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Inheritance of Two Chlorophyll Mutants in *Eucalyptus globulus*

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Summary

Mutations in two independent genes causing chlorophyll deficiency (*chl1* and *chl2*) were identified in two *Eucalyptus globulus* trees from remnant native forest. In each tree the proportion of albino progeny, following selfing, was found to be consistent with the segregation of a single gene. One of these genes (*chl1*) was found to be linked to the isozyme locus *Gpi-2*. The frequencies of the mutants were monitored in open-pollinated seed progeny and, from this, outcrossing rates were calculated. The rate of outcrossing in the *chl1* mutant tree was also determined using a multi-locus, maximum likelihood estimation based on three isozyme loci, this agreed closely with the *chl1* single locus estimate. This result highlights a role for rare, easily scored, mutants in seed orchards, where they can be utilized to monitor selfing rates.

Key words: outcrossing, selfing, albino, eucalypt, seed orchard.

Introduction

High levels of deleterious recessive mutations may accumulate, particularly in outbreeding species (BYERS and WALLER, 1999). Such alleles are exposed upon inbreeding, and abnormal phenotypes have been reported for many forest tree taxa (e.g. FRANKLIN, 1970; ELDRIDGE, 1970). The expression of these recessive mutations is believed to be the main cause of inbreeding depression in forest tree species (LEDIG, 1986), including eucalypts (GRIFFIN, MORAN and FRIPP, 1987; JAMES and KENNINGTON, 1993; HARDNER and POTTS, 1995). There are some reports of abnormal seedling phenotypes in eucalypts which are believed to be due to single recessive genes (ELDRIDGE, 1970; HODGSON, 1976; POTTS, 1990; POTTS and JORDAN, 1994). However, the genetic control of these abnormal phenotypes has been studied in very few cases as a result of long generation time and poor seed set following selfing. Such traits are of interest in forestry because easily scored mutants, controlled by single genes expressed at the seedling stage, allow a simple and cost-effective way to monitor outcrossing rates in open-pollinated seed (e.g. ELDRIDGE, 1970; HODGSON, 1976; POTTS, 1990).

During germination of *E. globulus* open-pollinated seed, two unrelated native trees were each found to produce some progeny that lacked chlorophyll (C. M. HARDNER, unpublished data). Both trees produced albinos that were discernable at the cotyledon stage and, initially, albino and non-albino phenotypes were equally vigorous. The albinos, however, were never observed to produce leaves and died a few weeks after germination. In one case (513) the albinos were yellow, and in the other (309) they were more pink, presumably the result of differing levels of anthocyanin. The aim of this study was to determine the genetic control of these albino phenotypes in *E. globulus* and to test their use in monitoring outcrossing rates.

Materials and Methods

The two trees described above (tree numbers 309 and 513) are located in southern Tasmania and are separated by a

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distance of six kilometres. Each tree was selfed, and a cross pollination between the two was also performed. Capsules arising from the assisted pollinations and some from open-pollination were then harvested. The seed samples were germinated and estimates of outcrossing rates determined, based upon the proportion of albino progeny present in a sample.

Outcrossing rates in eucalypts are more commonly estimated using isozymes (POTTS and WILTSHIRE, 1997). Sufficient allozyme polymorphism for estimating outcrossing rates was found in the progeny of tree 309 allowing an estimate to be calculated, based on three loci (*Pgd-1*, *Gpi-2* and *Aat-2*). The same sample of seedlings, used to estimate outcrossing by the frequency of the albino phenotype, was assayed for this purpose (as described in HARDNER, VAILLANCOURT and POTTS, 1996). An estimate of outcrossing was then calculated using the multilocus, maximum likelihood estimate of outcrossing rate program (RITLAND, 1990; http://genetics.forestry.ubc.ca/ritland/programs.html).

Results

The proportion of albino progeny, following assisted selfing, suggests that each of the albino phenotypes results from the expression of a single recessive gene for which the parent tree is heterozygous (*Table 1*). This is supported by Goodness of fit Chi-square tests which show no significant deviation from 3:1 ratios. A cross between the two trees resulted in no albino progeny out of a total of 66 and indicates that the similar phenotypes are caused by separate genes (*Table 1*). For the purposes of this study we have called the gene in tree 309 '*chl1*' and that of tree 513 '*chl2*'.

Table 1. – Progeny tallies from controlled crosses and the probability of the Chi squared goodness of fit for a 3:1 ratio are indicated.

Cross type	Number of seedlings			Chi squared
	green	albino	total	probability
309 self	15	10	25	0.083
513 self	18	4	22	0.460
309x513 F_1	66	0	66	2×10^{-6}

From the open-pollinated capsules of tree 309, 292 seedlings produced 37 albinos (12.7%) which, assuming albinos can be produced only by selfing, means that 12.7 x 4 is the estimated degree of selfing (50.8%), and therefore the outcrossing rate is 49.2%. A sample of 176 OP seed from tree 513 produced 26 albinos (14.8%) which gives an outcrossing estimate of about 41%.

Using the isozyme multi-locus estimation method for tree 309, an outcrossing rate of 52 ± 11% was obtained, this is in agreement with the albino estimate derived above (49.2%). A multi-locus estimate was also obtained for a sample of 55 albino seedlings from tree 309 and found to be zero, consistent with their proposed selfed origin. For this sample it was however, necessary to remove the *Gpi-2* locus from the analysis as it did not segregate independently of the albino phenotype, as a result of linkage. The maternal genotype at *Gpi-2* is heterozygous, however, from the 55 albino seedlings, 34 were found to be homozygous for the GPI-2^5 allozyme while only 2 were homozygous for GPI-2^1. An estimate of the recombination frequency, based on this sample, revealed that recombination between *chl1* and *Gpi-2* is occurring at a rate of 21% (23 out of 110 gametes involved a recombination event).

Discussion

This study has identified two *E. globulus* trees that carry mutations in separate genes, both of which result in chlorophyll deficient seedlings when the mutation is homozygous. Similar findings have been reported in other systems, for example, in the hazelnut *Corylus avella*, where two genes were also found to produce chlorophyll deficient seedlings (MEHLENBACHER and THOMPSON, 1991).

Single-locus morphological markers such as these may prove to be extremely useful for monitoring factors affecting outcrossing in eucalypts. A comparison between the frequency of albino seedlings and multi-locus isozyme estimates has previously been performed in *Pinus ponderosa* to determine population estimates of outcrossing and both methods were found to be in agreement (MITTON *et al.*, 1981). In this study also, the single locus and the multi-locus methods were in agreement when used to determine an outcrossing rate for an individual tree. In native forest it may be expected that the presence of bi-parental inbreeding (SKABO, VAILLANCOURT and POTTS, 1998) would downwardly bias the single locus estimate (BROWN, 1990), however, this does not appear to be happening in the present case, even though some neighbouring trees were also found to produce albino seedlings. Such a bias would be much less likely in seed orchards which are normally designed to maximise the distance between related trees and often have a large number of families represented by few trees. Selfing in seed orchards is expected to have a large economic impact on plantations since inbreeding depression from selfing results in nearly 50% reduction in stem volume when compared with outcrosses of *E. globulus* (HARDNER and POTTS, 1995). It is therefore important to closely monitor outcrossing rates, and this may be more easily implemented if trees can be found which harbour easily scored mutants. Screening for such parental trees in an orchard would not only be useful for outcrossing studies but may also reduce the additional nursery costs incurred in operational-scale sowing of seed containing lethal or semi-lethal genes.

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Buchbesprechung

Risikobewertung im Gentechnikrecht. Reihe Umwelt-Recht, Band 8. Von C. TÜNNESEN-HARMES. 2000. Erich Schmidt Verlag, Berlin, Bielefeld, München. 311 Seiten. 15,8 cm x 23,5 cm. Kartoniert. ISBN 3-503-05868-0. DM 108,–/€52,22/ öS 788,–/sFr 96,–.

Wie kaum eine andere Technologie hat sich die Bio- und Gentechnologie in den letzten 15 Jahren entwickelt. Kamen solche Methoden zunächst nur in den Laboratorien von Forschungsinstitutionen zur Anwendung, haben sie heute längst Einzug in die Praxis der Züchtung landwirtschaftlicher Kulturpflanzen, der Herstellung von Lebensmitteln in der Lebensmittelindustrie und der Erzeugung lebenswichtiger Inhaltsstoffe von Medikamenten gefunden. Die Nutzung der Gentechnik wird zudem für die Therapie unheilbarer Krankheiten sowie für diagnostische Zwecke diskutiert. Gleichermaßen mit dem potentiellen Nutzen der neuen Technologie sind Rufe zu möglichen Risiken laut geworden. Befürchtungen über unkontrollierbare Folgen haben zur Verunsicherung in weiten Teilen der Bevölkerung geführt. Der Gesetzgeber hat zwar versucht, mit strengen Verfahrensvorgaben für alle nur denkbaren Risiken eine hinreichende Vorsorge zu gewährleisten, dennoch sehen viele Kritiker unvorhersehbare bzw. noch unbekannte Risikopotentiale. Das vorliegende Buch von C. TÜNNESEN-HARMES soll einen Beitrag dazu leisten, die Wege der Entscheidungsfindung bei der Zulassung gentechnischer Vorhaben auf europäischer und nationaler Ebene transparenter zu machen und mögliche Schwachstellen der Risikobewertung aufzuzeigen. Die Untersuchung wurde im Rahmen einer Dissertation an der Rechtswissenschaftlichen Fakultät der Universität Münster durchgeführt. Der Umstand, dass die Promotion nicht im naturwissenschaftlichen Bereich angesiedelt wurde, belegt deutlich die Notwendigkeit zur interdisziplinarischen Zusammenarbeit bei der Bewältigung von Akzeptanzproblemen in der Gentechnik. Daher liegt in der vorliegenden Arbeit der Schwerpunkt weniger auf der Darlegung naturwissenschaftlicher Zusammenhänge als mehr auf der kritischen Beleuchtung verwaltungsrechtlicher Reglementierungen im Hinblick auf die Diskussion von „Chancen und Risiken" neuartiger Zukunftstechnologien. Somit werden zunächst die Bedeutung und Perspektiven sowie die möglichen Risiken der Gentechnik aufgezeigt. Für die Vereinheitlichung des Begriffs „Risiko" müssen die gebräuchlichen Definitionen des Risikobegriffs gegeneinander abgeglichen sowie die Methoden der Risikoermittlung und die der -bewertung dargelegt werden. Schließlich werden die verschiedenen Ebenen der Reglementierung auf nationaler und europäischer Ebene beschrieben. Das vorliegende Buch vermittelt somit Hintergrundinformationen zur Bewertung gentechnischer Vorhaben auf Basis der vorhandenen Regularien, die für den Antragsteller bereits im Vorfeld des Vorhabens hilfreich sein können.

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