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Gwendal Restoux, Daniel E. Silva, Fabrice Sagnard, Franck Torre, Etienne K. Klein, et al.. Life at the margin: the mating system of Mediterranean conifers. *Web Ecology*, 2008, 8, pp.94-102. hal-02665522

HAL Id: hal-02665522

<https://hal.inrae.fr/hal-02665522>

Submitted on 31 May 2020

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Life at the margin: the mating system of Mediterranean conifers

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Restoux, G., Silva, D. E., Sagnard, F., Torre, F., Klein, E. and Fady, B. 2008. Life at the margin: the mating system of Mediterranean conifers. – Web Ecol. 8: 94–102.

Mixed mating, where a single tree progeny results from a mixture of selfing and outcrossing, is widespread in conifers and could be an evolutionary advantage at ecological margins when mating partners become scarce. This study analyzes how the mating system responds to bioclimate and density variations. We surveyed published data on the mating system of *Abies*, *Picea* and *Pinus* species when information on bioclimate and stand density was available. Our survey revealed that Mediterranean species demonstrate a lower selfing rate than other species and that the proportion of selfed versus outcrossed progeny is not fixed within species. The highest variability in mating types within populations was found when stand density was the most variable.

To show how density affects the proportion of selfed versus outcrossed progeny, we used isozymes to genotype single tree seeds from a marginal *Abies alba* forest in Mediterranean France (Mont Ventoux) where low-to high-density stands are found. We then tested the adaptive potential of the different high and low density progenies by sowing them under controlled nursery conditions and measuring germination rate and seedling survival after 4 years under 3 different water regimes. Although the mean value of outcrossing rate was typical for mixed mating conifers ($t_m = 0.85$), individual outcrossing rates varied from 0.05 to 0.99 and were strongly correlated with stand type and density (t_m from 0.87 in high-density to 0.43 in low-density marginal stands). Significantly fewer seeds from the low density marginal stand germinated (32% vs 53% in the high density mature stand), although seedlings from the marginal stand had a better 4-year survival (81%) than seedlings from the high-density mature stand (63%) when the three water regimes (from least to most stressful) were averaged.

Trees from low density stands may be at a selective disadvantage because they produce more selfed progeny than trees from high-density stands. However, selfed seeds may be purged early on (at the embryo stage) and the remaining seeds tend to produce seedlings with better fitness. Variability of the selfing rate might thus be an adaptive strategy for species in highly contrasted environments where selection of both reproductive assurance and avoidance of inbreeding depression may be density dependent.

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The study of reproduction in plants encompasses a wide range of different processes, from flower and seed fecundity to mating barriers, from the scale and shape of pollen and seed flow to the frequency of long-distance dispersal (Griffin and Sedgley 1989). These processes are traditionally analyzed either from a population genetics or an ecological perspective (Cheptou and Schoen 2007). From the population genetics perspective, the mating system is a complex trait that affects the expression of selective forces. By increasing heterozygosity, outcrossing increases the effective rate of recombination by mixing non-related genomes (promoting the spread of beneficial alleles and consequently the purge of deleterious ones) while selfing increases the expression of genetic load (and subsequently the purge of deleterious recessive mutations). Mixed mating, in which a single tree progeny results from a mixture of selfing and outcrossing, is widespread in plants, although its maintenance remains an evolutionary puzzle (Goodwillie et al. 2005).

On the one hand, if selfing is advantageous (because two copies of the same genes are transmitted to the progeny instead of just one, Fisher 1941), alleles responsible for selfing should spread and become fixed in the population. On the other hand, if selfing is disadvantageous (because of inbreeding depression following the appearance of deleterious alleles at a homozygous state), selfing alleles should disappear from the population. However, because the genetic load is purged by selfing, either complete outcrossing or complete selfing should be evolutionary stable strategies depending on the relative fitness of selfed versus outcrossed progeny (Lande and Schemske 1985).

Several theories have been proposed to explain the widespread co-existence of both selfing and outcrossing within individuals, populations and species: spatial and temporal variability of inbreeding depression, pollen quantity-dependant outcrossing rate ('mass action' and 'pollen discounting') and reproductive assurance (Holsinger 1991, Porcher and Lande 2005; reviewed by Goodwillie et al. 2005, Cheptou and Schoen 2007).

The reproductive assurance hypothesis explains why mixed mating may be conserved states where there is a need to balance the cost of producing selfed seeds (suffering from inbreeding depression) with the cost of producing no seeds at all when partners (or pollinators) become scarce, while still maintaining the potential to produce outcrossed seeds (with limited inbreeding depression) when partners (or pollinators) are plentiful (reviewed by Cheptou and Schoen 2007). Empirical evidence demonstrating larger seed-sets for mixed mating versus strictly outcrossing individuals in low density populations is still very limited (Cheptou and Dieckmann 2002, Kalisz et al. 2004).

Mixed mating is widespread in trees, particularly in conifers (Mitton 1992). Although conifers have various life history traits, most are wind-pollinated and monoecious, which can lead to automatic selfing due to geitonogamy. Furthermore, they colonize a wide range of (often se-

vere) environments (from boreal to tropical) to which they have adapted since their emergence in the Carboniferous (DiMichele et al. 2001) and where their population densities have varied considerably. Therefore, they are a good model to test whether or not demography (and thus mate availability) can affect reproductive output and whether selfing contributes to reproductive assurance at low densities. For example, Robledo-Arnuncio et al. (2004) showed that low-stand density significantly increased both the proportion of selfed progeny and the rate of empty seeds due to inbreeding depression in the mixed mating conifer *Pinus sylvestris*.

Here, we test and discuss the hypothesis that reproductive assurance can be an evolutionary advantage at a species' ecological margin when and where potential mates become scarce (low census and/or low density). First, we analyze how bioclimatic and density variations can affect variability in the mating system using a set of published data compiled for conifers. We focused our attention on the Mediterranean area, where we expected to find strong local variations in population spatial structure due to the high geographical, climatic and environmental heterogeneity of this biome (Blondel and Aronson 1999). Second, we used original data to show how stand density affects the selfing rate, inbreeding depression and survival potential of *Abies alba*, a mixed mating European conifer that has numerous marginal populations under the Mediterranean climate (Quézel and Médail 2003).

Material and methods

We first conducted a survey of published data on the mating system of conifers, restricting our research to the three genera *Abies*, *Picea* and *Pinus* (where all species are mixed mating), where information on bioclimate (using 3 classes defined as Mediterranean, M, non-Mediterranean, nM, and non-Mediterranean but at the rear-edge of the natural distribution area, nM-r) and stand density were available. We collected 6, 30 and 10 studies dealing with M, nM and nM-r species, respectively. From these data, we computed the mean, minimum and maximum values of the average outcrossing rate (t_m) within each study (Table 1). We considered that t_m values greater than 1 were due to aberrations (MLTR or MLTF, Ritland 2002), and thus made them equal to 1 for all computations of outcrossing ranges and means. We attributed a density level to each population and classified studies according to species (Table 1). We separated density into 3 levels: high (3), medium (2) and low (1). We relied on authors' estimates of what they considered low, medium or high density for the species studied. For *Abies alba*, we considered that stand density was (i) low when adult trees were fewer than 25 per hectare, (ii) medium when adult trees were between 25 and 100 per hectare and (iii) high when density was over 100. The definition of these density classes is based on our own

Table 1. Comparison of mean outcrossing rates (t_m) in *Abies*, *Picea* and *Pinus* species in relation to bioclimate and mean population densities. Values of t_m in parenthesis are population extremes. An absence of population extremes indicate that only a single population was investigated. Values of t_m are estimated using isozyme or DNA markers and seed material, except Sorensen (1994) who used phenotypic markers. M = Mediterranean, nM = non-Mediterranean, nM-r = 'rear edge' Mediterranean distribution of a typically non-Mediterranean species, * = seed orchard.

Species	Bioclimate	Stand density	t_m	Geographic origin	Reference
<i>Abies alba</i>	nM-r	low to high	0.72 (0.43–0.87)	Medit. France	This study
<i>Abies alba</i>	nM	medium to high	0.89 (0.68–1.00)	German Alps	Schroeder 1989
<i>Abies amabilis</i>	nM	medium to high	0.82 (0.80–0.84)	W. Canada	El-Kassaby et al. 2003
<i>Abies balsamea</i>	nM	medium to high	0.89 (0.78–0.99)	E. USA	Neale and Adams 1985
<i>Abies borisii regis</i>	M	high	0.94	Greece	Fady and Westfall 1997
<i>Abies lasiocarpa</i>	nM	medium to high	0.89 (0.65–0.94)	Colorado, USA	Shea 1987
<i>Abies procera</i>	nM	high	0.94 (0.90–0.98)	Denmark (*)	Siegismund and Kjaer 1997
<i>Picea abies</i>	nM	medium to high	0.95 (0.95–0.96)	Italy	Morgante et al. 1991
<i>Picea abies</i>	nM	medium	0.83 (0.82–0.84)	Italy	Boscherini et al. 1993
<i>Picea breweriana</i>	nM-r	low to high	0.87 (0.75–1.00)	California, USA	Ledig et al. 2005
<i>Picea chihuahuana</i>	nM-r	low	0.08 (0.00–0.15)	Mexico	Ledig et al. 1997
<i>Picea engelmannii</i>	nM	medium to high	0.86 (0.78–0.96)	Colorado, USA	Shea 1987
<i>Picea glauca</i>	nM	low to high	0.90 (0.89–0.92)	Ontario, Canada	O'Connell et al. 2006
<i>Picea glauca</i>	nM	medium	0.73 (0.70–0.76)	E Canada	Innes and Ringius 1990
<i>Picea mariana</i>	nM	medium to high	0.72 (0.63–0.83)	Alberta, Canada	Sproule and Dancik 1996
<i>Picea mariana</i>	nM	high	0.93 (0.89–0.98)	Eastern Canada	Boyle and Morgenstern 1986
<i>Picea martinexii</i>	nM-r	low to medium	0.56 (0.40–0.69)	Mexico	Ledig et al. 2000
<i>Picea mexicana</i>	nM-r	low to high	0.68 (0.59–0.81)	Mexico	Ledig et al. 2002
<i>Picea omorika</i>	nM-r	medium to high	0.95 (0.84–1.02)	Serbia	Kuittinen and Savolainen 1992
<i>Picea rubens</i>	nM	low to high	0.60 (0.53–0.65)	E Canada	Rajora et al. 2000
<i>Picea sitchensis</i>	nM	medium to high	0.86 (0.65–0.97)	W Canada and USA	Mimura and Aitken 2007
<i>Pinus albicaulis</i>	nM	medium to high	0.86 (0.73–0.98)	W Canada and USA	Bower and Aitken 2007
<i>Pinus attenuata</i>	M	high	0.96	California, USA	Burczyk et al. 1996
<i>Pinus banksiana</i>	nM	high ?	0.89 (0.83–0.95)	Ontario, Canada	Fu et al. 1992
<i>Pinus brutia</i>	M	high	0.95 (*)	Turkey	Kaya et al. 2006
<i>Pinus cembra</i>	nM	low	0.69	Ukraine	Krutovskii et al. 1995
<i>Pinus cembra</i>	nM	high	0.81	Italy	Lewandowski and Burczyk 2000
<i>Pinus chiapensis</i>	nM	medium to high	0.87 (0.49–1.20)	Mexico	Del Castillo and Trujillo 2008
<i>Pinus contorta</i>	nM	high	0.95 (0.93–0.98)	Alberta, Canada	Perry and Dancik 1986
<i>Pinus greggii</i>	nM	high	0.97 (0.92–1.00)	Mexico	Parraguirre-Lezama et al. 2004
<i>Pinus jeffreyi</i>	nM	medium to high	0.94 (0.88–0.97)	Oregon, USA	Furnier and Adams 1986
<i>Pinus kesyia</i>	nM	low to high	0.83 (0.68–0.97)	Thailand	Boyle et al. 1991
<i>Pinus koraiensis</i>	nM	high	0.97 (0.92–1.03)	Far east Russia	Krutovskii et al. 1995
<i>Pinus leucodermis</i>	M	medium to high	0.77 (0.72–0.82)	Italy	Morgante et al. 1993
<i>Pinus maximartinezii</i>	nM-r	low to medium	0.82 (0.44–0.97)	Mexico	Ledig et al. 1999
<i>Pinus merkusii</i>	nM	high	0.87 (*)	Indonesia	Siregar and Hattemer 2001
<i>Pinus merkusii</i>	nM	low to high	0.47 (0.02–0.84)	Indonesia	Changtragoon and Finkeldey 1995
<i>Pinus pinaster</i>	M	high	0.98 (0.95–1.00)	Spain	De Lucas et al. 2008
<i>Pinus pinceana</i>	nM-r	medium	0.87 (0.84–0.90)	Mexico	Ledig et al. 2001
<i>Pinus ponderosa</i>	nM	high	0.91 (0.8–0.92)	NW USA	Sorensen 1994
<i>Pinus radiata</i>	M	medium to high	0.96 (0.9–1.00)	California and Mexico	Vogl et al. 2002
<i>Pinus sibirica</i>	nM	high	0.89 (0.82–0.98)	Siberia	Krutovskii et al. 1995
<i>Pinus strobus</i>	nM	medium to high	0.93 (0.87–0.99)	Canada	Rajora et al. 2002
<i>Pinus sylvestris</i>	nM-r	low to high	0.93 (0.75–0.99)	Spain	Robledo et al. 2004
<i>Pinus sylvestris</i>	nM	high	0.98 (0.96–0.99) *	Poland	Burczyk 1998
<i>Pinus washoensis</i>	nM-r	medium to high	0.86 (0.76–0.92)	Nevada, USA	Mitton et al. 1997

observations of natural stands in our study site (Mont Ventoux), where a significant spatial genetic structure was observed on a progeny when density was below 25 trees per ha (Sagnard 2001). From this data set, we estimated the variability of outcrossing rates between stands for each study by subtracting the minimum t_m value observed in the study from the maximum one. We also estimated the variability of stand density for each study: from one, when there was only one density class, to three, when density ranged from low to high. We tested the effect of bioclimate (M vs nM and nM-r either individually or grouped in a single non-Mediterranean class) on average outcrossing rate for each study, using a non-parametric ANOVA (Kruskal–Wallis rank sum test), because we could not test for variance equality. We also tested the relationship between stand density and the average outcrossing rates of stands using the same Kruskal–Wallis rank sum test.

Secondly, we used isozymes to investigate the genetic composition of individual progeny from a marginal *Abies alba* population growing under Mediterranean climate in a southeastern French mountain (Mont Ventoux, 44°10'N, 5°15'E). Mont Ventoux is characterized by stands of different ages, from old-growth relic stands to young stands where *Abies alba* has recolonized grazing areas abandoned in the late 19th century. Stands are also characterized by contrasting mature tree densities, from low (fewer than 25 trees per hectare in recolonized areas) to high (more than 100 trees per hectare in old, mature stands). We collected seeds from 52 trees from 4 different stands (Fig. 1): two high-density old-growth stands (HD1 and HD2), one medium-density old-growth stand at the periphery of an old-growth high-density stand, MD, and one low-density recolonizing stand (LD). A set of 7 polymorphic Mendelian isozymes developed for *Abies alba* was used on embryos, following the standard extraction, electrophoresis and scoring protocols of Fady et al. (1999). A total of 842 seeds were used to assess selfing rate and pollen pool composition using the MLTR software (Ritland 2002). We computed selfing rates for each sampled

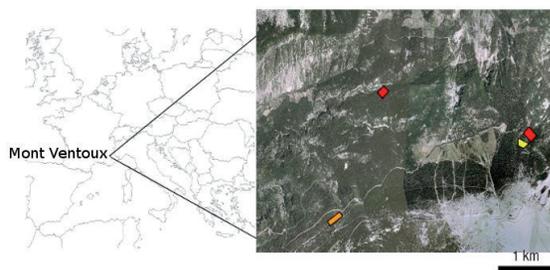


Fig. 1. Localization of Mont Ventoux at the European scale and localization of the stands analyzed within Mont Ventoux: high density mature stands HD1 and HD2 (in red), medium density peripheral stand MD (in orange) and low density recolonizing stand LD (in yellow).

individual in LD and MD stands but only for stand level in HD1 and HD2 stands.

Finally, we tested early inbreeding depression and survival potential of the different progenies by sowing them under controlled nursery conditions and measuring germination rate and seedling survival after a 4-year period. A subset of 32 seed trees originating either from the HD1 or LD stands was used for this experiment. Germination rate, i.e. the proportion of seeds in each progeny which produced a 5 mm long emerging radicle after stratification (6 weeks at +4°C in moist peat), was measured for all collected seeds (from 215 to 1790 seeds per tree). From these seed lots, 2400 germinated seeds (i.e. 75 seeds per seed tree) were planted in the nursery, using 9 complete blocks and a randomized progeny design within blocks. Three groups of 3 blocks each received different water regimes: drought (Wilting point, $W_p = 3-3.5$), light water stress ($W_p = 2.7$) and normal watering ($W_p = 1.5$). Progeny survival was measured each year for 4 years. Significance of germination rate and of survival was tested using a logistic regression within an analysis of deviance ('glm' function in R).

All statistical analyses were carried out using the R software, R Development Core Team.

Results

Our literature survey of conifer trees showed that the variability in the mating system of genera *Abies*, *Picea* and *Pinus* were highly variable among populations and species. The average t_m for each bioclimatic class was 0.92, 0.84 and 0.70 for Mediterranean (M), non-Mediterranean (nM) and non-Mediterranean rear-edge (nM-r) species or populations, respectively. There was a significant difference of t_m between the three bioclimatic classes ($\text{Chi}^2 = 9.13$, $\text{DF} = 2$, $p = 0.01$, Fig. 2). Furthermore, there was a significant difference between the average t_m over Mediterranean type species ($t_m = 0.92$) and over non-Mediterranean species, including both nM and nM-r species, ($t_m = 0.81$) ($\text{Chi}^2 = 5.68$, $\text{DF} = 1$, $p = 0.017$). A significantly higher outcrossing rate was found when stand density was higher ($\text{Chi}^2 = 23.81$, $\text{DF} = 4$, $p < 0.0001$, Fig. 3). Further, we found a general trend toward a higher t_m range in marginal populations (Fig. 4), although this could not be tested as this range was mainly dependent on the number of stands sampled in each study. Finally, a higher t_m range was observed when the density range was higher ($\text{Chi}^2 = 13.88$, $\text{DF} = 2$, $p = 0.001$, Fig. 5).

Our isozyme data showed that the mean multi-locus outcrossing rate (t_m) of the Mont Ventoux *Abies alba* population over the 4 stands was 0.72 (Table 1). However, although trees were in the same meso-environment, there were considerable differences in mean stand and individual outcrossing rates. Within the old growth stands HD1 and HD2 (tree density $> 100 \text{ ha}^{-1}$), average t_m were 0.85

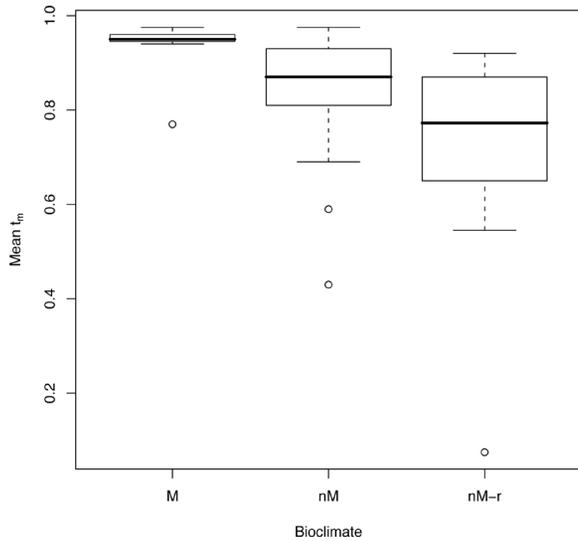


Fig. 2. Outcrossing rates over three bioclimates (Mediterranean, M, non-Mediterranean, nM, and rear edge non-Mediterranean nM-r) in *Abies*, *Picea* and *Pinus* species worldwide.

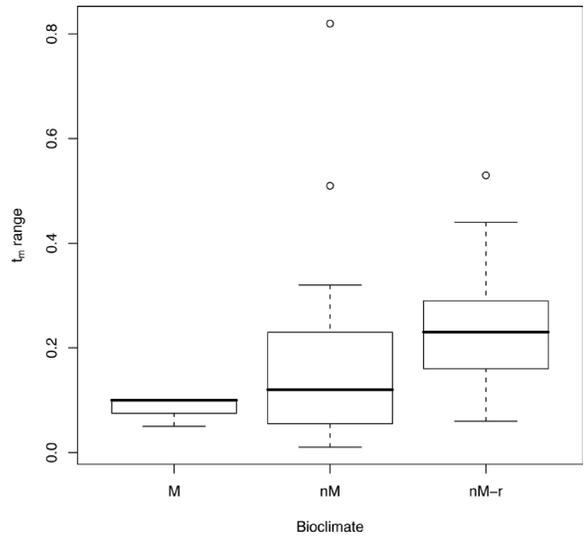


Fig. 4. Outcrossing rate variability among populations over three bioclimates (Mediterranean, M, non-Mediterranean, nM, and rear edge non-Mediterranean, nM-r) in *Abies*, *Picea* and *Pinus* species worldwide.

and 0.87, respectively. At stand edge, t_m decreased to 0.71 within the medium density mature stand (MD), with individual values ranging from 0.45 to 0.99. In the marginal low density recolonizing stand LD (density $< 30 \text{ ha}^{-1}$), t_m was 0.43 (individual values from 0.05 to 1.18). We found no difference in pollen pool genetic diversity between the different stands (H_e from 0.141 to 0.162).

Significantly fewer seeds from the low density marginal stand LD were able to germinate: 33.8% vs 52.3% in the

old-growth high-density stand HD1 ($F = 18.09$, $DF = 30$, $p = 0.0002$, Table 2). However, seedlings from LD had an overall better survival (81%) than seedlings from HD1 (63%) after 4 years ($p < 0.0001$). The analysis of deviance showed that water regime, stand density type and their interaction with progeny could explain differences in survival ($p < 0.0001$). Survival decreased with water stress for seeds originating from both LD and HD1 (survival rates: 82% and 73% for LD and HD1, respectively). Averaging

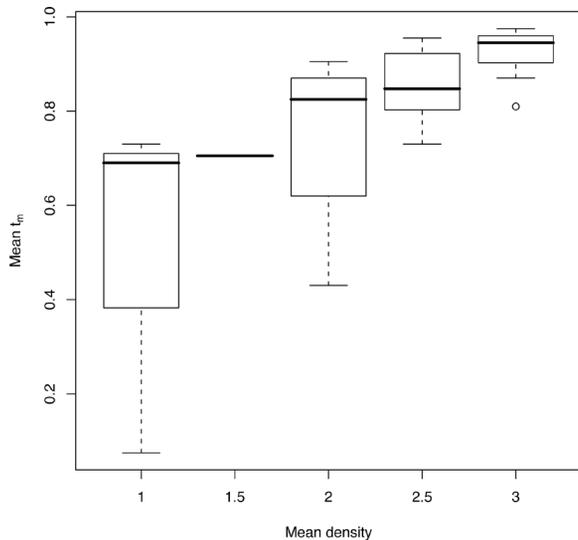


Fig. 3. Effect of density on outcrossing rate at the population level over three genera *Abies*, *Picea* and *Pinus*.

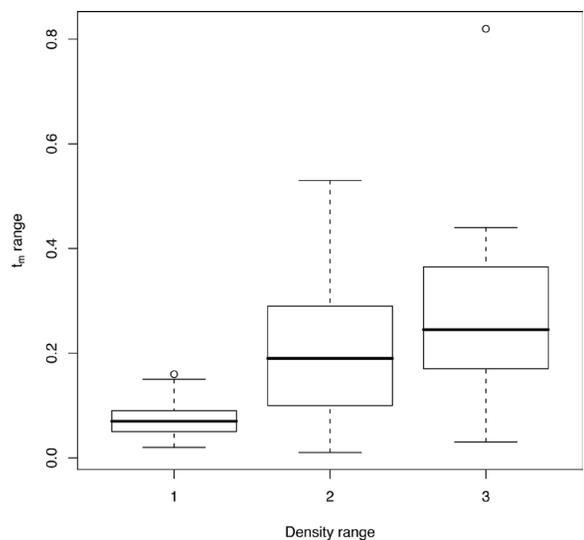


Fig. 5. Outcrossing rate variability versus density variability in *Abies*, *Picea* and *Pinus* species worldwide.

all three water treatments, LD seedlings had a better survival than HD1 ones. However, this difference is mainly due to the driest water regime (Table 2) and no significant difference between LD and HD1 seedlings was observed under the normal water regime.

Discussion

For reasons ranging from highly heterogeneous topography to the heterogeneity of land uses due to urbanization and agriculture in highly anthropogenic environments,

Table 2. Germination rate of the 32 seed lots tested according to stand density. Density type: HD = high density, LD = low density.

Name of seed tree	Density type	Total number of germinated seeds	Total number of seeds tested	Germination rate in %	Mortality in % after 4 years under high water stress
s 23 hp	HD	580	995	58.29	21.9
s 24 hp	HD	954	1790	53.30	46.7
s 25 hp	HD	838	1568	53.44	16.7
s 26 hp	HD	663	1251	53.00	39.3
s 27 hp	HD	323	577	55.98	29.6
s 28 hp	HD	561	1296	43.29	18.2
s 29 hp	HD	446	826	54.00	18.5
s 30 hp	HD	202	697	28.98	18.4
s 31 hp	HD	251	359	69.92	25.0
s 32 hp	HD	414	901	45.95	25.6
s 33 hp	HD	622	1147	54.23	41.5
s 34 hp	HD	355	1089	32.60	15.7
s 35 hp	HD	444	972	45.68	15.4
s 36 hp	HD	467	897	52.06	9.7
sem 21 hp	HD	796	1118	71.20	70.4
sp 22 hp	HD	857	1280	66.95	18.2
parc 5	LD	361	1367	26.41	2.5
parc 661	LD	134	698	19.20	11.8
parc 813	LD	158	627	25.20	23.8
s 18 hp	LD	338	1048	32.25	12.8
s 19 hp	LD	227	513	44.25	21.2
s 4 newhp	LD	162	460	35.22	7.1
sem 1 hp	LD	123	509	24.17	32.0
sem 12 hp	LD	152	435	34.94	14.8
sem 13 hp	LD	101	351	28.77	38.9
sem 14 hp	LD	463	907	51.05	9.5
sem 17 hp	LD	458	1212	37.79	23.1
sem 2 hp	LD	65	823	7.90	50.0
sem 20 hp	LD	304	794	38.29	4.3
sem 3 hp	LD	215	610	35.25	7.7
sem 6 hp	LD	591	1049	56.34	8.7
sem 8 hp	LD	208	595	34.96	20.0

species distribution areas are typically fragmented in the Mediterranean, thus leading to stands of highly contrasted densities, census and effective sizes (Blondel and Aronson 1999, Fady and Médail 2004). Out of the 9 *Abies* and 9 *Pinus* species found in Europe, 13 are Mediterranean endemics and an additional 3 have the 'rear-edge' of their distribution within the Mediterranean bioclimatic zone (Quézel and Médail 2003). Thirteen of these 16 species have patchy distribution areas under Mediterranean conditions. In such heterogeneous environments in terms of density and population size, pollen limitation may be a critical factor for reproduction at fine spatial scale (Restoux et al. unpubl.). Thus, a mixed mating system may be at an evolutionary advantage in such environments, since selfing ensures reproductive assurance when pollen is limited (Knight et al. 2005). On the other hand, pollen discounting, i.e. the cost of selfing due to pollen loss which is not dedicated to outcrossing, favors outcrossing when pollen is not limited (Holsinger 1991).

However, it is not because species live under Mediterranean conditions, characterized by heterogeneity in terms of density, that higher selfing or more variable mating types among and within populations are observed. Mediterranean-type conifers are typically major outcrossers, just like other conifers elsewhere (Table 1). The mean multi-locus outcrossing rate (t_m) found for *Pinus attenuata* in California by Burczyk et al. (1996), for example, is typical for that of mixed-mating conifers, with a low overall level of selfing. What leads to high selfing rates and a high variability in selfing rate is that populations and individuals within populations may experience low and highly variable levels of density, often because they are at the margin of the species distribution area. In *Abies alba* on the Mont Ventoux, not only does average selfing rate within a stand increase when stand density decreases, but also individual selfing rate variability increases when local density decreases (i.e. when spatial structure becomes more heterogeneous) as shown by Robledo-Arnuncio et al. (2004) for *Pinus sylvestris*.

Trees from low-density stands may face an uncertain demographic future because they produce both more selfed progeny, subject to inbreeding depression, and more empty seeds, due to pollen limitation and early expression of inbreeding depression, than trees from higher-density stands. However, selfed seeds may be purged early on (at the embryo stage, reviewed by Williams and Savolainen 1996) and the remaining seeds may produce seedlings with better fitness, possibly because of counter-selection of individuals with a higher genetic load (Pichot et al. 2006). This is just what we observed for *Abies alba* in the Mont Ventoux. Low-density stands (the younger stands) produced fewer viable seeds than high-density (mature) stands, but their fitness, as measured by seedling survival after 4 years in the nursery, was higher, especially under stressful conditions. Also, the fitness reduction at low density caused by inbreeding depression and pollen limitation

could be compensated by the demographic advantage of being in an open area where density dependence is weaker (e.g. fate of a gene from the colonization front, Edmonds et al. 2004).

Overall, the pattern observed here is typical of competing self-fertilization (Lloyd 1979), where self-pollen competes with outcross-pollen for pollination, leading to selfing rates governed by a 'mass-action law' (Holsinger 1991): selfing rate is proportional to the ratio of the amount of self-pollen over the total amount of pollen received by stigmas. As a consequence, individuals in low density stands might have a low outcrossing rate because their self-pollen is less diluted within the overall outcrossed pollen. The variability in the mating system in our study is due to obligate selfing in geitonogamous organisms, but we can wonder why such a mating system is conserved if pollen limitation does not occur, since it is costly in terms of fitness (Porcher and Lande 2005). One explanation could be the position of flowers on each tree which may decrease selfing rate because female flowers are located at the top of the crown and exposed to wind-dispersed pollen, whereas male flowers are much below, at the bottom of the crown. On steep slopes, such as those where mountain conifers grow, they are thus much closer to the female flowers of a neighboring seed tree than to their own female flowers. When density decreases, the benefit of selfing as a form of reproductive assurance is thus still possible.

We suggest that the mixed mating system has been conserved as a consequence of the contrasted environments where plants grow, particularly in Mediterranean environments. In such heterogeneous environments, a tradeoff between reproductive assurance, avoidance of inbreeding depression and possible pollen discounting may be selected at different times and places, leading to the maintenance of mixed mating as a viable reproductive strategy.

Acknowledgments – We wish to thank Patrice Brahic and the staff at the State Forest Nursery of Aix en Provence for preparing, installing and measuring the nursery test. This research was made possible by the grant 'Conservation des Ressources Génétiques Forestières' from the French Ministry of Agriculture to INRA. We are very grateful to Julia Fady-Welterlen for her English revision of the whole manuscript.

References

- Blondel, J. and Aronson, J. 1999. Biology and wildlife of the Mediterranean region. – Oxford Univ. Press.
- Bower, A. D. and Aitken, S. N. 2007. Mating system and inbreeding depression in whitebark pine (*Pinus albicaulis* Engelm.). – *Tree Genet. Gen.* 3: 379–388.
- Boyle, T. J. B. et al. 1991. Genetic studies in a tropical pine, *Pinus kesiya*: III. The mating system in four populations from northern Thailand. – *J. Trop. For. Sci.* 4: 37–44.
- Boyle, T. J. B. and Morgenstern, E. K. 1986. Estimates of outcrossing rates in six populations of black spruce in central New Brunswick. – *Silvae Genet.* 35: 102–106.

- Boscherini, G. et al. 1993. Mating system analysis in two Italian populations of Norway spruce. – *J. Genet. Breed.* 47: 45–48.
- Burczyk, J. et al. 1996. Mating patterns and pollen dispersal in a natural knob-cone pine (*Pinus attenuata* Lemmon.) stand. – *Heredity* 77: 251–260.
- Burczyk, J. 1998. Mating system variation in a Scots pine clonal seed orchard. – *Silvae Genet.* 47: 155–158.
- Changtragoon, S. and Finkeldey, R. 1995. Patterns of genetic variation and characterization of the mating system of *Pinus merkusii* in Thailand. – *For. Genet.* 2: 87–97.
- Cheptou, P. O. and Dieckmann, U. 2002. The evolution of self-fertilization in density-regulated populations. – *Proc. R. Soc. Lond. B* 269: 1177–1186.
- Cheptou, P. O. and Schoen, D. J. 2007. Combining population genetics and demographical approaches in evolutionary studies of plant mating systems. – *Oikos* 116: 271–279.
- Del Castillo, R. F. and Trujillo, S. 2008. Effect of inbreeding depression on outcrossing rates among populations of a tropical pine. – *New Phytol.* 177: 517–524.
- De-Lucas, A. I. et al. 2008. Mating system and pollen gene flow in Mediterranean maritime pine. – *Heredity* 100: 390–399.
- DiMichele, W. A. et al. 2001. Response of late Carboniferous and early Permian plant communities to climate change. – *Annu. Rev. Earth Plan. Sci.* 29: 461–487.
- Edmonds, C. A. et al. 2004. Mutations arising in the wave front of an expanding population. – *Proc. Natl Acad. Sci. USA* 101: 975–979.
- El-Kassaby, Y. A. et al. 2003. Genetic evaluation of alternative silvicultural systems in coastal montane forests: western hemlock and amabilis fir. – *Theor. Appl. Genet.* 107: 598–610.
- Fady, B. et al. 1999. Genetic differentiation in *Abies alba* populations from south-eastern France. – *For. Genet.* 6: 129–138.
- Fady, B. and Médail, F. 2004. Mediterranean forest ecosystems. – In: Burley, J. et al. (eds), *Encyclopedia of forest science*. Elsevier, pp. 1403–1414.
- Fady, B. and Westfall, R. D. 1997. Mating system parameters in a natural population of *Abies borisii regis* Mattfeld. – *Ann. For. Sci.* 54: 643–647.
- Fisher, R. A. 1941. Average excess and average effect of a gene substitution. – *Ann. Eugenics* 11:53–63.
- Fu, Y. B. et al. 1992. Pollen pool heterogeneity in Jack pine (*Pinus banksiana* Lamb) – a problem for estimating outcrossing rates. – *Theor. Appl. Genet.* 83: 500–508.
- Furnier, G. R. and Adams, W. T. 1986. Mating system in natural populations of Jeffrey pine. – *Am. J. Bot.* 73: 1002–1008.
- Goodwillie, C. et al. 2005. The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. – *Annu. Rev. Ecol. Evol. Syst.* 36: 47–79.
- Griffin, A. R. and Sedgley, M. 1989. Sexual reproduction of tree crops. – London Acad. Press.
- Holsinger, K. E. 1991. Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. – *Am. Nat.* 138: 606–622.
- Innes, D. J. and Ringius, G. G. 1990. Mating system and genetic structure of two populations of white spruce (*Picea glauca*) in eastern Newfoundland. – *Can. J. Bot.* 68: 1661–1666.
- Kalisz, S. et al. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. – *Nature* 430: 884–887.
- Kaya, N. et al. 2006. Mating system and pollen contamination in a *Pinus brutia* seed orchard. – *New For.* 31: 409–416.
- Knight, T. M. et al. 2005. Pollen limitation of plant reproduction: pattern and process. – *Annu. Rev. Ecol. Evol. Syst.* 36: 467–497.
- Krutovskii, K. V. et al. 1995. Isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pine species (subsection *Cembrae*, section *Strobi*, subgenus *Strobus*). – In: Baradat, P. et al. (eds), *Population genetics and conservation of forest trees*. SPB Acad. Publ., pp. 280–304.
- Kuittinen, H. and Savolainen, O. 1992. *Picea omarika* is a self fertile but outcrossing conifer. – *Heredity* 68: 183–187.
- Lande, R. and Schemske, D. W. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. – *Evolution* 39: 24–40.
- Ledig, F. T. et al. 1997. Recent evolution and divergence among populations of a rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. – *Evolution* 51: 1815–1827.
- Ledig, F. T. et al. 1999. Evidence for an extreme bottleneck in a rare Mexican pinyon: genetic diversity, disequilibrium and the mating system in *Pinus maximartinezii*. – *Evolution* 53: 91–99.
- Ledig, F. T. et al. 2000. The mating system and genetic diversity in Martinez spruce, an extremely rare endemic of Mexico's Sierra Madre Oriental: an example of facultative selfing and survival in interglacial refugia. – *Can. J. For. Res.* 30: 1156–1164.
- Ledig, F. T. et al. 2001. Genetic diversity and the mating system of a rare Mexican pinyon, *Pinus pinceana*, and a comparison with *Pinus maximartinezii* (Pinaceae). – *Am. J. Bot.* 88: 1977–1987.
- Ledig, F. T. et al. 2002. Genetic diversity, mating system and conservation of a Mexican subalpine relict *Picea mexicana*. – *Conserv. Genet.* 3: 113–122.
- Ledig, F. T. et al. 2005. Genic diversity, genetic structure, and mating system of Brewer spruce (Pinaceae), a relict of the Arcto-Tertiary forest. – *Am. J. Bot.* 92: 1975–1986.
- Lewandowski, A. and Burczyk, J. 2000. Mating system and genetic diversity in natural populations of European larch (*Larix decidua*) and stone pine (*Pinus cembra*) located at higher elevations. – *Silvae Genet.* 49: 158–161.
- Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. – *Am. Nat.* 113: 67–79.
- Mimura, M. and Aitken, S. N. 2007. Increased selfing and decreased effective pollen donor number in peripheral relative to central populations in *Picea sitchensis* (Pinaceae). – *Am. J. Bot.* 94: 991–998.
- Mitton, J. B. 1992. The dynamic mating systems of conifers. – *New For.* 6: 197–216.
- Mitton, J. B. et al. 1997. The pattern of inbreeding in Washoe pine and survival of inbred progeny under optimal environmental conditions. – *Silvae Genet.* 46: 215–219.
- Morgante, M. et al. 1991. Effects of stand density on outcrossing rate in two Norway spruce (*Picea abies*) populations. – *Can. J. Bot.* 69: 2704–2708.
- Morgante, M. et al. 1993. Selection against inbreds in early life-cycle phases in *Pinus leucodermis*. – *Ant. Heredity* 70: 622–627.
- Neale, D. B. and Adams, W. T. 1985. Allozyme and mating system variation in balsam fir (*Abies balsamea*) across a continuous elevational transect. – *Can. J. Bot.* 63: 2448–2453.

- O'Connell, L. M. et al. 2006. Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (*Picea glauca*) at the landscape level. – *Heredity* 97: 418–426.
- Parraguirre-Lezama, C. et al. 2004. Mating system in four natural populations of *Pinus greggii* Engelm. – *Agrociencia* 38: 107–119.
- Perry, D. J. and Dancik, B. P. 1986. Mating system dynamics of lodgepole pine in Alberta, Canada. – *Silvae Genet.* 35: 190–195.
- Pichot, C. et al. 2006. Déterminants et conséquences de la qualité génétique des graines et semis lors de la phase initiale de régénération naturelle des peuplements forestiers. – *Les actes du BRG* 6: 277–297.
- Porcher, E. and Lande, R. 2005. The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. – *J. Evol. Biol.* 18: 497–508.
- Quézal, P. and Médail, F. 2003. Ecologie et biogéographie des forêts du bassin méditerranéen. – Elsevier.
- Rajora, O. P. et al. 2000. Indicators of population viability in red spruce, *Picea rubens*. II. Genetic diversity, population structure, and mating behavior. – *Can. J. Bot.* 78: 941–956.
- Rajora, O. P. et al. 2002. Mating system and reproductive fitness traits of eastern white pine (*Pinus strobus*) in large, central versus small, isolated, marginal populations. – *Can. J. Bot.* 80: 1173–1184.
- Ritland, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. – *Heredity* 88: 221–228.
- Robledo-Arnuncio, J. J. et al. 2004. Increased selfing and correlated paternity in a small population of a predominantly outcrossing conifer, *Pinus sylvestris*. – *Mol. Ecol.* 13: 2567–2577.
- Sagnard, F. 2001. Dynamique de recolonisation des pinèdes pionnières par la hêtraie-sapinière: Etude de la structure des peuplements forestiers et de l'évolution génétique des populations de sapin pectiné (*Abies alba* Mill.) sur le Mont Ventoux. PhD thesis. – Paul Cézanne Univ., Aix-Marseille III, France.
- Schroeder, S. 1989. Outcrossing rates and seed characteristics in damaged natural populations of *Abies alba* Mill. – *Silvae Genet.* 38: 185–189.
- Shea, K. L. 1987. Effects of population structure and cone production on outcrossing rates in Engelmann spruce and subalpine fir. – *Evolution* 41: 124–136.
- Siegismund, H. R. and Kjaer, E. D. 1997. Outcrossing rates in two stands of noble fir (*Abies procera* Rehd.) in Denmark. – *Silvae Genet.* 46: 144–146.
- Siregar, I. Z. and Hattemer, H. H. 2001. Gene flow and mating system in a seedling seed orchard and a natural stand of *Pinus merkusii* (Jungh. et de Vriese) in Indonesia. – In: Muller-Starck, G. and Schubert, R. (eds), Genetic response of forest systems to changing environmental conditions. Kluwer, pp. 281–292.
- Sorensen, F. C. 1994. Frequency of seedlings from natural self-fertilization in Pacific Northwest ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.). – *Silvae Genet.* 43: 100–108.
- Sproule, A. T. and Dancik, B. P. 1996. The mating system of black spruce in north-central Alberta, Canada. – *Silvae Genet.* 45: 159–164.
- Vogl, C. et al. 2002. High resolution analysis of mating systems: inbreeding in natural populations of *Pinus radiata*. – *J. Evol. Biol.* 15: 433–439.
- Williams, C. G. and Savolainen, O. 1996. Inbreeding depression in conifers: Implications for breeding strategy. – *For. Sci.* 42: 102–117.