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How do genetic and ecological factors explain the number of empty seeds for a European conifer (*Abies alba*, Mill.) ?

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Introduction

Production of viable seeds is a key factor for recruitment and thus dynamics of natural populations. Seed set is the usual parameter used to assess the female fertility of individual plants, but may not be informative enough in conifers where production of empty seeds (no embryo but no visual difference with full seeds) is widespread. Measuring and explaining the proportion of empty seeds in addition to seed set should thus help to understand female reproductive success.

Empty seeds can result from a lack of pollen (Knight *et al.* 2005) or from the early expression of inbreeding depression (Savolainen *et al.* 1992). Both phenomena are likely to occur in marginal and / or recolonizing populations, such as the *Abies alba* populations of our study site. In this study, we compared both hypotheses at individual level by explaining the variation of empty seed rates among sampled trees. We confronted the hypothesis of pollen limitation by measuring individual pollen production and local neighborhood density, versus the hypothesis of inbreeding depression by estimating the individual selfing rate.

Methods

We monitored pollen production of 58 silver firs (46 in 2005 and 12 in 2004) from 2 sites in the Mediterranean French Alps. We estimated local density by counting the number of pollen donors within a 15m radius around each tree. For each tree we collected a sample of seeds (100 to 1800 seeds/tree) from which we estimated the proportion of empty seeds. In addition, 10 to 20 seeds per tree were genotyped using 4 paternally inherited chloroplast microsatellite markers (Vendramin *et al.* 1996). Individual selfing rates were estimated for each tree using its progeny haplotypes and a maximum likelihood approach. Finally, the relationships between empty seed rates, selfing rates, number of neighbors and pollen productions were investigated using linear and logistic regressions.

Results and Conclusions

The proportion of empty seeds was negatively correlated with the number of neighbors ($R^2=0.17$ p-value=0.004). This result suggested an effect of the quantity and/or of the quality (selfing) of available pollen. Furthermore, the individual quantity of pollen produced had a marginal positive effect on the rate of empty seeds (p-value = 0.08 in 2005) whereas the main effect affecting the rate of empty seeds was the global pollen production at the stand level (p-value = 0.01 for the year effect with a higher global production in 2004 than in 2005). No effect of selfing rate on the rate of empty seeds was detected. Our results show the importance of local density and support the effect of pollen shortage rather than the effect of early inbreeding depression, both widespread in tree species (Karkkainen *et al.* 1993 ; Kelly *et al.* 2002). Further investigations of this latter point were initiated with *in natura* controlled pollination experiments.

Finally, we detected no significant correlation between selfing rate and any variable characterizing the local environment and/or pollen production, which could suggest a genetic variability of selfing rate or a low precision of our estimation of this parameter.

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