population genetic and experimental analyses. Evolution **48**:952–964.

Eckman, J. E., D. O. Duggins, and A. T. Sewell. 1989. Ecology of understory kelp environments. I. Effects of kelps on flow and particle transport near the bottom. Journal of Experimental Marine Biology and Ecology **129**:173–187.

Gaylord, B., D. C. Reed, P. T. Raimondi, L. Washburn, and S. R. McLean. 2002. A physically based model of macroalgal spore dispersal in the wave and current-dominated nearshore. Ecology **83**:1239–1251.

Gaylord, B., D. C. Reed, L. Washburn, and P. T. Raimondi. *In press.* Physical–biological coupling in spore dispersal of kelp forest macroalgae. Journal of Marine Systems.

Graham, M. H. 2003. Coupling propagule output to supply at the edge and interior of a giant kelp forest. Ecology **84**: 1250–1264.

Graham, M. H., C. Harrold, S. Lisin, K. Light, J. M. Watanabe, and M. S. Foster. 1997. Population dynamics of giant kelp Macrocystis pyrifera along a wave exposure gradient. Marine Ecology Progress Series **148**:269–279.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. Journal of Theoretical Biology **12**:12–45.

Honys, D., and D. Twell. 2003. Comparative analysis of the Arabidopsis pollen transcriptome. Plant Physiology **132**: 640–652.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution **50**:54–70.

Jackson, G. A. 1984. Internal wave attenuation by coastal kelp stands. Journal of Physical Oceanography **14**:1300–1306.

Jackson, G. A. 1998. Currents in high drag environment of a coastal kelp stand off California. Continental Shelf Research **17**:1913–1928.

Jackson, G. A., and C. D. Winant. 1983. Effect of a kelp forest on coastal currents. Continental Shelf Research **2**: 75–80.

Jarne, P., and D. Charlesworth. 1993. The evolution of the selfing rate in functionally hermaphroditic plants and animals. Annual Review of Ecology and Systematics **24**:441–466.

Jones, D. F. 1917. Dominance of linked factors as a mean of accounting for heterosis. Genetics **2**:466–479.

Keller, L. F., and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolutionary Biology **17**:230–241.

Korpelainen, H., and M. Kolkkala. 1996. Genetic diversity and population structure in the outcrossing populations of *Equisetum arvense* and *E. hyemale* (Equisetaceae). American Journal of Botany **83**:58–62.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants I. Genetic models. Evolution **39**:24–40.

Lewis, R. J., and M. Neushul. 1994. Northern and southern hemisphere hybrids of *Macrocystis* (Phaeophyceae). Journal of Phycology **30**:346–353.

Lively, C. M., and D. G. Lloyd. 1990. The cost of biparental sex under individual selection. American Naturalist **135**: 489–500.

Luning, K., and D. G. Muller. 1978. Chemical interaction in sexual reproduction of several Laminariales (Phaeophyceae)—release and attraction of spermatozoids. Zeitschrift fuer Pflanzenphysiologie **89**:333–341.

Matsuda, H. 1987. Conditions for the evolution of altruism. Pages 67–80 *in* Y. Ito, J. L. Brown, and J. Kikkawa, editors. Animal societies: theories and facts. Japan Scientific Society Press, Tokyo, Japan.

Muller, D. G., I. Maier, and G. Glassmann. 1985. Survey on sexual pheromone specificity in Laminariales (Phaeophyceae). Phycologia **24**:475–477.

Nisbet, R. M., and J. R. Bence. 1989. Alternative dynamic regimes for canopy-forming kelp: a variant of density-population regulation. American Naturalist **134**:377–408.

Parker, I. M., R. R. Nakamura, and D. W. Schemske. 1995. Reproductive allocation and the fitness consequences of selfing in two sympatric species of *Epilobium* (Onagraceae) with contrasting mating systems. American Journal of Botany **82**:1007–1016.

Provosoli, L. 1968. Media and prospects for the cultivation of marine algae. Pages 63–75 *in* A. Watanabe and A. Hatori, editors. Cultures and collections of algae. Proceedings of the United States-Japan Conference, Hakone. Japanese Society of Plant Physiologists, Kyoto, Japan.

Reed, D. C. 1990. The effects of variable settlement and early competition on patterns of kelp recruitment. Ecology **71**: 776–787.

Reed, D. C., T. W. Anderson, and A. W. Ebeling. 1997. The role of reproductive synchrony in the colonization potential of kelp. Ecology **78**:2443–2457.

Reed, D. C., A. W. Ebeling, and T. W. Anderson. 1996. Differential reproductive responses to fluctuating resources in two seaweeds with different reproductive strategies. Ecology **77**:300–316.

Reed, D. C., D. R. Laur, and A. W. Ebeling. 1988. Variation in kelp dispersal and recruitment: the importance of episodic events. Ecological Monographs **58**:321–335.

Reed, D. C., P. T. Raimondi, M. H. Carr, and L. Goldwasser. 2000. The role of dispersal and disturbance in determining spatial heterogeneity in sedentary organisms. Ecology **81**: 2011–2026.

Reed, D. C., P. T. Raimondi, L. Washburn, B. Gaylord, B. P. Kinlan, and P. T. Drake. 2004*b*. A metapopulation perspective on patch dynamics and connectivity in giant kelp. *In* P. Sale and J Kritzer, editors. Marine metapopulations. Academic Press, *in press*.

Reed, D. C., S. C. Schroeter, and P. T. Raimondi. 2004*a*. Spore supply and habitat availability as sources of recruitment limitation in giant help, *Macrocystis pyrifera*. Journal of Phycology **40**:275–284.

Rose, M. R. 1991. The evolutionary biology of aging. Oxford University Press, New York, New York, USA.

Saito, Y. 1994. Is sterility by deleterious recessives an origin of inequalities in the evolution of eusociality? Journal of Theoretical Biology **166**:113–115.

Saito, Y., K. Sahara, and K. Mori. 2000. Inbreeding depression by recessive deleterious genes affecting female fecundity of a haplo-diploid mite. Journal of Evolutionary Biology **13**:668–678.

Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants II. Genetic models. Empirical observations. Evolution **39**:41–52.

Smith, R. H., and M. R. Shaw. 1980. Haploid sex ratios and the mutation rate. Nature **287**:728–729.

Soltis, P. S., and D. E. Soltis. 1988. Estimated rates of intragametophytic selfing in lycopods. American Journal of Botany **75**:248–256.

Soltis, D. E., P. S. Soltis, and R. D. Noyes. 1988. An electrophoretic investigation of intragametic selfing in *Equisetum arvense*. American Journal of Botany **75**:231–238.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.

Walsh, N. E., and D. Charlesworth. 1992. Evolutionary interpretations of differences in pollen tube growth rates. Quarterly Review of Biology **67**:19–37.

Williams, G. C. 1957. Pleiotropy, natural selection and and the evolution of senescence. Evolution **11**:398–411.

Wyatt, R. 1990. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae): V. Artificial crosses within and between populations. Systematic Botany **15**:363–369.

APPENDIX

An ANOVA table assessing zygote production and a repeated-measures ANCOVA table assessing sporophyte number are available in ESA's Electronic Data Archive: *Ecological Archives*: EO85-112-A1.