unknown etiology as well as in the well-characterized diseases such as systemic lupus erythematosus (*6*) and multiple sclerosis (*8*). In such conditions varying degrees of immune suppression or exhaustion are also demonstrated (*9*). With this in mind, the spleen cell responses to the T cell mitogens phytohemagglutinin M (PHA) and concanavalin A (Con A) and the B cell mitogen *Escherichia coli* lipopolysaccharide (LPS) (*4*) were examined (*10*). All mitogen experiments were performed in triplicate, three to six animals being used per experiment. With PHA, although the degree of responsiveness varied with the animal, the mean response of the arteriosclerotic animals was depressed as much as 400 percent from that of the controls. Similar results were obtained with Con A. No significant differences were noted with LPS (Table 1).

The mixed lymphocyte reaction, an indication of T cell responsiveness, was used to further characterize T cell reactivity (*11*). A mixed lymphocyte reaction would be expected to occur because Sprague-Dawley rats are not totally syngeneic. When the spleen cells of three arteriosclerotic animals were pooled, there was no increase in [^{3}H]thymidine uptake compared with the uptake in separate cultures. An approximate twofold increase in [^{3}H]thymidine uptake occurred in the pooled cells of three control animals. These findings give further indication of a depressed T cell function in the arteriosclerotic animal.

To verify normal B cell function in the LPS studies above, we assessed the antibody response of the arteriosclerotic animals to sheep RBC using the direct plaque-forming cell technique (*12*). Both arteriosclerotic and control animals were sensitized with 0.5 ml of a 10 percent suspension of sheep RBC and the number of plaques was counted on day 5. There was no significant difference between the mean response of the two groups of animals (Table 1). These results indicate that the T cell–dependent antibody response of the diseased animal was not altered. Since no decrease in activity was noted among the population of T cells involved in the antibody response to sheep RBC, whereas T cells involved in the mixed lymphocyte reaction and in the mitogen response were significantly suppressed, this indicates that different populations of T cells were involved.

The arteriosclerotic animals manifested significant T cell depression. It may be postulated that the suppression is due to multiple pregnancies that increase the amount of suppressor substances (*13*); however, the stress, which is known to increase cortisone production (*3*), may also deplete the spleen of functional T cells. Depression of T cells has been demonstrated in aging mice (*14*), autoimmune disease (*9*), virus infections (*15*), and in some experimental tumor models (*11*). The antigen-antibody complexes demonstrated in the vessel wall cannot be attributed to an autoimmune phenomenon to arterial tissue, but we advance the hypothesis that these complexes might include viral, environmental, or other tissue antigens, since similar deposits have been demonstrated in other models (*16*).

We suggest that this model of arteriosclerosis might be included in the same category of disorders as some autoimmune or viral conditions that manifest immune complex deposition in tissues as well as significant immune suppression. However, it should be emphasized that our data demonstrate that the stress of multiple pregnancies results in immune suppression.

EDMUND C. LATTIME
HELEN R. STRAUSSER
Department of Zoology and Physiology, Rutgers University–Newark, Newark, New Jersey 07102

References and Notes

1. B. C. Wexler, *J. Atheroscler. Res.* **4**, 57 (1964); *Circ. Res.* **14**, 32 (1964); *ibid.* **15**, 485 (1964); *Atherosclerosis* **11**, 383 (1970).
2. ———, *J. Atheroscler. Res.* **9**, 267 (1969).
3. H. Folch and B. Waksman, *J. Immunol.* **113**, 127 (1974); A. A. Monjan and M. I. Collector, *Science* **196**, 307 (1977).
4. The animals were purchased from ARS Sprague-Dawley Co.; peroxidase-labeled rat antibody to rabbit IgG from Cappell Labs; mitogens from Gibco; [^{3}H]thymidine from New England Nuclear.
5. C. Minick and W. Campbell, *J. Exp. Med.* **124**, 635 (1966).
6. C. Cochrane and F. Dixon, in *Textbook of Immunopathology*, P. A. Miescher and H. J. Muller-Eberhard, Eds. (Grune & Stratton, New York, 1976), p. 137.
7. P. Nakane and G. Pierce, *J. Cell Biol.* **33**, 307 (1967).
8. T. Tachovsky, H. Koprowski, R. Lisak, A. Theofilopoulos, F. Dixon, *Lancet* **1976-II**, 997 (1976).
9. N. Talal, *Transplant. Rev.* **31**, 240 (1976).
10. For the mitogen assays, 0.1 ml (6×10^6 viable nucleated spleen cells) was added to each well of a microculture plate (96 wells; Falcon). Medium (0.1 ml) containing the various mitogens was added to each well and the plates were incubated at 37°C in an atmosphere containing 5 percent CO_2. After 24 hours the cultures were exposed to 0.5 μc of [^{3}H]thymidine (6.7 c/mmole) in 50 μl of medium. The cultures were harvested with a Mash II (Microbiological Associates) 20 hours after the addition of thymidine. The radioactivity collected on the filters was counted in a Beckman liquid scintillation counter.
11. For the mixed lymphocyte reaction, spleens from three animals were combined, and 0.1 ml (6×10^6 viable nucleated spleen cells) was added to the wells of a microculture plate. Medium (0.1 ml) was added to each well and the culture was incubated at 37°C in 5 percent CO_2. After 24 hours the cultures were exposed to 0.5 μc of [^{3}H]thymidine. The cultures were then incubated and harvested as in (*10*).
12. D. Dresser and M. Greaves, in *Handbook of Experimental Immunology*, D. M. Weir, Ed. (Blackwell, Oxford, 1973), p. 27.2.
13. M. R. Harrison, *Scand. J. Immunol.* **5**, 881 (1976).
14. K. Hirokawa and T. Makinodan, *J. Immunol.* **114**, 1659 (1975).
15. M. Oldstone and F. Dixon, in *Textbook of Immunopathology*, P. Miescher and H. Muller-Eberhard, Eds. (Grune & Stratton, New York, 1976), p. 310.
16. M. Oldstone and F. Dixon, *J. Exp. Med.* **134**, 32s (1971); D. F. Davies, *J. Atheroscler. Res.* **10**, 253 (1969).
17. We thank B. P. Sonnenblick for editorial advice and J. Kalman and M. Rosenstein for expert technical assistance. Supported in part by Busch Memorial Foundation and NIH biomedical grant RR 7059.

3 June 1977

Paternity and Genetic Heterogeneity in the Polygynous Bat, *Phyllostomus hastatus*

Abstract. *Wild colonies of greater spearnose bats were marked, censused regularly, and genotyped at three polymorphic allozyme loci. While adult composition of social units is very stable and strong polygyny results in marked changes in gene frequencies between generations, dispersal of offspring is sufficient to prevent significant genetic heterogeneities between social units. Kin selection cannot explain social cohesiveness in these highly social mammals.*

Social organization in animal populations can reduce genetically effective population sizes and restrict gene flow between adjacent social units. These effects and their evolutionary consequences are maximized when three features act in concert: (i) a low exchange rate of adults between groups; (ii) a preferential recruitment of juveniles into their parental groups; and (iii) a restriction of mate selection to members of the same social unit, coupled with a large disparity in mating success among members of one sex. Recent treatments support the argument that the combined effects of these features are to generate substantial genetic heterogeneity among social units, result in rapid fixation of initially rare genetic characters, and perhaps lead to reproductive isolation and speciation (*1*). Since these latter effects are all important stages in evolutionary processes, it seems particularly important to know (i) how frequently the three social features are acting in concert and (ii) how easily a dissonant feature can counter the effects produced by two sympathetic features.

Turning the argument around, it has become fashionable in sociobiology to invoke genetic similarity through descent as a cause, as well as a result, of social organization. In particular, all the three features noted above, when acting in concert, can generate a high degree of genetic relatedness among members of the same social group. Through kin selection, this may in turn lead to increased cooperation among group members, more stable social units, modification of reproductive schedules, and other shifts in individual behaviors (*2*). From the sociobiological point of view, we may thus ask: How often is close genetic relatedness among individuals a necessary condition for the evolution of stable and cohesive social units?

With these issues in mind, we present field data on paternity and genetic heterogeneity of social units using genetic markers in the Neotropical bat, *Phyllostomus hastatus*. Prior studies on this bat in Trinidad showed that wild populations are divided into colonies of several hundred to several thousand individuals that roost during the day in limestone caves (*3*). Each colony is subdivided into clusters of from 10 to 100 bats, and each cluster consists either of annually stable harems (adult females, recent young, and one adult male), or of bachelor groups (males of all ages and occasionally a few juvenile females). Compositional stability of harem females is high, with an annual turnover rate (32 percent per year) comparable to independent estimates of annual adult female mortality (*4*). Female composition in harems is independent of the identity of the current harem male and, in captive colonies, is even independent of the presence of any male. Harem males vigorously defend their harems against other males, are quickly replaced when removed experimentally, and are the only males observed to mate with females in the day roosts (*4*). It is clear that these bats exhibit two of the critical social features noted above, namely, partitioning of the population into stable and cohesive social units and polygynous mating.

Because of the tight clustering and relative temerity of this species, we have been able to capture entire clusters, mark and sample all group members, and release the bats with little subsequent disruption of social behavior or group compositions. When these collections were made during the annual and synchronous parturition period (late April to early May), between 55 and 77 percent of all mother-youngster pairs could be correctly matched for each harem. Marked colonies were then monitored every 2 weeks for 9 months to determine compositional changes, juvenile dispersal, and harem male turnovers.

The genotypes of all captured animals were determined with respect to three polymorphic allozymes obtained from blood. Samples were obtained by piercing a wing vein with a hypodermic needle and collecting blood in heparinized capillary tubes. Plasma and cellular fractions were separated by microhematocrit centrifugation, and the two fractions were stored separately in Dry Ice. Horizontal starch gel electrophoresis followed general procedures, buffers, and stains (*5*). Two of the allozyme systems were isolated from red blood cells: indophenol oxidase (IPO) is diallelic with an average frequency of 82 percent for the common allele, and phosphoglucomutase (PGM) is triallelic with an average frequency of 90 percent for the common allele. The third system, isolated from plasma, is a triallelic transferrin (Tff) with the common allele at an average frequency of 72 percent. Variation of all three systems fits a single locus multiallelic model, the loci are not linked, and null alleles either do not occur or are present at very low frequencies (*6*).

Table 1. Probability of detecting a bastard. The probability of detecting a bastard (PDB) (*7*) is calculated as the likelihood that we would detect it had a male other than the harem male fathered a particular baby. Thus, the PDB for the first mother-baby pair in this table is .13 at the IPO locus (none of the alternative BB fathers could be detected, but we would expect one-half of the AB fathers, or .13 of the total males, to reveal themselves); 0.0 at the PGM locus (BB or BC fathers would always give the expected allele); and .67 for the Tff locus (all CC, AC, and AA plus one-half of the BC and AB fathers would be detected). The total PDB is the sum of the PDB's for each locus minus the chance that detection would occur concurrently in two or three loci. The probability of not detecting a bastard (PNDB) is 1 − total PDB. A running product of the ranked PNDB's is taken, starting with the largest PNDB. The number of PNDB's needed to reach .05 is (*K*), the maximum number of bastards that could exist in the harem with a 5 percent chance that none would be detected.

IPO mother	Baby	PDB	PGM mother	Baby	PDB	Tff mother	Baby	PDB	Total PDB	Total PNDB
BB	BB	.13	BB	BC	0.0	CC	BC	.67	.71	.29
BB	BB	.13	BB	BB	0.0	BC	BC	.33	.42	.58
BB	BB	.13	BB	BB	0.0	AB	BB	.67	.71	.29
AB	AB	.06	AB	BC	0.0	BC	BC	.33	.38	.62
AB	AB	.06	AB	BB	0.0	CC	BC	.67	.68	.32
AB	BB	.06	BB	BB	0.0	BC	BB	.33	.38	.62
BB	BB	.13	BB	BB	0.0	BC	BC	.33	.42	.58
BB	BB	.13	BB	BC	0.0	BC	BB	.33	.42	.58
BB	BB	.13	BB	BC	0.0	AC	BC	.67	.71	.29
BB	BB	.13	BB	BB	0.0	BC	BC	.33	.42	.58
AB	AB	.06	AA	AB	0.0	CC	BC	.67	.68	.32
BB	BB	.13	BB	BB	0.0	AC	AA*	"Bastard"		
BB	BB	.13	BB	BC	0.0	CC	BC	.67	.71	.29
BB	BB	.13	BB	BB	0.0	CC	BC	.67	.71	.29
AB	AB	.06	BB	BC	0.0	CC	BC	.67	.68	.32
Harem male, BB			Harem male, BC			Harem male, BB				

Genotype of bachelor male population (N = *30*)

IPO:	AA	AB	BB
	0	8	22
f =	0	.27	.73

PGM:	BB	BC	CC
	29	1	0
f =	.97	.03	0

Tff:	AA	AB	BB	BC	CC	AC
	0	0	4	10	12	1
f =	0	0	.15	.37	.44	.04

N, number of mother-baby pairs; *B*, number of detected bastards; *K*, maximum number of bastards at the .05 confidence level.

Leakage to other males:
Minimum = *B*/*N*
Maximum = (*B* + *K*)/*N*

Harem male reproductive control:
Minimum = 1 − maximum leakage × 100
Maximum = 1 − minimum leakage × 100

For this harem: *N* = 15, *B* = 1, and *K* = 6; the reproductive control was 93 percent maximum and 53 percent minimum.

*The only one whose phenotype was totally incomparable with the current harem male.

Beginning in April 1976, a total of 331 *P. hastatus* were marked, their genotypes were determined, and their social status was ascertained. Data were collected from three limestone caves located in parallel valleys in Trinidad's Northern Range. In Guanapo Valley cave, three harems and two bachelor groups, constituting more than 80 percent of the cave population, were sampled (56 adult females, 30 adult males, 33 youngsters matched to known mothers, and 15 unmatched youngsters). In Lopinot Valley (Darceuil) cave, four harems and one bachelor group, about 70 percent of the cave population, were sampled (61 adult females, 10 adult males, 26 youngsters matched to mothers, and 22 unmatched youngsters). In September 1976, the Caura Valley cave was sampled, and two harems, (about 30 percent of the cave population) were examined (54 adult females, 2 adult males, and 21 weaned and unmatched juveniles). Lopinot cave is approximately 8 km west of Guanapo cave, and Caura cave is about 6 km west of Lopinot cave.

Of the three social features that promote genetic structuring of social groups, adult female compositional stability was already known to be high. We thus used the genetic marker data to determine (i) whether there is sufficient polygyny to make harem offspring more genetically homogeneous than the population as a whole and (ii) whether this genetic structuring of progeny is incorporated into parental groups. Three types of calculation are needed to determine the degree of polygyny in these bats.

1) It was necessary to demonstrate that mating was indeed nonrandom. We thus examined the four harems that contained seven or more matched mother-baby pairs. In each harem, the rarest allele that could have been contributed by the current harem male was identified, and we computed the probability that as many offspring as are observed to carry it should do so if matings were random among all cave males. In these four harems, mating was found to be significantly nonrandom (binomial test, all cases with $P < .005$).

2) We establish an upper limit on paternity that can be ascribed to the current harem male. This is the fraction of sampled offspring in a harem which is genetically consistent with the current harem male as a father. These estimates of paternity range from 85 to 100 percent for the four harems with seven or more matched mother-baby pairs, and 75 to 100 percent for all seven harems. The average upper estimate of paternity for the seven harems is 90 percent. Finally, because genetic compatibility between harem male and progeny does not prove paternity (since other possible paternal genotypes are also present in the cave), a lower limit on paternity is needed, if we assume the worst. The worst is that several "bastards" are indeed present but have gone undetected (*7*). If the genotypes of each youngster, its mother, and its presumed father, are noted, as well as the genotype frequencies of the males in the cave, it is possible to compute a probability for each youngster that it was a bastard, but we would not have detected it. Youngsters in a harem were ranked according to decreasing probabilities of being undetected bastards and the cumulative product of these probabilities was computed until it reached 5 percent. The number of youngsters required to reach 5 percent is the maximum number of undetected bastards likely to occur in the harem. This number plus the number of obvious bastards was used to compute a minimal paternity rate in each harem. For the four harems with seven or more matched mother-baby pairs, the upper and lower estimates of paternity were (N, numbers of pairs examined): 93 to 53 percent ($N = 15$), 85 to 38 percent ($N = 13$), 100 to 22 percent ($N = 9$), and 100 to 0 percent ($N = 7$). It is probably significant that the upper and lower estimates converge for larger sample sizes. Averaging these values, a typical harem male accounts for at least 28 percent and at most 94 percent of the offspring in his harem. Given a 2-year stint as harem male and an observed average harem size of 22 females, this results in the fathering of from 12 to 41 offspring per male.

This degree of polygyny should make the harem male very important in determining the genetic composition of the next generation. As evidence of his impact, we observe substantial allele frequency changes between maternal and filial generations in six of the seven harems (Table 2), and in four of the cases, these changes are statistically significant. Polygyny in these bats is thus sufficient to alter within-harem gene frequencies between generations.

These changes in genetic composition could be incorporated into the adult social units either by preferential recruitment of female offspring into parental units or by the formation of new harems exclusively by females having the same father. Analysis of genotypes of adult females in nine harems in the three study caves shows that, in fact, there is no significant genetic heterogeneity between either harems in the same cave or different colonies in different caves (Table 3). While we do not yet have sufficient data on the generation of new harems, censuses taken in March 1977 of colonies marked a year earlier indicate that nearly all female offspring leave their parental

Table 2. Allele frequency changes within harems between mother and filial generations.

Cave	Harem	Locus	Allele	"f" mothers	N	"f" babies	N
Guanapo	O	Tff	b	.20	25	.58	19*
Guanapo	O	PGM	c	.00	25	.18	19
Guanapo	LG	Tff	a	.07	22	.25	18*
Guanapo	W	IPO	b	.78	9	1.00	7
Lopinot	Or	Tff	c	.58	13	.77	11
Lopinot	Wh	IPO	a	.16	22	.44	17*
Lopinot	Y	PGM	a	.08	13	.45	10*

*$P < .05$, G test.

Table 3. *Phyllostomus hastatus*; genetic heterogeneity in 1976.

Cave	Locus	G	d.f.	P*	N
Among harems within caves					
Lopinot-Darceuil (four harems)	Tff	3.8	3	> .1	62
	IPO	1.6	3	> .5	62
	PGM	1.9	3	> .5	62
Guanapo (three harems)	Tff	5.4	2	> .05	56
	IPO	0.6	2	> .5	56
	PGM	0.1	2	> .9	56
Caura (two harems)	Tff	0.3	1	> .5	54
	IPO	1.6	1	> .05	54
	PGM	0.9	1	> .1	54
Among colonies in different caves (Lopinot-Darceuil, Guanapo, and Caura caves)					
	Tff	1.8	4	> .5	172
	IPO	0.7	2	> .5	172
	PGM	3.5	2	> .1	172

*P calculated by G test for heterogeneity.

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 (19 → 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 (19 → 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

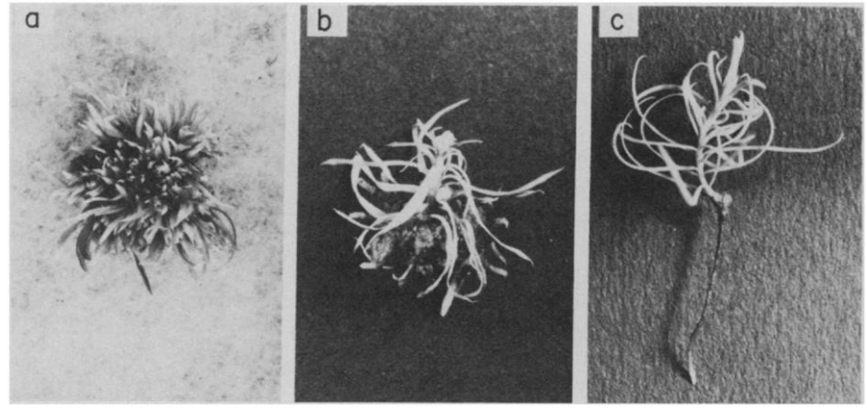

Fig. 1. Regeneration of plantlets in culture from cotyledons of Douglas fir. (a) Adventitious buds produced from cotyledon explants cultured for approximately 4 weeks on the surface of a fabric tissue support saturated with liquid medium containing 5 μM BAP plus 5 nM NAA, and subsequently cultured for 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).