Mating patterns in plant populations have a crucial role in establishing spatio-temporal patterns of genetic diversity within populations as well as in their evolutionary dynamics (Barrett, 2003, Glémin et al., 2006). Mating system and pollen dispersal patterns have a direct influence on individual reproductive ability, effective population size, and degree of population subdivision; affecting the levels of inbreeding, genetic variation, genetic structure, and speciation (Barrett, 2003, Charlesworth, 2003). Mating system is a complex trait that reflects the interaction between floral traits, demography, genetics, population structure, and a number of environmental factors that affect pollination (Barrett and Eckert, 1990, Ritland 2002). Understanding a species’ mating system is crucial for a meaningful analysis of the genetic diversity among its natural populations and for successful long term conservation efforts (Charlesworth, 2003).

Mangrove swamps are highly productive ecosystems that provide numerous environmental services, including protection from flood and coastal erosion, habitat for reproduction, and nurseries for a variety of organisms – many of which are critical for fisheries maintenance and biodiversity conservation (Polidoro et al., 2010). Because of their high productivity, mangrove swamps are a nutrient source for other coastal and marine ecosystems such as marine prairies and coral reefs (Duke et al., 2007). The black mangrove, *Avicennia germinans* (L.) L. (Avicenniaceae), is one of the most important mangrove tree species in the western hemisphere; this species is distributed extensively along tropical and subtropical coasts of the eastern Pacific and the Atlantic coasts of the American Continent and West Africa (Tomlinson, 1986). *Avicennia germinans* is a cryptoviviparous species with buoyant propagules, permitting effective long distance dispersal (Rabinowitz, 1976; Nettel and Dodd, 2007). The perfect flowers of *A. germinans* are bee pollinated; self-fertilization is probably avoided in individual flowers by the early development of male organs and late development of female organs (Tomlinson, 1986). Studies on floral biology and pollination system in *Avicennia marina* (Forssk.) Viehr.– the main representative of the genus in East Africa, Australia, and Asia – found indications of partial self-incompatibility (Clarke and Myerscough, 1991). Furthermore, genetic studies on *A. germinans* and *A. marina* suggest that tropical populations tend to be outcrossed (Arnaud-Haond et al., 2006; Giang et al., 2003; Cerón-Souza et al., 2005). However, direct evidence of the proportion of outcrossing and self-pollination are lacking for the genus. Moreover, little is known about the effect of population substructure and inbreeding caused by mating with relatives (biparental inbreeding). The pre-sent study aims to analyze the mating system of *A. germinans* populations using progeny arrays and microsatellite genetic markers.

During Fall 2005, we collected fruiting bodies (one propagule per fruiting body) and leaves from mother trees at three localities along the Chiapas, Mexico tropical coastline: (1) La Cigüeña [CIG] (14° 37’ 00” N, 92° 19’ 00” W), close to the border with Guatemala, in an area highly impacted by population expansion, road constructions, and agricultural activity;

### Table 1. Genetic diversity and inbreeding estimates of three *Avicennia germinans* populations from Chiapas, Mexico. n, number of adult trees; $H_s$, observed heterozygosity; $H_e$, expected heterozygosity; $R_t$, allelic richness; $F_{is}$ inbreeding coefficient. Standard error in parenthesis.

<table>
<thead>
<tr>
<th>Location</th>
<th>Code</th>
<th>n</th>
<th>$H_s$ (0.04)</th>
<th>$H_e$ (0.04)</th>
<th>$R_t$ (0.04)</th>
<th>$F_{is}$ (0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>La Cigüeña</td>
<td>CIG</td>
<td>32</td>
<td>0.40</td>
<td>0.41</td>
<td>3.46</td>
<td>0.04</td>
</tr>
<tr>
<td>Santa Catalina</td>
<td>CAT</td>
<td>30</td>
<td>0.39</td>
<td>0.43</td>
<td>3.17</td>
<td>0.07</td>
</tr>
<tr>
<td>El Paraíso</td>
<td>PAR</td>
<td>32</td>
<td>0.44</td>
<td>0.43</td>
<td>4.42</td>
<td>-0.04</td>
</tr>
</tbody>
</table>
(2) Santa Catalina [CAT] (15° 18’ 00” N, 92° 52’ 00” W, found within the Biosphere Reserve of La Encrucijada, this area presents the best developed mangrove forests in Mexico with trees reaching 30 meters in height; and (3) Paraíso [PAR] (15° 57’ 00” N, 93° 49’ 00” W), located in the extreme northwest of Chiapas, this location has a drier regime and mangroves are less developed, reaching just five to eight meters tall. At each locality, we collected leaves from around 30 potential parental trees and leaves and fruiting bodies from five to nine mother trees; selected trees were located at least 30 m apart. The number of fruiting bodies per mother tree analyzed ranged from 14 to 20 at CIG, from 7 to 20 at CAT, and from 13 to 14 at PAR; average number of fruiting bodies analyzed per mother tree was 18, 16, and 14, respectively. Plant material was dried using silica gel and a herbarium oven. Genomic DNA from leaves was extracted using the CTAB method (Cullings, 1992); genomic DNA from cotyledons was extracted using the method described by Thangjam et al. (2003). We PCR amplified six microsatellites designed for the black mangrove (Nettel et al., 2005) in three groups: (a) Te1, (b) Di13, Te8, Te9, and (c) Di6t, Te4t. PCR cocktail included 1X PCR buffer, 2.0 mm MgCl₂, 0.2 mm of each dNTP, 25 mg/mL BSA, 250 µm each forward and reverse primer (forward primers were fluorescently labeled), 1U of Taq Gold Polymerase (Applied Biosystems), and 5 ng of template DNA for a final 20 µL volume. Amplification and electrophoresis conditions followed Nettel et al. (2005), performing an annealing temperature of 58 °C for group (a) and 50 °C for groups (b) and (c). Results were analyzed with GENESCAN 3.7 and GENOTYPER 3.7 software (Applied Biosystems).

Gene diversity of parental trees was evaluated at each location by estimating expected heterozygosity (Hₑ), observed heterozygosity (Hₒ), and allelic richness (R) using the FSTAT ver 2.9.3.2 (Goudet et al., 2002) software. We also estimated the inbreeding coefficient and tested for deviations from Hardy-Weinberg expectations with FSTAT. Mating system analyses were performed with MLTR ver 3.2 (Ritland, 2002). MLTR takes advantage of multilocus information to infer levels of true selfing, of biparental inbreeding, and the effects of intrapopulation genetic substructure on population mating patterns. We estimated the following parameters: multilocus population outcrossing rate (tm); inbreeding coefficient of mother trees (F); minimum estimate of the apparent selfing due to biparental inbreeding (tₑ – tₛ); the proportion of siblings that share the same father (rₛ); and the effect of population substructure on outcrossing events [rₛ (s) – rₛ (m)]. Default values were used for initial conditions (tm = 0.9, F = 0.1, and rₛ = 0.1). Standard errors were calculated by 100 bootstrap repetitions, re-sampling individuals within families.

Estimated levels of genetic diversity were intermediate in Avicennia germinans sampled populations compared to those detected with microsatellite markers for A. marina (Arnaud-Haond et al. 2006); allelic richness ranged from 3.17 (CAT) to 4.42 (PAR) and observed and expected heterozygosity ranged from 0.39 (CAT) to 0.44 (PAR) and 0.41 (CIG) to 0.43 (CAT and PAR), respectively. Estimates of inbreeding coefficients were close to zero, ranging from -0.04 (PAR) to 0.07 (CAT); none of the studied populations presented significant deviations from Hardy-Weinberg equilibrium.

The estimated outcrossing rate (tₑ) ranged from 0.583 (Standard Error, SE = 0.09) in CIG to 0.774 (SE = 0.09) in CAT (Table 2). The detected level of biparental inbreeding was very low, ranging from 0.062 (SE = 0.03) in CAT to 0.013 (SE = 0.04) in PAR. The proportion of siblings that share the same father was low but present in CIG and CAT (0.18 [SE = 0.05] and 0.23 [SE = 0.08], respectively). Estimates for the effect of population substructure on outcrossing events were negative for all localities, indicating that outcrossing events were not influenced significantly by genetic substructuring among pollen donors. Our results confirm with direct evidence that Avicennia germinans populations are predominantly outcrossing but that they can support moderate levels of self-fertilization; up to almost 50% in the impacted population CIG and about 30% in the preserved population CAT. Our results are consistent with previous inferences regarding the mating system of Avicennia; a mixture of outbreeding and selfing in tropical populations, where outcrossing events predominate but are mostly at random with very little effect of population substructure and biparental inbreeding.

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<table>
<thead>
<tr>
<th>Code</th>
<th>Families/propagules n</th>
<th>F</th>
<th>tₑ</th>
<th>tₛ</th>
<th>rₛ (s) – rₛ (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIG</td>
<td>8/159</td>
<td>0.049 (0.09)</td>
<td>0.583 (0.09)</td>
<td>0.043 (0.04)</td>
<td>0.180 (0.05)</td>
</tr>
<tr>
<td>CAT</td>
<td>9/157</td>
<td>-0.200 (0.07)</td>
<td>0.774 (0.09)</td>
<td>0.062 (0.027)</td>
<td>0.231 (0.08)</td>
</tr>
<tr>
<td>PAR</td>
<td>5/74</td>
<td>-0.200 (0.09)</td>
<td>0.770 (0.12)</td>
<td>0.013 (0.038)</td>
<td>0.056 (0.14)</td>
</tr>
</tbody>
</table>

Table 2. Mating system parameters of three populations of Avicennia germinans from Chiapas, México. Families/propagules n, total number of parental trees and total number of seedlings examined per population; F, inbreeding coefficient of mother trees; tₑ, multilocus outcrossing rate; tₛ, bilateral inbreeding; rₛ (s), proportion of siblings that share the same father; rₛ (s) – rₛ (m), effect of population substructure on outcrossing events. Standard errors in parentheses.
Literature cited


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