units prior to their first birthdays. That recruitment into new harems and replacements in existing ones do draw randomly on the full pool of new offspring is evidenced by the lack of genetic differences between social units.

Our results suggest two lessons which may have general significance. (i) Even when two of the social features promoting local genetic homogeneity are present (in our case, stable adult composition of groups and a strong skew in male mating success), dissonant values for the third can completely dilute out the effects of the first two features. It is possible that other combinations of two features can equally be opposed by the third. Hence, to understand genetic effects of social structure, it is clearly mandatory to know the nature of all three features in the system studied. (ii) Even though these bats form very stable and cohesive groups of females (the males being primarily appendages and unrelated to the determination of group composition or stability), they need not be more closely related to each other than they are to females in the population as a whole. This suggests that kin selection is not a necessary condition, although in other species it may be a sufficient one, for the evolution of stable social units. The current tendency to explain all complicated social behavior as the outcome of kin selection should perhaps be tempered with more caution.

GARY F. MCCRACKEN
JACK W. BRADBURY
Department of Biology, C-016, University of California at San Diego, La Jolla 92093

References and Notes

1. G. L. Bush, *Annu. Rev. Ecol. Sys.* **6**, 339 (1975); A. C. Wilson, G. L. Bush, S. M. Case, M. C. King, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5061 (1975); R. K. Selander, *Am. Zool.* **10**, 53 (1970).
2. B. C. R. Bertram, in *Growing Points in Ethology*, P. P. G. Bateson and R. A. Hinde, Ed. (Cambridge Univ. Press, Cambridge, 1976), p. 281; M. J. W. Eberhard. *Q. Rev. Biol.* **50**, 1 (1975); W. D. Hamilton, *J. Theor. Biol.* **7**, 1 (1964); *Annu. Rev. Ecol. Syst.* **3**, 193 (1972); R. L. Trivers and H. Hare, *Science* **191**, 249 (1976); E. O. Wilson, *Sociobiology: The New Synthesis* (Harvard/Belknap Press, Cambridge, Mass., 1975), pp. 117–129.
3. G. G. Goodwin and A. M. Greenhall, *Bull. Am. Mus. Nat. Hist.* **122**, 187 (1961).
4. T. C. Williams and J. M. Williams, *Anim. Behav.* **18**, 302 (1970); J. Bradbury, L. Emmons, B. Tannenbaum C. Kagarise, in preparation.
5. R. K. Selander, M. H. Smith, S. V. Yang, W. E. Johnson, J. B. Gentry, *Stud. Genet.* **6**, 49 (1971) (University of Texas Publ. 7103).
6. G. F. McCracken, in preparation
7. The term "bastard" is used to denote a baby born into a harem but not in fact fathered by the current harem male.
8. We thank Dr. J. L. Price, B. Ramlal, and M. Pereira of Simla, Asa Wright Nature Centre, Trinidad, West Indies, and Dr. J. S. Kenny and the staff of the Department of Biological Sciences, University of the West Indies, St. Augustine, Trinidad, for suggestions and logistical support. Research supported by NSF grant BNS 76-04400 to J.W.B.

16 May 1977

Regeneration of Douglas Fir Plantlets Through Tissue Culture

Abstract. *Douglas fir plantlets were produced in tissue culture under defined conditions from cotyledon explants obtained from 2- to 4-week-old seedlings. Tissue pieces were cultured on the surface of a fabric tissue support (100 percent polyester) saturated with liquid nutrient medium; this facilitated periodic changes of the medium to meet the requirements at successive developmental stages without transfer of cultured tissues. Plant growth regulators were needed to stimulate adventitious bud formation. Plantlets were regenerated by rooting excised shoots at 19°C on agar-solidified medium containing sucrose and the auxin naphthalene-2-acetic acid. After root initiation, plant growth regulators were removed; this resulted in stimulation of root elongation and the subsequent development of plantlets, which were then established in soil.*

Adventitious buds have been produced in vitro from cotyledons of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] (*1*). We now report adventitious root formation on shoots derived from these buds and describe methods for high-frequency regeneration of plantlets from the cotyledons. A tissue culture system showing potential for use in mass clonal propagation is also described.

Douglas fir cotyledons, derived from 2- to 4-week-old seedlings, were the source of material for plantlet regeneration. Open pollinated seeds were sown directly in a soil mixture consisting of 60 percent sorghum peat moss and 40 percent vermiculite (Meca-Peat, Langley Ltd., Fort Langley, British Columbia, Canada), germinated, and subsequently grown in a growth chamber maintained at 25°C during an 18-hour photoperiod at a light intensity of 1000 footcandles (~ 11,000 lu/m²) followed by 19°C during a 6-hour dark period. When the seedlings were 2 to 4 weeks old, they were excised at the upper region of the hypocotyl and sterilized as follows. Plant materials were submerged with slight agitation in 6 percent Clorox (5.25 percent NaOCl) for 8 minutes and then rinsed three times with sterile distilled water until they were free of NaOCl. Before the establishment of cotyledons in culture, the NaOCl-treated plant tissue was placed for 3 to 6 days on an agar-solidified nutrient medium containing the plant growth regulators N_6-benzylaminopurine (BAP) and naphthelene-2-acetic acid (NAA) at concentrations of 5 μM and 5 nM, respectively. At the end of this treatment, contaminated and injured tissues were eliminated; only vigorously growing cotyledons were used for in vitro experiments.

The tissue culture system consists of a culture vessel and a fabric tissue support made of 100 percent polyester fleece 3 mm thick (Pellon Corp., Lowell, Massachusetts). A plastic petri dish (either 60 by 15 mm or 100 by 20 mm), layered with a circular disk of polyester fleece, was filled with liquid nutrient medium to such a level that the fabric tissue support was well moistened and served as a bridge between tissue explants and nutrient. Aseptic cotyledons were sliced cross-sectionally at approximately 3-mm intervals, and these explants (14 for the small petri dish and 25 for the large one) were then cultured on the surface of the fabric

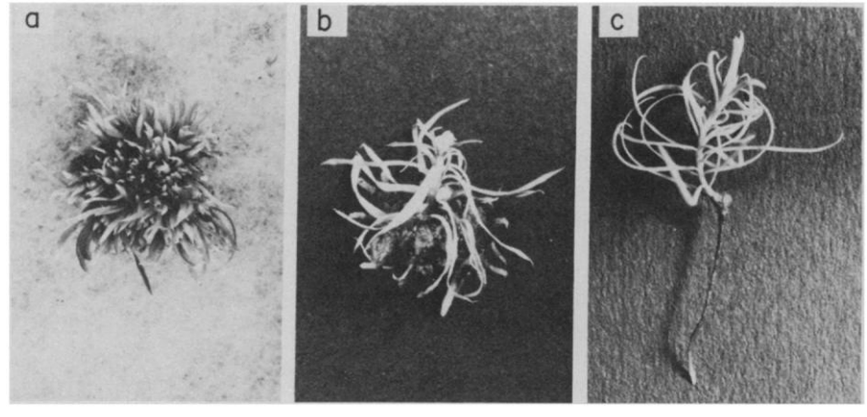

Fig. 1. Regeneration of plantlets in culture from cotyledons of Douglas fir. (a) Adventitious buds produced from cotyledon explants cultured for approximately 4 weeks on the surface of a fabric tissue support saturated with liquid medium containing 5 μM BAP plus 5 nM NAA, and subsequently cultured for 2 weeks on a new medium containing no plant growth regulators. Plantlets were regenerated by rooting shoots excised from the shoot cluster (a) on an agar-solidified medium containing 0.25 μM NAA at incubation temperatures of 24°C (b) and 19°C (c).

tissue support. To change the nutrient medium, the old medium was siphoned off and the new medium was added to the same petri dish. The composition of the basal medium, the plant growth regulator supplements optimal for initiation of adventitious bud formation and subsequent bud growth, and the culture environment have been described (*1*). The plant growth regulators used included auxins [NAA, indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA)] and a cytokinin (BAP). To secure the production of adventitious buds on cotyledon cultures, combined cytokinin and auxin (5 μM BAP plus 0.5 to 5.0 nM NAA, or 5 μM BAP plus 2.5 to 5 μM each of IBA and IAA) was added to the basal nutrient medium. To obtain sufficient numbers of adventitious buds, a cultivation period of 4 to 6 weeks was necessary. Subsequently, bud growth was stimulated by replacing the medium containing plant growth regulators with basal medium. The continued presence of the growth regulators (especially auxin) resulted in inhibition of bud development because of competition by callus growth on tissue in direct contact with the nutrient. Removal of the growth regulators stopped callus growth and resulted in stem elongation and needle expansion of adventitious buds (Fig. 1a). Shoots large enough to be easily excised were separated from the callus mass and grown on basal medium.

To produce Douglas fir plantlets, the excised shoots (approximately 2 cm long) were placed in individual culture tubes (2.5 by 15 cm) containing 20 ml of agar-solidified nutrient medium and incubated at 19°C. The basal medium was identical to that used for bud formation except for the concentration of sucrose. Studies performed with varying concentrations of sucrose and NAA in the nutrient medium showed that the optimal concentrations of these two compounds for promotion of root formation were 0.5 percent sucrose and 0.25 μM NAA. With this medium, a high frequency (~ 80 percent) of plantlet production was obtained (Table 1). Failure to produce plantlets occurred when a higher concentration of either NAA or sucrose was used; high levels of NAA caused prolific callus growth at the basal end of the stem and excess sucrose reduced the vitality of the shoots. Approximately 4 weeks after the excised shoots were subjected to the rooting conditions, root primordia started to emerge from the cut surface; subsequent withdrawal of NAA from the medium resulted in rapid root elongation (Fig. 1c) and plantlet growth. When similar experiments were performed at an incubation temperature of 24°C, relatively few plantlets (~ 30 percent) were produced; furthermore, those produced were abnormal, exhibiting a discontinuity in their anatomical structure caused by a proliferation of friable callus at the transition region between stem and root (Fig. 1b). In contrast, the plantlets produced at 19°C had a normal morphological appearance (Fig. 1c) and were subsequently established in soil with > 90 percent survival.

Table 1. Effect of various concentrations of NAA and sucrose on regeneration of Douglas fir plantlets in culture. The basal nutrient medium (minus sucrose) was supplemented with NAA and sucrose at the concentrations indicated. The growth chamber was maintained at 19°C with a 16-hour photoperiod (200 foot-candles) followed by an 8-hour dark period.

NAA (μM)	Sucrose (%)	Shoots cultured	Shoots producing roots
2.50	0.5	10	0
0.50	0.5	10	0
0.25	0.5	10	8
0.25	1.0	10	4
0.25	3.0	10	1
0.25	5.0	10	0
0.25	8.0	10	0

These experimental results demonstrate that the mode of action of auxin (in this case, NAA) is influenced by changes in incubation temperature. Since cells cultured at the higher temperature (24°C) are expected to be metabolically more active than those cultured at the lower temperature (19°C), we conclude that auxin affects two different cell types. Thus, the hormonal effect of auxin (NAA) on cultured cells is expressed either as (i) stimulation of adventitious root formation at the lower temperature, or (ii) stimulation of unorganized cell proliferation at the elevated temperature.

Regeneration of plantlets in vitro has been reported for only two economically important conifer species, longleaf pine (*Pinus palustris* Mill.), for which no quantitative data were presented (*2*), and western hemlock (*Tsuga heterophylla*), for which an undefined rooting medium (soil) was used (*3*). In the work reported here, we used chemically defined media and achieved high-frequency regeneration of plantlets from tissues of Douglas fir. The reproducibility we obtained should encourage application of this method in tree improvement programs. We believe that this is an important step in developing tissue culture as a tool for use in the domestication of wild tree species.

TSAI-YING CHENG*
THANH H. VOQUI
Department of Chemistry and Biochemical Sciences, Oregon Graduate Center, Beaverton 97005

References and Notes

1. T. Y. Cheng, *Plant Sci. Lett.* **5**, 97 (1975); *ibid.* **9**, 179 (1977).
2. H. E. Sommer, C. L. Brown, P. P. Kormanik, *Bot. Gaz.* **136**, 196 (1975).
3. T. Y. Cheng, *Plant Cell Physiol.* **17**, 1167 (1976).
4. Research was supported by a grant from the Weyerhaeuser Corp., Tacoma, Wash. Douglas fir seeds were supplied by the Weyerhaeuser Forestry Research Center, Centralia, Wash. We thank Drs. G. Doyle Daves, Jr., and John H. Rediske for their support in this research, and Faith Henning for technical assistance.
* Reprint requests should be sent to T.-Y.C.

26 April 1977

Diazepam Maintenance of Alcohol Preference During Alcohol Withdrawal

Abstract. *After forced intragastric intubation of alcohol, rats will show a greatly increased tendency to self-administer alcohol in a free-choice situation. Diazepam (Valium) dosage (5 milligrams per kilogram of body weight) during the period of withdrawal serves to maintain undiminished such alcohol self-administration. Without such diazepam dosage the tendency to self-administer alcohol returns to control levels.*

Although there is at present little evidence that most pharmacological treatment is of benefit in the treatment of alcoholism (*1*), some success has been claimed with the use of benzodiazepines (*2*). In an effort to investigate the use of such drugs, we administered diazepam (Valium, injectable, Hoffmann–La Roche) to rats in which elevated intakes of alcohol had been produced (*3–5*) by forced intragastric intubation of ethanol through implanted gastric fistulas. Once the period of forced intubation was over, the rats were given a choice between two neutral flavors. The choice of one of the flavors was paired with direct intragastric intubation of an equal volume of 20 percent alcohol (*3, 4*). Whereas rats not pretreated with alcohol tend to avoid the flavor paired with it, rats pretreated with alcohol will drink 70 to 80 percent of the fluid paired with alco-