Natural hybridization between Pinus halepensis and Pinus brutia in Greece

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Introduction

Aleppo pine (Pinus halepensis Mill.) is widely distributed in the Mediterranean region ranging from Morocco to the main land of Greece. It grows also on some small islands of the west Aegean sea, while in Asia Minor is rarely found, only in one locality above Adana. It is native in coastal region of Syria and is also found in Israel and Jordan.

Pinus brutia Ten., which was treated as a variety of Aleppo pine is at present considered to be a well established species (Mirov 1955). To the same conclusion came Nahal (1962) after morphological, geographical, biochemical, and ecological studies of the two species. This species has a more restricted range and grows from Greece to Iraq. A detail map of distribution of the species is given by Chirchfield (1966).

From fossil record it appears that Pinus brutia in Tertiary had a larger distribution than today, while P. halepensis occupied the same region with a considerable northern distribution in latitude (Nahal 1962).

In Greece both species occur, P. halepensis on the west part of the country and P. brutia on the east and the main islands of the Aegean sea and Crete (Fig. 1).

There is a well defined spatial isolation of the two species, the shortest distance being about 50 Kilometres, between the natural populations of Aleppo Pine in Chalkidiki (peninsula of Holly mountain), and those of P. brutia on island Thassos.

Isolated occurrences of the one species inside the range of the other have been reported by Papaioannou (1935, 1936, 1954) and Moulopoulos (1951) (Fig. 1, points C, D and G). According to Papaioannou the two species form natural hybrids, when they come in contact. In NE. Chalkidiki (Fig. 1, Point C) there is a natural overlap of the two species. In this particular area, Papaioannou (1936) identified natural hybrids. He described them as intermediate forms between the two species and gave them a specific name (Pinus goliana sp. nov.). In central Greece (Fig. 1. Point D) a stand of Pinus brutia occurs inside the range of Pinus halepensis, as Papaioannou states (1954), there is enough evidence that it was established artificially one hundred years ago. In island Rodos (Fig. 1, Point G) P. halepensis occurs inside the range of P. brutia which again is considered by the author mentioned previously, as an outcome of human interference, going back some hundred years. At this point only the species today are intermixed and there is also a continuous population of the native species. In the two other places (Points C and D) the populations described are isolated from stands of the native species by a distance ranging from 5 to 14 Kilometers.

Artificial crossings between the two species were performed for the first time in 1948 by Moulopoulos and Basiotis. Thirteen years later (1961) they report that the crossings were only successful when P. brutia was the female parent and not reciprocally.

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The F₁ generation obtained was fertile and exhibited morphological characteristics more or less intermediate, and hybrid vigor for cone dimensions and height growth.

The same crossing was performed by Basshott (1972) and he obtained the same results. When irradiated pollen (800 r) of *P. brutia* was used to pollinate *P. halepensis*, he was able to obtain a few seeds from the reciprocal crossing and a small number of putative hybrid seedlings.

According to the results obtained from the artificial crossings performed and considering that there exists an overlapping of pollen shedding from the two species (Papatou 1954) the production of natural hybrids is to be expected whenever the two species come in contact.

One objective of this study is to analyse all the populations of the two species at the points of contact and to determine hybridization and its nature using more efficient methods than these simple ones above described. The second objective is the study of populations adjacent to those where active hybridization is supposed to take place, in order to specify if introgression occurs and its extent.

**Material and Methods**

In the Spring of 1974 mature cones of current year, and foliage samples were collected separately from 18 to 33 mother trees of seven populations. Three of the sampled populations are those where the two species come in contact (Points C, D, G of Fig. 1), while the other four represent stands of *P. halepensis* (Points B, E and F) and *P. brutia* (Point A). Systematic sampling was chosen because only this method was considered as the most suitable for studying hybridization and elucidating patterns of variation.

Within each population, mother trees were selected randomly, but with restrictions: (1) to be dominants or co-dominants, being not less than 40 years old, because it was found out that young trees of *P. brutia* have not developed resin ducts enough in their needles, until the age of 20–25 years, (2) possessing mature cones of the current year, because *P. halepensis* is exhibiting serotiny, bearing closed cones of various age with obvious colour and malformations of the peduncle related to their age, (3) being at least 50 metres apart and to have one or more neighbors within 30 metres. In those areas where the two species meet or overlap, no attempt was made to select one or the other species because it was felt that such selection would prevent the possibility of determining the population structure.

From each mother tree, 5 vigorous branches were collected, bearing 10 to 15 closed mature cones, from the middle upper and outer portions of the crowns and also 5 branch shoots. Upon transfer to Athens, 10 unopened cones not presenting any defect or insect attack were selected from each mother tree. Lengths, diameters (across the broadest portion), peduncle lengths angle between the axis of the cone and the branch bearing them were measured. (See Fig. 2 for details of angle measurement). Afterwards cones were dried in the open air; then the seeds were extracted and wing length, wing width, (across the broadest portion), seed length, seed width and seed thickness from 10 seeds of each mother tree measured, using a micrometer. One thousand seeds were then counted, weighed and stored in a refrigerator of approximately 4°C until to be used.

Branch shoots were handled as follows: Two fascicles of two years old needles were taken from each branch shoot (10 fascicles per mother tree). The length of the fascicle sheaths and the length of the needles were measured.

![Fig. 2. — Measurement of the angle (in degrees) between the axis of the cone and the branch bearing it.](image)

Needle width and thickness at the middle portion of 10 needles (one from each fascicle) were also measured using an eyepiece micrometer.

Preserved needle specimens were then used to count number of teeth and number of resin canals. Counts of teeth were made with a binocular microscope (∗100) using reflected light for illumination. The number of teeth was counted on 1 cm. segments taken from the mid point of ten needles from each mother tree. Number of resin canals was counted on cross sections through the mid point of ten needles of each mother tree. Freehand cross sections were cut with a razor blade and mounted with distilled water on slides. These then were examined under a microscope (∗100) and the number of resin ducts was recorded. The mean of all measurements, per mother tree, of each character was used for the analyses performed in this investigation.

**Results**

Thirteen characters were scored and their means and standard deviations for each population sampled appear in Tab. 1.

All possible combinations of characters were tried and those which best discriminated the parental species were used to prepare — by methods outlined by Anderson (1949, 1953) — pictorialized scatter diagrams for the parents, for the putative hybrid populations, as well as for populations occurring in close distance to the points of contact of the two species.

Fascicle sheath length and angle of cone axis to branch axis bearing the cone were chosen to give the main axes of the scatter diagrams, because they showed a wide range, did not overlap between the parents and yet could be measured accurately. Fascicle length proved to be a strong diagnostic trait to separate *Pinus halepensis* from *P. brutia* even in young age before the initiation of reproductive organs. The same character has been used for scatter diagrams' preparation by Margen et al. (1969) in their analysis of Lobolly × Shortleaf pine hybrids. De Vail (1946) considered fascicle sheath length as a strong diagnostic character, to separate Slash and Longleaf pines, being unaffected by climate, soil type, age, etc.

Five other characters that were scored are indicated by rays from each circle (representing one plant) in the scat-
<table>
<thead>
<tr>
<th>Cone length (cm)</th>
<th>Peduncle length (cm)</th>
<th>Number of resin ducts</th>
<th>Number of teeth per cm</th>
<th>Needle length (cm)</th>
<th>Seed thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Mean of 13 trees</td>
<td>Mean of 13 trees</td>
<td>Mean of 13 trees</td>
<td>Mean of 13 trees</td>
<td>Mean of 13 trees</td>
</tr>
<tr>
<td>P. brutia</td>
<td>6.79</td>
<td>15.13</td>
<td>2.20</td>
<td>6.25</td>
<td>0.020</td>
</tr>
<tr>
<td>Pop. 1</td>
<td>4.87</td>
<td>13.15</td>
<td>2.30</td>
<td>5.35</td>
<td>0.018</td>
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<tr>
<td>Pop. 2</td>
<td>6.14</td>
<td>12.84</td>
<td>2.35</td>
<td>6.04</td>
<td>0.018</td>
</tr>
<tr>
<td>Pop. 3</td>
<td>7.22</td>
<td>11.76</td>
<td>2.40</td>
<td>6.75</td>
<td>0.024</td>
</tr>
<tr>
<td>Pop. 4</td>
<td>7.97</td>
<td>10.79</td>
<td>2.50</td>
<td>7.54</td>
<td>0.028</td>
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<tr>
<td>Pop. 5</td>
<td>8.97</td>
<td>9.80</td>
<td>2.60</td>
<td>8.42</td>
<td>0.032</td>
</tr>
<tr>
<td>Pop. 6</td>
<td>9.97</td>
<td>8.80</td>
<td>2.70</td>
<td>9.32</td>
<td>0.036</td>
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<td>Pop. 7</td>
<td>10.97</td>
<td>7.80</td>
<td>2.80</td>
<td>10.22</td>
<td>0.040</td>
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<td>Pop. 8</td>
<td>11.97</td>
<td>6.80</td>
<td>2.90</td>
<td>11.12</td>
<td>0.044</td>
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<tr>
<td>Pop. 9</td>
<td>12.97</td>
<td>5.80</td>
<td>3.00</td>
<td>12.02</td>
<td>0.048</td>
</tr>
<tr>
<td>Pop. 10</td>
<td>13.97</td>
<td>4.80</td>
<td>3.10</td>
<td>12.92</td>
<td>0.052</td>
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<td>Pop. 11</td>
<td>14.97</td>
<td>3.80</td>
<td>3.20</td>
<td>11.82</td>
<td>0.056</td>
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<td>Pop. 12</td>
<td>15.97</td>
<td>2.80</td>
<td>3.30</td>
<td>10.72</td>
<td>0.060</td>
</tr>
<tr>
<td>Pop. 13</td>
<td>16.97</td>
<td>1.80</td>
<td>3.40</td>
<td>9.62</td>
<td>0.064</td>
</tr>
<tr>
<td>Pop. 14</td>
<td>17.97</td>
<td>.80</td>
<td>3.50</td>
<td>8.52</td>
<td>0.068</td>
</tr>
<tr>
<td>Pop. 15</td>
<td>18.97</td>
<td>0.80</td>
<td>3.60</td>
<td>7.42</td>
<td>0.072</td>
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<tr>
<td>Pop. 16</td>
<td>19.97</td>
<td>0.80</td>
<td>3.70</td>
<td>6.32</td>
<td>0.076</td>
</tr>
<tr>
<td>Pop. 17</td>
<td>20.97</td>
<td>0.80</td>
<td>3.80</td>
<td>5.22</td>
<td>0.080</td>
</tr>
<tr>
<td>Pop. 18</td>
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<td>0.80</td>
<td>3.90</td>
<td>4.12</td>
<td>0.084</td>
</tr>
<tr>
<td>Pop. 19</td>
<td>22.97</td>
<td>0.80</td>
<td>4.00</td>
<td>3.02</td>
<td>0.088</td>
</tr>
<tr>
<td>Pop. 20</td>
<td>23.97</td>
<td>0.80</td>
<td>4.10</td>
<td>2.02</td>
<td>0.092</td>
</tr>
<tr>
<td>Pop. 21</td>
<td>24.97</td>
<td>0.80</td>
<td>4.20</td>
<td>1.02</td>
<td>0.096</td>
</tr>
<tr>
<td>Pop. 22</td>
<td>25.97</td>
<td>0.80</td>
<td>4.30</td>
<td>0.02</td>
<td>0.100</td>
</tr>
</tbody>
</table>

By this choice of characters good separation was obtained between the parents. Trees that were identified in the field as either Pinus halepensis or Pinus brutia were confirmed by the analysis; and the putative hybrids that were labelled as such in the forest fell in the hybrid category.

Interpretation of the scatter diagrams

The scatter diagrams prepared for each sampled population (Fig. 3-8) verify the presence of variation originally detected, and show more clearly that hybridization between the two species has contributed to it.

Population A and B (Fig. 3) represent the parental species, with P. brutia on the upper right side and P. halepensis on the lower left side of the scatter diagram. The samples were taken from island Samos and Holly mountains respectively. As it becomes evident from the scatter diagram, as well as from Table 1, the species are discriminating very efficiently by the characters used. Comparisons of the two means, of each one of the seven characters of the two parental species by the t-test, showed that all characters used to prepare the scatter diagrams, differ significantly at the 5 and 1 per cent level of significance. Some
characters of *P. brutia* identified on a number of trees of population B can be explained either as the result of introgression, or as the usual variation of that species.

![Diagram](image1)

*Fig. 4.* Population C, of N.E. Chalkidiki.

Population C (Fig. 4) growing in N.E Chalkidiki appear to be a hybrid swarm between the species. Trees resembling *P. brutia* are found mostly on higher elevations (up to 490 m), while trees with *P. halepensis* phenotypic appearance occur mainly close to the coast. Intermediates are present in both sites. This population, today is well isolated from any other natural stand, for a distance of at least 14 Kilometers. The soil where the hybrid swarm grows, has been derived from diorite syenitic rocks and amphibolites. Considering the fact that fire is one of the main factors controlling the distribution of these species it is probable that in the past a continuous distribution of *P. halepensis* existed along the coast, while *P. brutia* as a relic occupied the higher points where the climate is more cold, not suitable for Aleppo pine.

Population D of mountain Pelion (Fig. 5) in Central Greece, appears to be *P. brutia* introgressed by *P. halepensis* with few intermediates and occasionally trees looking more like *P. halepensis*. This population is a small one, not more than 100 ha, growing on elevation 250—350 meters on soil derived from mica schist and serpentine with pH 6.0 to 6.5. The understory is composed of *Arbutus unedo*, *Erica arborea*, *Phillyrea media*, and also trees of *Castanea vesca* are scattered in the area, indicating that the climatic zone is not that of the distribution of *P. halepensis*. In this general area Aleppo pine stands can be found at a distance 5 to 7 kilometers ranging from the coast up to 200 meters on calcareous soil with pH 7.5—8.0. Well documented information gathered from people living in the area states that a few trees of *P. brutia* has been introduced and planted out almost one hundred years ago. Natural regeneration of the initial trees from seeds and their progeny gave rise to the population existing there today.

![Diagram](image2)

*Fig. 5.* Population D, of mountain Pelion (Neochorion) in Central Greece.

Population E (Fig. 6) grows close to population D at a distance of at least 5 kilometers. The analysis performed on the sample taken from this particular population shows that characters have been introgressed by *P. brutia* and even some have an intermediate appearance.

![Diagram](image3)

*Fig. 6.* Population E, of mountain Pelion (Promyrion) in Central Greece.

![Diagram](image4)

*Fig. 7.* Population F, of Attica near Athens.
Population F (Fig. 7) grows in the province of Attica near Athens, on soil derived from lime stone with PH around 8. The analysis performed shows that this population exhibits a variation which cannot be explained as usual for Aleppo pine, but as a result of infiltration of *P. brutia* germ plasm into *P. halepensis*. In this area, being near to the capital of Greece the Aleppo pine stands have been opened for building of houses, gardens, playgrounds etc, while in the same time trees of *P. brutia* were planted. In this way conditions were created not only for contact of the two species but also for the establishment of their hybrids.

![Graph](attachment:image.png)

*Fig. 8. — Population G, of Island Rodos.*

Population G of island Rodos (Fig. 8), as it can be seen from the analysis performed, shows a distinct pattern of variation. It represents two groups of trees, one consists of specimens with pure *P. halepensis* appearance and a number of introgressant forms (Lower left side of the scatter diagram), a second group consists of specimens of pure *P. brutia* phenotypes and a number of introgressant forms (upper right corner of the scatter diagram). There are also a few intermediates, probably F1,S. On the ground all trees with *P. halepensis* phenotypes grow at the N.W site of the island 60—80 meters above sea-level on marls with PH around 8.0. Trees also with prevailing *P. halepensis* characters or intermediates grow in the same area or in the vicinity not further than 3 to 4 km and on soil of the same nature or derived from calcareous flyash, with PH around 8.0. Trees of *P. brutia* phenotypes can be found all over the area and inside the center of *P. halepensis* distribution. It should be emphasized, however, that pure *P. brutia* were sampled from an area at a distance 20 to 25 km away from *P. halepensis* and 300 to 400 meters above sea-level in the center of the island growing on soil derived from lime stones with PH around 7.0. The introgressant forms of *P. brutia* are present only in the vicinity of the contact of the two species and not farther than 4 to 5 kilometers and 100 to 160 meters above sea-level. The pattern of variation which came out from the analysis, shows clearly the existence of hybridization and active introgression in two directions, simultaneously. It seems that the small area initially occupied by *P. halepensis* has been invaded, not long time ago, by *P. brutia*, with the result that hybridization and subsequent introgression occurred to both species. Available evidence, even today shows that the two species were well isolated by a broad belt of natural stands of *Cupressus sempervirens* L. Agricultural cultivation, fires and extensive exploitation of cypress wood, not only opened up and partially removed the isolation zone of *C. sempervirens* but also created new habitats and ecological niches not previously available.

**Discussion**

The two low elevation Mediterranean Pine species, *P. halepensis* and *P. brutia* are recognised as well established species of the genus. Morphological and anatomical studies carried out in the course of this investigation (Tab. 1) show clearly that they are distinguished by a number of independent characters, whose range of variation of each one of them is entirely separate and distinct.

It is also clear that the two species, besides spatial isolation existing among them (Fig. 1), they also exhibit ecological and partial mechanical isolation. *P. brutia* is a species more resistant to low freezing temperatures than *P. halepensis* (PAPAIOANNOU 1936, MOLIPOULOS 1951) and can also better withstand climatic fluctuation of extreme nature regarding temperature and soil moisture. Artificial crossings show clearly the existence of a partial mechanical isolation expressed by a prevention of fertilization when *P. halepensis* is used as female parent. From the intraspecific crossings performed, 90% filled seeds were obtained, while between species 30% (BASSOYI 1972), which shows that also a partial sterility exists between the two species. The F1 is quite fertile, but there is no data of the behavior of F2, S trees and back crossing. Such a study might reveal that incompatibility and sterility are involved between certain trees of such a population.

After these findings it becomes clear that introgression of *P. brutia* characters into *P. halepensis* by means of pollen dispersal is impossible unless trees of the former species or hybrids are being introduced inside the range of the latter. This is exactly the case of hybridization and introgression detected by the analysis performed in populations D and E in central Greece. A few trees of *P. brutia* were planted one hundred years ago—two of them are still there which gave a population of some hundred hectares by natural seeding and regeneration. It is very interesting to follow up the expansion of the population and the succession of events. Older trees resemble more *P. brutia* while the youngest are identified as F1, S or resembling more *P. halepensis*. Taking into consideration that a generation of these species takes at least twenty years, it shows how fast hybridization and introgression spread out between the two species.

After the first hybrids were established only then it was possible to start the opposite infiltration of *P. brutia* genes to *P. halepensis* native forests. The two scatter diagrams (Fig. 5 and 6) show that the degree of hybridization and introgression of *P. brutia* population is more advanced than the respective native population.

This procedure is expected to be followed up in all cases where *P. brutia* is introduced inside the range of *P. halepensis*, if the results of the artificial crossings reported, have application to natural crossings as well.

In the case of *P. halepensis* introduction into the range of *P. brutia*, natural hybridization and introgression will spread out fast, if no other barriers besides spatial isolation are operating, preventing hybridization and establishment of hybrids products.

On this basis the question is raised whether this phenomenon is limited today to the areas reported in this investigation, or whether it is more common and intensive,
and if this is really true what is going to be the evolution of natural forest in Greece.

According to PAPADOPOULOS (1954) hybridization has been limited so far due to the existence of well defined spatial isolation. The recent introduction, however, of P. brutia for a variety of purposes into the range of Pinus halepensis will have as consequence extensive hybridization and introgression of Aleppo pine forests. He states that after a century or so, it will be rare to find pure stands of that species in the main land of Greece. He suggests removal of introduced specimens in order the integrity of Aleppo pine to be maintained. This species is the main resin producer of the country with production twice or more than P. brutia.

Considering population E and F and the analysis performed one can support these views. On the other hand the same cannot be concluded in the case of population B of Holly mountain, where is no evidence of recent introgression in spite of the fact that in close distance a hybrid swarm exists at least for some centuries ago. The same can be said for the island Rodos (Pop G) where hybridization and introgression are localized and hybrids can be found in the areas previously occupied by cypres forest or abandoned cultivated fields, i.e. on new environments for both species. Extensive fires of P. brutia forests in the island might spread out P. halepensis genes and especially those controlling cone serosity, a character which seems not to be present in P. brutia (MOULOPOULOS 1961), although it was observed that while in southern populations, as in island Rodos a few trees keep closed cones, in Thrace almost all trees maintain closed cones with ripe seeds, at least one year after maturity. This particular character is of vital importance for natural regeneration after fire in season without available ripe seeds, of the current year, on the trees.

On this ground it is anticipated that introduction of P. brutia into the range of Aleppo pine, and in areas where pollen of the native species is in abundance will have as consequence natural hybridization following the steps already discussed. The establishment, however, of hybrids will be successful only in case where open space will be available and the environment better suitable for them than either of the two species. Hybrids are also expected to occur and to be established in artificial plantations, out of the range of the two species, in cases where both are planted in mixture. A third possibility is the introgression of P. brutia genes into P. halepensis forests in areas where it is not the optimum of the distribution of that species, but it occupies the area because spatial isolation prevented the presence of P. brutia.

On the other hand, introduced trees of P. halepensis into the range of P. brutia, and in areas with mild climate, and dry summers with high incidence of fires, as in Crete, Rodos and some of the main islands of the Aegean sea, might very well be potential donors of germ plasm into the native pine forests. The same is not expected for northern populations as in Thrace, where low freezing temperatures will be a strong selective factor against the establishment of P. halepensis and probably hybrids.

Finally hybridization might be a useful method of breeding for these pine species, since hybrids exhibit hybrid vigor, at least in young age, better stem form than P. halepensis (MOULOPOULOS and BASSIOUS 1961) and may combine other useful physiological and anatomical characters of the parental species. The use of hybrids should be confined in environments where natural hybrids were detected and studied in this investigation, unless experiments show their superiority in other environments. In any case plantations of hybrids or specimens of the opposite species should be kept away from the best stands of either species in order to maintain integrity and thereby the adaptive peak represented by the two species.

**Summary**

Seven populations of P. brutia and P. halepensis were sampled and thirteen morphological and anatomical characters were scored from each mother tree selected within each sample. Seven of these characters that best discriminated the two species were used to prepare pictorialized scatter diagrams, for each one of the populations sampled. It was found that in all cases where the two species come in contact, natural hybridization occurs, a fact which verifies the results obtained by artificial crossings performed by others previously.

Reciprocal infiltration of germ plasm of one species into the other was also detected. The implication of this phenomenon to the evolution of the species involved is discussed and especially for the areas where the primary isolation is considered to be spatial. It seems certain that hybridization and introgression detected is the outcome of secondary intergradation. The two species were previously well isolated and only recently came into contact by artificial plantations or distraction of previously existing natural isolation zones.

**Key words:** Pinus halepensis, Pinus brutia, natural hybridization, introgression, evolution.

**Zusammenfassung**

Im Frühjahr 1974 wurden in 7 Vorkommen von Pinus halepensis Mill. und Pinus brutia Ten. in Griechenland, von der Halbinsel Chalkidike bis zur Insel Rhodos, an Hand von Zapfen- und Nadelproben von 33 Einzelbäumen Untersuchungen sowohl zur Prüfung morphologischer und anatomischer Merkmale im Hinblick auf eine Trennung der Arten als insbesondere auf zu vermutende Hybridformen zwischen diesen beiden Arten angestellt. Hierbei konnte gefunden werden, daß der Hybridcharakter in Kontaktzonen zwischen den beiden Arten besonders hervortritt, woraus auf Introgreflvische Hybridisation geschlossen wird.

**Literature Cited**