Gender Expression in *Abies pinsapo* Boiss., a Mediterranean Fir

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Gender expression was recorded for three geographically distinct populations and two stand densities of pinsapo fir (*Abies pinsapo*) in southern Spain, during 4 consecutive years (1990–1993). During this period the trees only flowered in 1991 and 1992, and thus only two cone crops could be quantified. No significant geographical variation in flowering intensity or in cone crop production was found. The most extreme variations were correlated with stand density: flowering intensity and cone crop were always greater in low density trees. *Abies pinsapo* is a monoecious species but all the populations studied showed a predominance of female plants, with a few male and monoecious individuals, thus indicating a functionally subdioecious breeding system. No differences in gender expression between populations were recorded. Differences in sex expression of individuals at two different stand densities were found, but these were only significant in 1991. In dense stands of pinsapo firs branches were limited to the top of trees and thus, they produced mainly female cones, whereas isolated trees had branches from ground level and as a consequence they were predominantly male, with a higher total reproductive effort. The results indicate that relative investment in male and female reproductive structures by *A. pinsapo* individuals is a response to plant architecture, plant resource status and environmental variation.

Key words: *Abies pinsapo*, plant density, gender variation, reproductive biology, monoecy, Mediterranean fir.

INTRODUCTION

*Abies* is a large genus with approx. 46 species distributed in the northern hemisphere. *Abies pinsapo* Boiss., is a narrow relict fir occurring in the Sub-Betic mountains of southern Spain. Characteristics of *A. pinsapo* biology, such as seed germination, seedling survival, phenology, pollen dispersal capacity and viability, and seed crop production have been reported elsewhere (Arista, Herrera and Talavera, 1992; Arista, 1993, 1994; Arista and Talavera, 1994a, b; Arista and Talavera, 1996). Among *Abies* species, monoecy is the predominant breeding system, with female strobili at the top of the crown and male ones on the lower branches of the tree. Gender variation has been reported in other monoecious conifers such as *Pinus* (Fouler, 1965; Smith, 1981), *Taxus* (Allison, 1991) and *Juniperus* (Jordano, 1991), but it has not previously been reported in the genus *Abies*.

The phenotypic expression of gender depends on the distribution of resources to male and/or female functions in each reproductive episode (Goldman and Willson, 1986). According to the local mate theory, in those situations where parental fitness gains through pollen are small (because pollen dispersal is very localized) allocation ratios should shift in favour of female function. In *Abies pinsapo* populations the pollen dispersal capacity is very low (Arista and Talavera, 1994a), resulting in a large number of empty seeds and favouring endogamy depression in low density zones (Arista and Talavera, 1996). The purpose of this study was to assess between-population, between-density, and interannual variation of both cone production and gender expression in *Abies pinsapo* within the context of tree size and morphology.

MATERIALS AND METHODS

Study sites

The main study site was in the Sub-Betic mountains of the Sierra de Grazalema, Cádiz province (Sierra de Grazalema Nature Park; 600–1600 m above sea level), mean annual temperature is 15 °C and mean annual rainfall is approximately 2000 mm. At this site there is considerable variation in stand density, some trees grow at low density (150 trees ha⁻¹), while others grow in a dense forest of 3400 trees ha⁻¹. In addition, data were collected from another two populations; the Sierra de las Nieves and the Sierra Bermeja. The site in the Sierra de las Nieves, Málaga province (Sierra de las Nieves Nature Park; 900–1900 m above sea level) has a mean annual temperature of 15 °C, and has a drier winter (572 mm rain year⁻¹ on average) than Grazalema. The site in the Sierra Bermeja, Málaga province (Sierra Bermeja Nature Paraje; 1100–1449 m above sea level) has a mean annual temperature of 17 °C and a yearly rainfall of 961 mm.

Cone production and gender expression

Production of male and female cones was studied from 1991 to 1992 at all three sites. A total of 163 marked trees, haphazardly selected after flowering, were sampled in Sierra de Grazalema (SG), 28 growing at low density and 135 at high density. In addition 34 trees were monitored in Sierra de las Nieves (SN), and 35 in the Sierra Bermeja (SB).

Production of both male and female cones was estimated for each tree during April and May. The counts were conducted with the aid of binoculars along one complete side of the tree. The number of female cones was counted.
directly on the tree. To estimate the pollen cone production, the number of apical branches bearing male cones was counted and multiplied by the mean number of male cones per branch \(8.6\) \((n = 87)\) in 1991 and \(8.9\) \((n = 30)\) in 1992. To prevent possible confusion between reproductively immature trees and non-flowering, mature trees, the youngest tree in flower in 1991 was measured (in each of the three populations) and all trees smaller than this individual were subsequently rejected from the sample.

Based on the number of male and female cones, the functional sexuality of each tree was described according to methods proposed by Lloyd (1980b). Estimates of phenotypic gender were derived from the expression:

\[
G_i = g_i /[g_i + (a_i \times E')]; E' = \Sigma g_i / a_i
\]

where \(g_i\) is the total number of female cones produced on one side of tree \(i\) and \(a_i\) is the number of pollen cones. \(E'\) is an equivalence factor that equates the probability of androdioecial \(rs\) gynoecial units contributing genes to the next generation (Lloyd, 1979, 1980a). In this procedure \(G_i\) expresses the functional ‘femalesness’ of an individual tree, and ranges from 0 to 1. Each sampled tree was assigned to a gender category following Lloyd (1981): ‘male’ \(G_i < 0.2\), ‘inconstant male’ \(0.2 < G_i < 0.4\), ‘monoecious’ \(0.4 < G_i < 0.6\), ‘inconstant female’ \(0.6 < G_i < 0.8\) and ‘female’ \(0.8 < G_i < 1\).

Production of ripe seed cones has not been used because in *Abies pinsapo* the level of seed cone production varies directly with pollen availability, and fully developed cones with zero seed set were found (Arista and Talavera, 1996).

To determine the relationship between cone production and tree size, the height, trunk diameter at breast height (DBH), trunk circumference and the maximum crown diameter, were recorded for as many trees as possible. The size data for the three sites were pooled. The height of the sampled trees ranged from 4.5 to 25 m (\(\bar{x} = 11\) m, \(n = 136\)), with a crown diameter of 3.5 to 12 m (\(\bar{x} = 6\) m, \(n = 105\)) and trunk circumference of 34 to 271 cm (\(\bar{x} = 130\) cm, \(n = 108\)). Of the variables considered trunk circumference correlated best with cone production, and so differences in circumference of trees with both female and male cones and those with female-only cones were examined.

### Statistical analyses

For the statistical analyses, samples with non-normal distributions were normalized by adding 0.01 to each value and taking its logarithm (base 10). Percentage data were arcsin transformed before statistical analysis. Samples that could not be normalized were tested with the Kruskall–Wallis non-parametric test, and for inter-year comparisons the Wilcoxon non-parametric test \((Z)\) by repeated samples was used.

### Geographic variation

Little variation was found in the three geographically isolated *pinsapo* populations. Flowering intensity in each population was similar in both years \((P > 0.05)\), and intersite differences in female cone crop were not significant in any year (Table 1). Pollen cone crop was only significantly higher in the SG population.

Likewise gender expression did not vary between populations \((F = 2.75, \text{2 d.f.,} \ P = 0.065)\; \text{Fig. 1}\). In 1991 the percentage of functional female plants (‘female’ and ‘inconstant female’ pooled) in the SG population was \(77.6\%\), in SN \(88\%\) and in SB \(65\%\). In general, percentages of male plants were very low, usually not exceeding \(16\%\). In SG, the percentage of male plants was double that in SN. All populations had more than \(60\%\) of plants with \(G_i > 0.40\), and between 34 and 73\% of the trees were strictly female \((G_i = 1, \text{without any male cones})\). Not a single tree with exclusively male cones was found. Mean female gender was \(0.78 \pm 0.02\) \((n = 114)\) in SG, \(0.86 \pm 0.04\) \((n = 26)\) in SN and \(0.70 \pm 0.06\) in SB. There were no significant differences between populations in average female gender \((F = 2.75, \ P = 0.06)\). In 1992, mean female gender was also very similar with \(0.65 \pm 0.02\) \((n = 137)\) in SG, \(0.69 \pm 0.05\) \((n = 31)\) in SN and \(0.64 \pm 0.05\) \((n = 28)\) in SB \((F = 0.76, \ P = 0.465)\).

### Variation with density

Extreme variations in flowering intensity and cone production were found in trees growing at two markedly

<table>
<thead>
<tr>
<th>Year</th>
<th>Site</th>
<th>(\bar{x} \pm \text{s.e.})</th>
<th>Q1</th>
<th>M</th>
<th>Q3</th>
<th>(n)</th>
<th>(\bar{x} \pm \text{s.e.})</th>
<th>Q1</th>
<th>M</th>
<th>Q3</th>
<th>(n)</th>
<th>% trees not flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>SG</td>
<td>73.3 (\pm 10^a)</td>
<td>17</td>
<td>22</td>
<td>106</td>
<td>163</td>
<td>13.8 (\pm 4^b)</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>158</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>35.6 (\pm 6^a)</td>
<td>28</td>
<td>33</td>
<td>70</td>
<td>34</td>
<td>0.4 (\pm 0.3^c)</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>34</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>31.0 (\pm 7^a)</td>
<td>8.5</td>
<td>11</td>
<td>72</td>
<td>35</td>
<td>0.6 (\pm 0.2^c)</td>
<td>0</td>
<td>0</td>
<td>1.1</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>1992</td>
<td>SG</td>
<td>72.9 (\pm 6^a)</td>
<td>26</td>
<td>52</td>
<td>99</td>
<td>148</td>
<td>19.6 (\pm 3^d)</td>
<td>0</td>
<td>10</td>
<td>25.2</td>
<td>141</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SN</td>
<td>99.3 (\pm 16^a)</td>
<td>38</td>
<td>55</td>
<td>160</td>
<td>34</td>
<td>4.4 (\pm 1^d)</td>
<td>0</td>
<td>1</td>
<td>64</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>SB</td>
<td>65.7 (\pm 14^a)</td>
<td>18</td>
<td>32</td>
<td>94</td>
<td>35</td>
<td>1.1 (\pm 0.3^d)</td>
<td>0.07</td>
<td>0.2</td>
<td>2.2</td>
<td>32</td>
<td>11.5</td>
</tr>
</tbody>
</table>

(*) in thousands.

For each character, sites sharing the same superscript did not differ significantly \((P > 0.05\) using Kruskall–Wallis test). Q1 and Q3, upper and lower quartiles of the cone crop distribution; M is the median crown size; \(n\), number of trees.

### Table 1. Mean pollen and seed cone production of *Abies pinsapo* in Sierra de Grazalema (SG), Sierra de las Nieves (SN) and Sierra Bermeja (SB) forest, during the flowering period of 1991 and 1992.
different stand densities in the Sierra de Grazalema. The production of female cones was approx. twice as high in low density trees (mean of 139 and 108 cones in 1991 and 1992, respectively, Table 2) than in high density trees (mean of 59 and 64 in 1991 and 1992, respectively). Male cone production was also markedly higher in the low density population than in the high density population (Table 2). In both years the differences between stands were significant, except in the male cone crop of 1991.

Gender expression was different between stand densities (Fig. 2). In both years, the percentage of functional female trees was higher in the high density stand than the low density stand. In contrast, the proportion of functional male trees was higher in the low density than in the high density stand. In the low density stand, male cone production was

<table>
<thead>
<tr>
<th>Year</th>
<th>Stand density</th>
<th>Seed cones</th>
<th>Pollen cones*</th>
<th>% trees not flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x} \pm s.e$</td>
<td>Q1</td>
<td>M</td>
</tr>
<tr>
<td>1991</td>
<td>low</td>
<td>139.6 $\pm 28^a$</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>59.5 $\pm 10^b$</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>1992</td>
<td>low</td>
<td>108.3 $\pm 17^a$</td>
<td>45</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>high</td>
<td>64.9 $\pm 6^c$</td>
<td>20</td>
<td>45</td>
</tr>
</tbody>
</table>

* in thousands.
Densities within each character having the same superscript do not differ significantly at $P > 0.05$ using the Kruskall-Wallis test. Q1 and Q3, lower and upper quartiles of the cone crop distribution; M, median crop size; n, number of trees.
Between F density and between years (to 0 flowering seasons, but the decrease in G proportion of functional female trees during the two 1991 at both densities (proportion of functional male trees in 1992 compared to 1992). There was a significant inter-year increase in the

\[ P_{4 \text{ d.f.},} \] these differences were significant only in 1991 (proportion of functional male trees in 1992 compared to 1992).

\[ \bar{G} \] ± 020 in 1992. In general, there was a greater male effort in isolated trees. But 

\[ \bar{G} \] ± 023 in 1991, decreasing ± 005 for 1991 and 1992, respectively). The female cone crop increased only slightly with trunk circumference, whereas the correlation between male cone crop production with increasing tree circumference was marked. For the same tree circumference, the male cone production was higher than the female cone production. In both years, the slopes of both tree circumference-cone production regression lines were significantly different, indicating a higher investment in male cones in larger trees (ANCOVA analysis in 1991, \( F = 7.13, P < 0.001; \) in 1992 \( F = 9.345, P < 0.001)\). In strictly-female trees (\( G_i = 1 \)) trunk size was not correlated with female cone production in either year (\( r = 0.268 \) and \( r = -0.002, P > 0.05 \) for 1991 and 1992, respectively).

**DISCUSSION**

Remarkable reproductive similarities between the three *A. pinsapo* populations were recorded in 1991 and 1992. In the four year period from 1990–1993, the three populations flowered only in 1991 and 1992 with a similar intensity. Such synchrony suggests the existence of an internal rhythm in the species. Observations over a longer period (12 years) indicate that *A. pinsapo* cone crops occur in alternate years and only occasionally in two consecutive years (Arista and

<table>
<thead>
<tr>
<th>Gender in 1992</th>
<th>n</th>
<th>F</th>
<th>iF</th>
<th>Mo</th>
<th>iM</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>88</td>
<td>31(35-2)</td>
<td>20(22-7)</td>
<td>19(21-5)</td>
<td>12(13-6)</td>
<td>6(6-8)</td>
</tr>
<tr>
<td>iF</td>
<td>20</td>
<td>6(4-0)</td>
<td>4(4-0)</td>
<td>5(4-5)</td>
<td>3(4-5)</td>
<td>0(0-0)</td>
</tr>
<tr>
<td>Mo</td>
<td>13</td>
<td>2(1-5)</td>
<td>4(3-0)</td>
<td>7(4-0)</td>
<td>2(1-5)</td>
<td>1(1-6)</td>
</tr>
<tr>
<td>iM</td>
<td>9</td>
<td>2(2-2)</td>
<td>2(2-2)</td>
<td>3(3-3)</td>
<td>1(1-1)</td>
<td>1(1-1)</td>
</tr>
<tr>
<td>M</td>
<td>14</td>
<td>2(1-4)</td>
<td>0(0)</td>
<td>3(2-1)</td>
<td>6(4-8)</td>
<td>3(2-4)</td>
</tr>
</tbody>
</table>

The percentage data are shown in parentheses. \( n \) is the size of each category in 1991. F, female; iF, inconsistent female; Mo, monoecious; iM, inconsistent male; and M, male (Lloyd, 1981).

markedly higher than the female cone production in each tree, indicating a greater male effort in isolated trees. But these differences were significant only in 1991 (\( G = 29.8, 4 \text{ d.f.}, P < 0.01 \); and \( G = 7.51, 4 \text{ d.f.}, P > 0.05 \) in 1992). There was a significant inter-year increase in the proportion of functional male trees in 1992 compared to 1991 at both densities (\( F = 5.46, 1 \text{ d.f.}, P = 0.02 \) for low density and \( F = 16.11, 1 \text{ d.f.}, P < 0.001 \) for high density).

**Between-year variation**

Since no variation was found in gender expression between populations, the data for the three sites were pooled. There were marked differences in the average gender between years (\( F = 13.7, 1 \text{ d.f.}, P < 0.001 \)). The mean functional female was \( 0.79 \pm 0.023 \) in 1991, decreasing to \( 0.659 \pm 0.020 \) in 1992. In general, there was a greater proportion of functional female trees during the two flowering seasons, but the decrease in \( G \) in 1992 indicates a trend towards increased maleness compared to 1991. In fact, the number of functional male plants (‘male’ and ‘inconstant male’ pooled) increased from 23 in 1991 to 35 in 1992 (Table 3).

If only changes exceeding 20% are considered as a sex change (Freeman, McArthur and Harper, 1984), approximately 57% of the trees showed sexual constancy in the two years, while 43% changed sexual expression (\( G = 0.5, P > 0.05, 2 \text{ d.f.} \)). About 35% of female plants remained female in consecutive years. Changes to a different gender were recorded only eight times (six of these were from female to male, Table 3), indicating that only a small proportion of trees increases or decreases markedly the production of male or female structures.

On the other hand, most of the non-flowering trees of 1991 flowered in 1992. Gender expression of these trees was predominantly female (68%; \( n = 59 \)) or inconsistent female (22%), and only one tree was male (1.7%).

**Gender variation with size**

Seed and pollen cone production increased gradually with tree size. In 1991 and 1992 strong positive correlations between the diameter of trees and cone production (male and female) were found (in 1991, \( r = 0.584 \) and 0.588 for female and male cones, respectively, \( n = 48, P < 0.01 \) and in 1992, \( r = 0.338 \) and 0.587 for female and male respectively, \( n = 78, P < 0.01; \) Fig. 3). The female cone crop increased only slightly with trunk circumference, whereas the correlation between male cone crop production with increasing tree circumference was marked. For the same tree circumference, the male cone production was higher than the female cone production. In both years, the slopes of both tree circumference-cone production regression lines were significantly different, indicating a higher investment in male cones in larger trees (ANCOVA analysis in 1991, \( F = 7.13, P < 0.001; \) in 1992 \( F = 9.345, P < 0.001 \)). In strictly-female trees (\( G_i = 1 \)) trunk size was not correlated with female cone production in either year (\( r = 0.268 \) and \( r = -0.002, P > 0.05 \) for 1991 and 1992, respectively).
A similar situation has been recorded in other fir species (Powell, 1977; Singh and Owens, 1981).

In *A. pinsapo* a strong relationship between cone crop and tree size was recorded; cone crop increases in larger trees, presumably due to the greater resource status of larger individuals. The most extreme cone crop differences however were found at different densities. Trees growing in open stands, relatively free of competition have a greater photosynthetic surface area and can invest more resources in reproduction. These isolated trees have a higher cone production (male and female), and even a higher fruit set (Arista and Talavera, 1996).

The relative investment in male and female reproductive structures by *A. pinsapo* individuals directly affects the male to female strobilus ratio and therefore sex expression. In some conifers the sex ratio of a given genotype can be affected by environmental conditions, or by hormone applications (Pharis et al., 1970; Freeman et al., 1981; McArthur and Freeman, 1982; Freeman and Vitale, 1985).

In other conifers, gender variation extends to individuals that produce only female cones, especially in young trees (Smith, 1981; Lloyd and Bawa, 1984). In *A. pinsapo*, when young individuals reach reproductive maturity they produce exclusively female cones, and only when the female branches have produced several cone crops do they start to produce male cones. However, although the young *A. pinsapo* trees are always female, the gender variation also affects older individuals. In 1991, between 65% and 88% of the trees were functionally female and of these, between 22% and 56% bore exclusively female cones. A similar situation was found in 1992. Thus, although *Abies pinsapo* is a monoecious species, gender expression modification gives rise to female, male and monoecious plants, thereby producing a subdioecious population. This kind of phenomenon has been widely recorded in other monoecious species (Lloyd 1975, 1980a, b; Willson, 1979; Freeman et al., 1984; Freeman and Vitale, 1985; Allison, 1991; Jordano, 1991), but not in any fir species to date.

The main component of the gender variation found in older individuals is due to a relatively larger increase in male cone crop with tree size, as a consequence of tree architecture. As in all species of *Abies*, male cones tend to be produced on lower, older branches whereas female cones are produced on the younger branches nearest the top of the tree. As tree size increased, the male cone crop increment was always higher than that of the female cone crop (see Fig. 3), a situation that reflected the differential increment of the male and female crown proportions. In larger trees, male cone crop was markedly greater than female cone crop and the result, in gender quantification, was a tendency towards ‘maleness’. Thus, in trees with two kinds of cone, the smallest tend to be functionally female and the largest male.

Variation in gender expression was also recorded between populations of different density. Once again differences could be explained as a consequence of tree architecture (female and male cone position), in combination with the growth form. At low density, trees are short and broad with branches from ground level which tend to produce male cones. In contrast, trees growing at high density are tall with branches limited to the top half of the tree, and they tend to ‘femaleness’. A similar situation has been found in other conifers such as *Picea* (Caron, Wang and Schooky, 1990) or *Pinus* (Smith, Hamrick and Kramer, 1988).

These situations suggest that in *A. pinsapo* gender modification may be a consequence of plant architecture and of its associated resource status. The resource status of a plant is a combination of its size and vigour (which are in turn affected by its age). But plant architecture, particularly the male and female cone position, seems to have a greater importance in the gender variation of pinsapo fir.

The cone position pattern is probably due to a gradient of hormone concentration along the stem (Owens, 1969; Heslop Harrison, 1972; Jackson and Sweet, 1972; Powell, 1972; Yin and Qinn, 1992). But this situation could also have some advantages because the cones are placed in optimal positions for pollen removal and receipt (Smith, 1981; Willson, 1984), and because this pattern also permits a labile sex expression that could give some selective advantage to the individual in some circumstances (Freeman et al., 1981). In evolutionary terms, a plant should allocate its resources in such a way as to maximize its fitness (Silvertown and Lovett-Doust, 1993). According to Willson (1979), if a plant is growing in conditions that make pollen donation unlikely, investment in male flowers is probably disadvantageous. So, under a dense conspecific canopy where wind is a poor carrier of pollen, male cone production is probably unfavourable and therefore, a typically wind-pollinated monoecious species shifts entirely to the production of female flowers. On the other hand, pinsapo pollen has a low dispersal capacity (Arista and Talavera, 1994b), and so, in low density stands pinsapo pollen cannot travel far and a higher production of male cones could increase the reproductive success.

Nevertheless, gender variation (and the higher proportion of female trees) contributes to guarantee a higher proportion of outcrossing progeny, as has been found in *Abies pinsapo* populations (Arista and Talavera, 1996). In small populations that are geographically and reproductively isolated such as in *A. pinsapo* (there are only four important populations, and pollen flow between these is not possible; Arista and Talavera, 1994a), a minimal outcrossing rate will be very important to avoid endogamy depression. The local mate competition theory states that selection favours a parent biasing the sex ratio toward females in small mating populations (Hamilton, 1967; McArthur and Freeman, 1982) as in fact occurs in *A. pinsapo*.

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LITERATURE CITED


