Male-specific AFLP markers reveal extreme female-biased sex-ratios in the surfgrass *Phyllospadix torreyi* (Zosteraceae)

Final Technical Summary

Final Study Report
Male-specific AFLP markers reveal extreme female-biased sex-ratios in the surfgrass *Phyllospadix torreyi* (Zosteraceae)

Final Technical Summary

Final Study Report

Authors

Douglas S. Bush
Daniel C. Reed
Sally J. Holbrook
Scott A. Hodges
Principal Investigators

Prepared under MMS Cooperative Agreement No. 14-35-0001-31063 by
Coastal Marine Institute
Marine Science Institute
University of California
Santa Barbara, CA  93106-6150
Disclaimer

This report has been reviewed by the Pacific Outer Continental Shelf Region, Minerals Management Service, U.S. Department of the Interior and approved for publication. The opinions, findings, conclusions, or recommendations in this report are those of the authors, and do not necessarily reflect the views and policies of the Minerals Management Service. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use. This report has not been edited for conformity with Minerals Management Service editorial standards.

Availability of Report

Extra copies of the report may be obtained from:

U.S. Dept. of the Interior
Minerals Management Service
Pacific OCS Region
770 Paseo Camarillo
Camarillo, CA 93010
Phone: 805-389-7621

A PDF file of this report is available at:
http://www.coastalresearchcenter.ucsb.edu/CMI/

Suggested Citation

The suggested citation for this report is:

**Table of Contents**

FINAL TECHNICAL SUMMARY........................................................................................................ 1

FINAL STUDY REPORT ..................................................................................................................... 3
  Introduction ..................................................................................................................................... 3
  Methods .......................................................................................................................................... 4
    Study organisms .......................................................................................................................... 4
    Collection of samples .................................................................................................................. 4
    DNA extraction & AFLP amplification ......................................................................................... 5
    Cloning and sequencing of male markers ..................................................................................... 5
    Determination of genotypes ........................................................................................................ 6
  Results ........................................................................................................................................... 6
    Identification of male markers ..................................................................................................... 6
    Sequence analysis of male markers .............................................................................................. 8
    Determination of population sex-ratios ....................................................................................... 9
  Discussion ....................................................................................................................................... 10

ACKNOWLEDGEMENTS .................................................................................................................... 13

REFERENCES .................................................................................................................................... 14

APPENDICES .................................................................................................................................... 17
List of Tables

Table 1.  The frequency of AFLP male markers ........................................... 6
Table 2.  Characterization of ten male-specific AFLP markers ....................... 9
Table 3.  The frequency of males in 22 natural populations .......................... 10

List of Figures

Figure 1.  Map of Santa Barbara, California County coastline ........................ 5
Figure 2.  The frequency of AFLP male markers ........................................... 7
Figure 3.  The frequency of co-occurrence of the ten male markers ................... 8

List of Appendices

Appendix 1.  Frequency of male genotypes .................................................... 17
Studия: Population genetics of surfgrass (*Phyllospadix torreyi*) for use in restoration

**Report Title:** Male-specific AFLP markers reveal extreme female-biased sex-ratios in the surfgrass *Phyllospadix torreyi* (Zosteraceae)

**Contract Number:** 14-35-0001-31063

**Sponsoring OCS Region:** Pacific

**Applicable Planning Area(s):** Southern California

**Fiscal Year(s) of Project Funding:** FY 00/01, FY 01/02, FY 02/03

**Completion Date of Report:** June 2007

**Cost(s):** FY 00/01: $67,067; FY 01/02: $53,451; FY 02/03: $8,461

**Cumulative Project Cost:** $128,979

**Project Manager:** Russell J. Schmitt

**Address:** Coastal Research Center, Marine Science Institute, University of California, Santa Barbara, CA 93106-6150

**Principal Investigators:** Douglas S. Bush¹, Daniel C. Reed², Sally J. Holbrook¹,² and Scott A. Hodges¹*

**Addresses:** ¹ Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106-9610; ² Marine Science Institute, University of California, Santa Barbara, CA 93106-6150;  *Author for correspondence

**Keywords:** Intertidal ecology, sex determination, male/female ratio, genetic markers

**Background:** The surfgrass *Phyllospadix torreyi* is a clonal marine angiosperm that occurs in dense persistent stands in the intertidal and shallow subtidal zones of moderate to high energy coastlines of the northern Pacific Ocean. Surfgrasses are among the most productive seagrass communities yet studied and their stands form the basis for a thriving intertidal community. Because surfgrass populations have been and may be disturbed through oil and gas exploration, understanding how to restore populations is an important goal. Complicating these efforts is the fact that surfgrass is a dioecious species consisting of separate male and female plants. Natural populations appear to be strongly biased towards females and thus replicating sex-ratios may be important in restoration efforts.
OBJECTIVES: Our goal was to determine if sex determination in surfgrass was due to genetic factors and then to determine the actual frequency of male and female individuals. Because surfgrass forms dense mats it is impossible to identify genetic individuals. Furthermore, we sought to determine if there are microhabitat correlates with where each sex occurs.

DESCRIPTION: Using a DNA fingerprinting technique, amplified fragment length polymorphisms (AFLP), we scanned the genome of known males and females to identify genetic markers associated with sex. We also used these AFLP markers to identify unique genetic individuals in random samples.

STUDY RESULTS: We identified a number of genetic markers that were highly correlated with sex. Using a combination of these markers we were able to conclusively sex individuals that were non-flowering. All of the markers we identified were male-specific markers suggesting that surfgrass has an XY sex-determination system. The genetic markers also reveal that the male-determining Y region is likely accumulating transposable elements as has been hypothesized for the evolution of Y-chromosomes. After identifying unique genetic individuals from random samples across the Santa Barbara Co. coastline, we determined that the frequency of males was only about four percent. We did not find any correlation with the frequency of males and habitat.

SIGNIFICANT CONCLUSIONS: We conclude that individual sexes in surfgrass are determined by an XY genetic system. Significantly, we find that males of surfgrass are exceptionally rare. This suggests that great care should be used to establish male plants during restoration to ensure that the population can sustain reproduction.

STUDY PRODUCTS:

Published work:

Presentations:
Introduction

Dioecious species are predicted to have unbiased sex-ratios due to negative frequency dependent selection (Fisher 1930). In dioecious plants however, equal sex-ratios account for only 29 percent of species and 57 percent of species have male-biased ratios (Delph 1999). A number of factors have been suggested to account for male-biased sex-ratios, in particular, differences in the cost of reproduction between the sexes (Lloyd & Webb 1977). If females invest a higher proportion of resources to reproduction than males, trade-offs in other life-history traits can result. For instance, stress may cause females to have higher mortality (Lloyd 1974, Meagher 1981) or flower less frequently (Cipollini & Stiles 1991).

In contrast to male-biased sex-ratios, female-biased sex-ratios are rare in plants, accounting for only 14 percent of species (Delph 1999). However, among plants with sex chromosomes, female-biased sex-ratios are particularly common (Lloyd 1974). A number of hypotheses have been developed to account for this genetic association including selfish genetic elements (Taylor 1999), the relative competitive ability of pollen tubes carrying different sex chromosomes, termed certation (Correns 1917, Stehlik & Barrett 2006), as well as local competition (de Jong & Klinkhamer 2002) or differential herbivory between the sexes (Agren et al. 1999). For example, in Silene latifolia, sex-ratio distorters have been identified (Taylor 1994, 1999) and in Rumex, strong evidence for certation has recently been reported (Stehlik & Barrett 2006).

The association between sex chromosomes and female-biased sex-ratios suggests that the sex chromosomes themselves cause the lower frequency of males. In most plants studied to date with sex chromosomes, males are the heterogametic sex though many dioecious species have not been studied (Charlesworth 2002, Vyskot & Hobza 2004). Because the Y chromosome does not recombine with the X, the Y chromosome can accumulate deleterious mutations. This accumulation is accelerated due to the lower effective population size of the Y chromosome compared to autosomes and results in degeneration of gene function (Rice 1987, Charlesworth 2002, Vyskot & Hobza 2004). This degeneration of the Y-chromosome could then lead directly to impaired performance of males (Charlesworth 2002) and may account for the poorer performance of Y-bearing pollen (Stehlik & Barrett 2006).

Among the few reports of strong female-biased sex-ratios in plants, those for the marine angiosperm surfgrass (Phyllospadix torreyi) are exceptional (Dudley 1893, Cox et al. 1992, Williams 1995). Williams (1995) reported a population average of approximately 85 percent females on Santa Catalina Island, California. However, these numbers were based on the number of observed flowering shoots and may be biased by a number of factors. Phyllospadix torreyi grows as a rhizome and forms dense clonal mats, which over time can become fragmented due to disturbance making the identification of single individuals essentially impossible. Thus, reported population sex-ratios based on flowering represent data on ramets rather than genetic individuals. Problems associated with using flowers to estimate population sex-ratios will be exacerbated if the sexes differ in the extent of clonal growth, mortality, age of reproduction, or the frequency and magnitude of flowering (Webb 1999). Without
knowledge of the identity of unique genotypes, it is impossible to determine the sex-ratio of adult plants.

While *Phyllospadix torreyi* populations appear strongly female-biased, it is equivocal whether this species possesses sex chromosomes (Stewart and Rudenberg 1980). The lack of clear evidence of sex chromosomes in *P. torreyi* is interesting because in plant species with sex chromosomes, both the X and the Y-chromosomes are usually larger than the autosomes (Parker 1990) and therefore should be easy to recognize. The reported extreme female-biased sex-ratios and the lack of clear evidence for sex chromosomes provided the underlying motivation for our study. We sought to identify genetic markers that are linked to gender determination. Once we identified male-specific markers for *P. torreyi* we used them to determine the sex-ratio of genetically distinct individuals in a large number of natural surfgrass populations.

**Methods**

**Study organism:** The surfgrass *Phyllospadix torreyi* (Family Potamogetonaceae; den Hartog 1970) is a clonal marine angiosperm that occurs in dense persistent stands in the intertidal and shallow subtidal zones of moderate to high energy coastlines of the northern Pacific Ocean (Phillips 1979, Turner 1985, Stewart 1989). Surfgrasses are among the most productive seagrass communities yet studied (Ramirez-Garcia et al. 1998, 2002) and are unique among seagrasses in that they grow on rocks in wave-swept areas (Phillips & Menez 1988). Their thin leaves and flowering shoots emerge from creeping rhizomes that are solidly attached to the hard substrate by long branching root hairs, following germination and early growth during a period of attachment to a host alga (Blanchette et al. 1999). *Phyllospadix* is dioecious; both male and female plants produce long reproductive shoots (rhapidia) that support spikes of flowers (spadices), which are most prevalent during the summer and early fall (Williams 1995, authors, unpublished data).

**Collection of samples:** Samples obtained from flowering individuals were used to screen for gender specific markers in *Phyllospadix torreyi* (N= 140 male and 280 female). These samples were collected from 21 localities distributed along a 260 km stretch of coast near Santa Barbara, California, USA. Samples obtained from non-flowering individuals were used to genetically determine the population sex-ratios at 22 spatially discrete sites located within a 159 km segment of the 260 km study region, which encompassed the Point Conception biogeographic transition zone (Briggs 1974; Figure 1).
For analysis we divided the 22 sites into three geographic regions: sites north of Point Conception (Region 1), sites in the western Santa Barbara Channel (Region 2) and sites in the eastern Santa Barbara Channel (Region 3). We collected a total of 591 samples in July 2001 from the low intertidal zone at each of the 22 sites during low tides and from the shallow subtidal zone at 10 of the sites by divers using scuba.

**DNA extraction & AFLP amplification:** DNA was extracted from the basal portion of fresh leaves using the DNeasy kit (Qiagen). Each extraction was carried out twice as single extractions proved to contain inhibitors to the AFLP reactions. The AFLP protocol followed a modified version of Vos et al. (1995) with a 5X digestion with EcoRI and MseI and a fluorescently labeled E-primer in each selective amplification. Reaction products were separated on a LiCor 4200 DNA sequencer and variable bands were scored with SAGA MX AFLP scoring software (LiCor).

**Cloning and sequencing of male markers:** Once specific AFLP markers were identified, we ran the reactions again but used $^{33}$P-labeled primers. The reactions were separated on polyacrylamide gels, dried and exposed to X-ray film. Subsequently, we extracted the male-marker bands from the gel, recovered the DNA and PCR amplified them using the appropriate AFLP primers. The products were then cloned using pGEM T-Easy cloning kit (Promega). Colonies with the correct insert size were identified with PCR amplification of colonies using M13 primers. The amplification products were used directly in simultaneous bi-directional sequencing following the protocol for the Thermosequenase kit (Amersham) with internal
primers T7 and pGEM+46 (5'-CCGCGGGAATTCGAT-3') containing fluorescent IRD labels 800 and 700 (Li-Cor), respectively. Sequencing was conducted on a Li-Cor 4200 sequencer.

**Determination of genotypes:** Because *Phyllospadix* has extensive growth by rhizomes, samples collected at any one site may be ramets and thus not genetically distinct individuals. To determine the repeatability of AFLP genotyping from single individuals, we collected two samples from some ramets, isolated the DNA and ran the AFLP from each sample separately. After scoring, we determined the percentage of bands that were scored identically from these replicate samples. We then applied this percentage as a cutoff for identifying different genotypes among samples from each site.

**Results**

**Identification of male markers:** We screened known male and female samples for AFLP markers that were in high frequency in one or the other sex. We used 20 pairs of primers, which generated 450 variable bands. Of these, we found 10 bands that were highly abundant in male samples (Table 1) but not in females; we refer to these bands as male markers.

**Table 1.** The frequency of AFLP male markers in *Phyllospadix torreyi* in the three study regions along the coast of California. The frequency of each marker is listed as a proportion of individuals having that marker and was determined from samples of known males and females collected from the three regions shown in Figure 1. Sample sizes (n) are given in parentheses.
We did not find any bands that were abundant in females but not in males. While the male markers were at high frequency in males, no single marker was present in every male (Table 1, Fig. 2). Furthermore, seven of the ten markers were found in some females (Table 1, Fig. 2). We then determined the total number of male markers present in all individuals where all markers had been scored. We found that over 80% of females had none of the male markers and never more than two (Fig. 3). In contrast, nearly half of the males had all 10 male markers and never fewer than six. Thus, we classified non-flowering individuals as males when they had six or more male markers and as female if they had two or less.

Figure 2. The frequency of AFLP male markers in flowering male and female individuals of *Phyllospadix torreyi* in the Santa Barbara region. The presence or absence of each marker was determined for all samples. The sample size for each marker ranged from 130 to 140 for males and 180 to 280 for females. Male marker characteristics are shown in Table 1.
Number of male markers

Proportion of individuals

- **males**
- **females**

Figure 3. The frequency of co-occurrence of the ten male markers in male and female *Phyllospadix torreyi*. Data are the proportion of male and female individuals having zero to all ten of the male markers. The number of male markers was determined for individuals of known gender (as identified by flowers) where all ten male markers could be scored. Sample sizes were 112 and 157 for males and females, respectively.

Sequence analysis of male markers: Male markers varied from 124 to 835 bp in length, including the AFLP primers, which added a total of 38 bp to the native sequence (Table 2). The native sequences were then subjected to tBlastx searches at the NCBI website. The four longest sequences (338 – 797 bp) had very strong hits to retroelements (Table 2). The smaller fragments did not have significant hits to the databases.
Table 2. Characterization of ten male-specific AFLP markers in *Phyllospadix torreyi*. The oligonucleotides used to generate the AFLP bands that are associated with maleness (AFLP Primers) and the size of the marker in basepairs (Size) are given. Size includes the AFLP primers which add a total of 38 bp to the native sequence. Significant homologues identified by a search of the NCBI database using the protein translation of the marker DNA sequence are listed.

<table>
<thead>
<tr>
<th>Male-Marker Number</th>
<th>AFLP Primers</th>
<th>Size</th>
<th>Homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M-CTG, E-ACT</td>
<td>161</td>
<td>None identified</td>
</tr>
<tr>
<td>2</td>
<td>M-CTT, E-ACA</td>
<td>124</td>
<td>None identified; 98% Identical to Band 3</td>
</tr>
<tr>
<td>3</td>
<td>M-CAT, E-ACA</td>
<td>125</td>
<td>None identified</td>
</tr>
<tr>
<td>4</td>
<td>M-CTC, E-AAC</td>
<td>218</td>
<td>None identified</td>
</tr>
<tr>
<td>5</td>
<td>M-CTC, E-AAG</td>
<td>376</td>
<td>Pol Polyprotein Reverse Transcriptase</td>
</tr>
<tr>
<td>6</td>
<td>M-CTC, E-AAG</td>
<td>835</td>
<td>Pol Polyprotein Retrotransposon</td>
</tr>
<tr>
<td>7</td>
<td>M-CAA, E-AAC</td>
<td>176</td>
<td>None identified</td>
</tr>
<tr>
<td>8</td>
<td>M-CCA, E-AAG</td>
<td>655</td>
<td>Pol Polyprotein Retroelement</td>
</tr>
<tr>
<td>9</td>
<td>M-CAG, E-AAG</td>
<td>513</td>
<td>Pol Polyprotein Retroelement</td>
</tr>
<tr>
<td>10</td>
<td>M-CCC, E-AAC</td>
<td>270</td>
<td>None identified</td>
</tr>
</tbody>
</table>

**Determination of population sex-ratios:** We analyzed a total of 594 samples that were collected from plants that were not flowering at the 22 sites. We found that replicate samples from the same individual resulted in < 5% difference in scoring and thus used this as a conservative estimate of the number of genotypes in our sample. Using this cutoff we identified a total of 482 genotypes. For these unique genotypes, we then determined the number of male-specific bands and assigned gender to each sample as outlined above. We identified 21 male plants, representing only 4.36 percent of all genotypes. Chi-Square analyses revealed that the proportion of males did not differ significantly among the three coastal regions ($\chi^2 = 1.9882$, DF = 2, P = 0.39) or between the intertidal and subtidal zones ($\chi^2 = 0.0905$, DF = 1, P = 0.77; Table 3).
Table 3. The frequency of males in 22 natural populations in (A) the three study regions summed across the intertidal and subtidal zones, and (B) the intertidal and subtidal zones summed across the three study regions. Values are the number of samples, genotypes and males for each category. The percentages of genotypes that were male are shown in parentheses.

(A)

<table>
<thead>
<tr>
<th>Region</th>
<th>Samples</th>
<th>Genotypes</th>
<th>Males</th>
<th>Males (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>153</td>
<td>119</td>
<td>3</td>
<td>2.4%</td>
</tr>
<tr>
<td>2</td>
<td>219</td>
<td>190</td>
<td>9</td>
<td>4.2%</td>
</tr>
<tr>
<td>3</td>
<td>219</td>
<td>163</td>
<td>10</td>
<td>6.0%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>591</td>
<td>472</td>
<td>22</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

(B)

<table>
<thead>
<tr>
<th>Depth</th>
<th>Samples</th>
<th>Genotypes</th>
<th>Males</th>
<th>Males (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intertidal</td>
<td>461</td>
<td>351</td>
<td>17</td>
<td>4.8%</td>
</tr>
<tr>
<td>Subtidal</td>
<td>130</td>
<td>121</td>
<td>5</td>
<td>4.1%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>591</td>
<td>472</td>
<td>22</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

Discussion

In this study, we determined the gender of adult plants in populations of the marine angiosperm, *Phyllospadix torreyi*, using a combination of molecular markers. We were able to identify the gender of plants, regardless of their flowering state, and found an exceptionally high frequency of females (0.96 over all samples), the highest for any reported flowering plant. Previously Williams (1995) reported a slightly lower frequency of females (0.86) on Santa Catalina Island, California based on flowering shoots. The differences between Williams’ findings (1995) and ours may reflect actual differences in sex-ratios among populations on Santa Catalina Island and those along the Santa Barbara Co. coast. Alternatively, these populations may have similar frequencies of females, but differ in the frequency that males and females flower or in the numbers of flowering shoots they produce. Regardless, *P. torreyi* maintains extreme female-biased populations over a wide-geographic area.

One mechanism that can influence adult sex ratios is performance differences between the two sexes (Delph 1999). Among dioecious plants, females generally suffer greater mortality, presumably at the expense of providing greater resources to their offspring (Delph 1999). Interestingly, Williams (1995) found that males of *Phyllospadix torreyi* were inferior to
females in their ability to adhere to rocky substrates. She also found that males increased in abundance (based on flowering) in subtidal habitats and concluded that they experienced greater mortality in intertidal habitats due to strong wave action. Like Williams (1995), we also noted a tendency for a greater frequency of flowering males in subtidal, compared to intertidal, habitats near Santa Barbara (Reed & Holbrook, unpub. data). However, using genetic markers, we found no difference in the sex-ratio between these two habitats. This result suggests that males may not have differential mortality across the habitats but, rather, that they may be more likely to flower, or that females are less likely to flower, in the subtidal region.

Though we did not find evidence for differential survival of the sexes in different habitats, differential mortality may still contribute to the extreme sex-ratio found in adult plants. Information on the sex-ratios of earlier life stages is needed to determine if males have higher rates of mortality than females. Only then will it be possible to determine if sex-ratios change through time and thus whether there are performance differences between the two sexes. For instance, in *Rumex nivalis*, Stehlik & Barrett (2005) determined that sex ratios became more female biased from seed to adult suggesting that males had greater mortality than females. Such a finding would impact restoration efforts of *Phyllospadix torreyi*, particularly if early life-history stages are utilized (Bull et al. 2004). Unfortunately, we were unable to amplify DNA extracted from seeds of *Phyllospadix torreyi* and thus could not determine if a similar pattern is happening in this species.

Like most other plant species with a genetic sex-determination system (Charlesworth 2002), we found that males of *Phyllospadix torreyi* are the heterogametic sex. Despite this finding, it is equivocal whether *P. torreyi* has differentiated sex chromosomes. Stewart & Rudenberg (1980) found occasional meiotic preparations in *P. torreyi* with chromosomes that appeared or behaved unusually. However all chromosome counts were $n = 9$ and the authors were equivocal whether the unusual preparations were evidence for sex chromosomes (Stewart & Rudenberg, 1980). Harada (as reported in Kuo 2001) found that the Japanese species of *Phyllospadix* (*P. iwatensis* and *P. japonicus*) had $2n = 16, 17, 18, or 20$ which he interpreted as a system of multiple sex chromosomes. However, Uchiyama (1993) found that both sexes in these two species had $2n = 20$. Thus, while male *P. torreyi* are the heterogametic sex, the sex-determining region may well be only a portion of one of the chromosome pairs.

When heteromorphic sex chromosomes exist in plants, the Y-chromosome is often larger than the X (Parker 1990) and this size difference is thought to be due to the accumulation of transposable elements in the non-recombining region of the Y-chromosome (Charlesworth et al. 2005). Consistent with this hypothesis, we only found high sequence similarities to transposable elements for the male-markers in *Phyllospadix torreyi*. The high frequency of transposable elements coupled with the lack of differentiated sex chromosomes suggests that *Phyllospadix* is relatively early in the evolution of sex chromosomes. However, we do not know whether frequency of transposable elements in the male determining region is higher than that found in the corresponding regions of females or in autosomal regions and thus whether the Y-region is accumulating elements at a particularly high rate. Identifying early stages in the evolution of sex chromosomes is of wide interest (Liu et al. 2004), and future research in this area on *Phyllospadix torreyi* may prove to be particularly enlightening. Not
only would *P. torreyi* provide an interesting system for comparison, but its particularly small genome size (approximately 370 Mbp/1C, Hodges unpub. data) may be useful for identifying the genes underlying sex determination.

The presence of a genetic mechanism of sex determination in *Phyllospadix torreyi* suggests that this system itself may influence sex ratios. A non-recombining region of a Y-chromosome can accumulate deleterious mutations resulting in degeneration of the genes in this region (Charlesworth 2002). Such degeneration could occur because of, or in concert with the accumulation of transposable elements (as we have found here for *P. torreyi*) and may affect all functions associated with genes in the non-recombining region. Haploid pollen carrying a Y-chromosome would be especially affected by such degeneration (Smith 1963, Lloyd 1974, Charlesworth 2002). This in turn may make pollen carrying a Y-chromosome less competitive (also known as certation) as has been recently shown in *Rumex nivalis* (Stehlik & Barrett 2006), another strongly female-biased dioecious plant. In *P. torreyi*, pollen is produced as long strands that are easily tangled and form mats, suggesting that pollen competition may be common. In fact, seed/ovule ratios appear to be high as each flower contains only a single ovule and fruit set is high (Williams 1995, Reed & Holbrook, unpub. data). Controlled pollination experiments would be extremely useful for determining whether strong pollen competition occurs in *P. torreyi*. However, the wave-swept environment where this species occurs will make such studies quite challenging.
ACKNOWLEDGEMENTS

We thank M. Anghera for his help and encouragement in all phases of this work. The research was supported by the National Science Foundation (OCE 99-82105) and the Minerals Management Service of the U.S. Department of the Interior (MMS Agreement No. 14-35-00011-30761). The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the U.S. Government.
REFERENCES


Male-Specific AFLP Markers Reveal Extreme Female-Biased Sex-Ratios


Vyskot, B. and R. Hobza. 2004. Gender in plants: sex chromosomes are emerging from


systems. Pp 149-174 in M.A. Geber, T.E. Dawson and L.F. Delph, eds. Gender and
sexual dimorphism in flowering plants. Springer-Verlag, Berlin, Germany.

Williams, S.L. 1995. Surfgrass Phyllospadix torreyi reproduction: Reproductive phenology,
APPENDIX A

Frequency of male genotypes in the intertidal and subtidal zones at the 22 study sites. Distance - distance (km) of each site from the most northern site sampled (Purisima Point). Data include two specimens whose habitat status (intertidal, subtidal) is unknown.

<table>
<thead>
<tr>
<th>Site</th>
<th>Distance</th>
<th>Tot # Smps</th>
<th>Tot # Genotypes</th>
<th>Tot # Males</th>
<th>Total # Inter Smps</th>
<th>Tot # Inter Geno</th>
<th># Inter Male</th>
<th>Tot # Sub Smps</th>
<th>Tot # Sub Genotypes</th>
<th>Tot # Sub males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purisima Point</td>
<td>R 0</td>
<td>20</td>
<td>14</td>
<td>0</td>
<td>20</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Site 4</td>
<td>S 1</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stairs</td>
<td>T 2</td>
<td>18</td>
<td>10</td>
<td>0</td>
<td>18</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lompoc Landing</td>
<td>Q 4</td>
<td>30</td>
<td>25</td>
<td>0</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Wall Beach</td>
<td>U 6</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Honda Point</td>
<td>P 18</td>
<td>16</td>
<td>13</td>
<td>0</td>
<td>16</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Boat House</td>
<td>O 28</td>
<td>30</td>
<td>28</td>
<td>3</td>
<td>20</td>
<td>18</td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><strong>Region 1 Total</strong></td>
<td></td>
<td><strong>153</strong></td>
<td><strong>119</strong></td>
<td><strong>3</strong></td>
<td><strong>133</strong></td>
<td><strong>99</strong></td>
<td><strong>3</strong></td>
<td><strong>20</strong></td>
<td><strong>20</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>San Augustine</td>
<td>M 61</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>19</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drakes</td>
<td>F 66</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>20</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alegria</td>
<td>A 70</td>
<td>70</td>
<td>65</td>
<td>1</td>
<td>45</td>
<td>42</td>
<td>1</td>
<td>25</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Caliente (C)</td>
<td>C 72</td>
<td>30</td>
<td>25</td>
<td>1</td>
<td>20</td>
<td>15</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>San Onofre</td>
<td>N 79</td>
<td>30</td>
<td>24</td>
<td>1</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Arroyo Hondo</td>
<td>B 84</td>
<td>20</td>
<td>17</td>
<td>4</td>
<td>20</td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arroyo Quemado (D)</td>
<td>D 86</td>
<td>30</td>
<td>29</td>
<td>2</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td><strong>Region 2 Total</strong></td>
<td></td>
<td><strong>219</strong></td>
<td><strong>190</strong></td>
<td><strong>9</strong></td>
<td><strong>164</strong></td>
<td><strong>139</strong></td>
<td><strong>8</strong></td>
<td><strong>55</strong></td>
<td><strong>51</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>Hendry's (G)</td>
<td>G 130</td>
<td>30</td>
<td>25</td>
<td>3</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Light House</td>
<td>I 132</td>
<td>20</td>
<td>13</td>
<td>2</td>
<td>20</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shore Line</td>
<td>SH 133</td>
<td>50</td>
<td>46</td>
<td>0</td>
<td>25</td>
<td>23</td>
<td>0</td>
<td>25</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Leadbetter (H)</td>
<td>H 135</td>
<td>30</td>
<td>27</td>
<td>2</td>
<td>20</td>
<td>17</td>
<td>2</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Miramar</td>
<td>K 143</td>
<td>30</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Loon point</td>
<td>J 148</td>
<td>20</td>
<td>13</td>
<td>0</td>
<td>20</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Casitas</td>
<td>E 158</td>
<td>19</td>
<td>10</td>
<td>1</td>
<td>19</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rincon</td>
<td>L 159</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>20</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Region 3 Total</strong></td>
<td></td>
<td><strong>219</strong></td>
<td><strong>166</strong></td>
<td><strong>10</strong></td>
<td><strong>164</strong></td>
<td><strong>113</strong></td>
<td><strong>6</strong></td>
<td><strong>55</strong></td>
<td><strong>50</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>
The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the Offshore Minerals Management Program administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS Royalty Management Program meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.