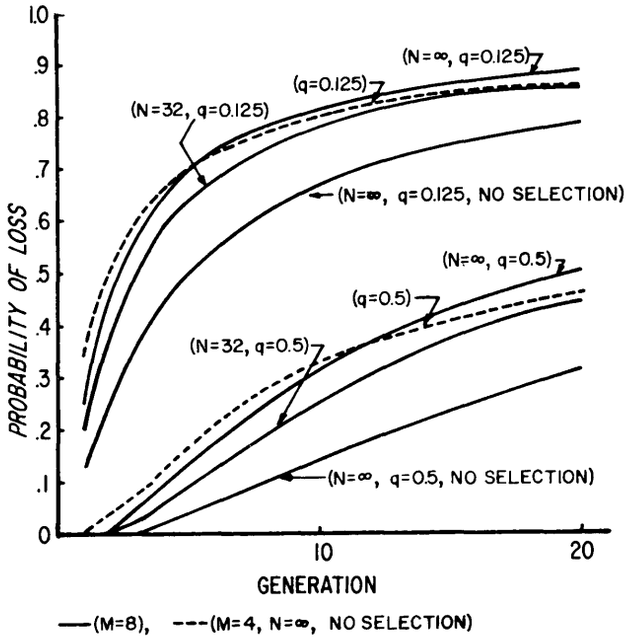

Fig. 8. — Probabilities of loss for M = 8 under declining selection pressure with restricted progeny populations.

the effect of selection and can overcome drift even when initial gene frequencies are low. For example, if M = 8, the probability of loss for $q^{|0|} = 0.125$ and r = 0.5 is eventually lower than for r = 0.0625 *(Fig. 4)*. This effect is more noticeable for M = 16, since the larger population sizes allow the selection effects of the linkage to be more influential *(Fig. 5)*.

The effect of tight linkage in increasing the likelihood of losing the allele of future interest can also be seen with alleles at higher initial gene frequencies, $q^{|0|} = 0.5$ *(Fig. 6)*. Selection effects for the larger population sizes (M = 8) do overcome the inertia of drift for M = 4 and hence more strongly force the loss of the allele of interest.

When the progeny population size is restricted and selection of the next parental generation is consequently limited, the effectiveness of selection is reduced. When this selection is indirect and of rapidly diminishing strength (r = 0.5), the loss of alleles is also slower. In 4-parent breeding, restricting the progeny population to 16 for a selection proportion of 0.25 instead of 0.10 almost halves the differences in loss probabilities between the cases of selection vs. no selection when $q^{|0|} = 0.5$, but the reduction in the effectiveness of selection is less when $q^{|0|} = 0.125$. For 8-parent breeding with a progeny population size of 32, the loss probabilities are similarly affected *(Fig. 8)*.

Breeding Alternatives

When it is necessary to breed in small or subdivided populations, the loss of alleles of a neutral locus (locus A) is rapid and is substantially increased if the alleles are under negative selection pressure. For parental populations of 8 or more, the short-run loss of alleles is a serious problem mainly for low frequency alleles. However, simple expedients such as doubling the parental population size or reducing the progeny population size can diminish the effects of selection. In later generations, however, tight linkage and large parental population size can increase the ultimate probabilities of loss of the alleles of interest.

If subdivided or otherwise small breeding populations are planned, it therefore seems wise to approximately double the size which would give acceptable risks of allelic loss as would be accepted under no selection. For the h^2 and r levels studied, M = 8 gives reasonable probability of loss for early and late selection. Some economies in maintaining these programs may be affected by limiting the progeny population size but at some cost in selection advance.

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Changes in the Protein Bands in Pollen grains of Populus ciliata during Storage and its effect on their Viability and Germination

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Summary

Disc electrophoresis studies on the pollen grains of *Populus ciliata* revealed the presence of a variety of protein bands depending on the pH of the buffer. Pollen grains on storage at 4° C started losing their viability after three months which also coincided with the simultaneous appearance of new protein bands. It seems that the loss of pollen germinability depends on the appearance of one or, a few inhibitory principles which are probably protein-like.

Key words: *Populus ciliata*, Pollen grains, Proteins, Storage, Germination, Viability.

Zusammenfassung

Plattenelektrophorese-Studien von Pollenkörnern bei *Populus ciliata* zeigten das Vorhandensein von verschiedenen Proteinbändern in Abhängigkeit vom pH-Wert des Puffers. Bei Pollenkörnern, die bei 4° C gelagert wurden begann die Lebensfähigkeit nach 3 Monaten abzunehmen, was auch der gleichzeitigen Erscheinung neuer Proteinbänder entspricht. Es scheint, daß der Verlust der Pollenkeimfähigkeit auf der Erscheinung eines oder einiger weniger hemmender Grundbestandteile beruht, die wahrscheinlich proteinähnlich sind.

Introduction

In spite of several reports, of successful preservation of pollen grains at low temperatures (Bredemann, Garber, Hartech and Suhr, 1947; Griggs, Vassell and Iwakiri, 1953; Khan, Hyene and Goss, 1971), no report deals with the total duration of viability as influenced by the endogenous behaviour of different proteins. Studies on pine pollen, with regard to their amino acid analysis and appraisal of their proteins showed no significant difference in protein band pattern with age and hence no correlation between viability and protein fraction (Bingham, Krugman, and Estermann, 1964). However, similar phytochemical characterization of protein fractions of *Populus ciliata* pollen is lacking. The information thus attained through electrophoresis of pollen of *P. ciliata* in polyacrylamide gels might help to explain the physiological causes of deterioration in viability of *P. ciliata* pollen grains after a period of storage even at low temperature.

Materials and Methods

Mature male catkins were harvested during the month of March—April, 1980. They were air-dried for 5—7 days and then de-lipified by the method of Lee and Fairbrothers (1969) and stored at 4° C. Pollen was prepared for electrophoresis as described by McCowan, Beck and Hall, (1968) and Novacky and Hampton, (1968). Pollen grains (4 g fresh weight) were ground in a cold morter in one of the three buffers i.e., (a) Tris/HCI buffer, pH 8.0 (b) B-alanine/acetic acid buffer, pH 4.6 or (c) glycine/ acetic acid pH 3.8 containing 8M urea, 0.1% ascorbic acid and 0.1% cystein hydrochloride. Pollen was strained through fine acetate cloth and re-extracted twice with each buffer and centrifuged at 10,000 g for one hour when supernatant was used for electrophoresis as described by Davis (1964). All gels were cooled to 4° C before the protein extract was applied and all subsequent steps were conducted at this temperature. The gels were larger than standard, the length of running gel being 8.5 cm. This was prepared and run in plexiglass tubes 10 cm long and slightly less than 7 mm ID. The plexiglass tubes allowed easier removal of the gels and were more convenient to handle than standard glass tubes. Electrophoresis was conducted at 3mA/tube for two hours and was repeated twice. Gels were stained by the method of Weber and Osborn (1969). They were dipped in solution containing 1% Coomassie blue, 25% isopropanol, and 10% acetic acid and stained for 20 min. followed by destaining for 24 hours in 15% methanol and 7.5% acetic acid. For studying the protein bands at different intervals of storage, the analysis was carried out only in buffer A.

Pollen viability was tested using 1% safranin and taking shrivelled pollen grains as non-viable, while for studying germination percentage, the hanging drop method was used (Snyder, 1961; Kirby and Stanley, 1976). Pollen was cultured in 1%, 5%, 10%, 15% sucrose and 1% agar. The data were analysed statistically by analysis of variance.

Results and Discussion

The electrophoretic band pattern of protein extracts from freshly harvested pollen obtained with different buffers, is shown in *Fig. 1.* The soluble proteins separated into ten bands in buffer A *(Fig. 1A).* The number of protein bands were reduced to four in buffer B while the same were reduced to a mere two in buffer C *(Fig. 1, B, C).* It seemed that the pH of the buffer or the capacity of urea to unfold the protein molecules at a specific pH was the major governing factor in separating various soluble proteins. Because of the better resolution in buffer A, subsequent studies on pollen proteins at different periods of storage were restricted only to this buffer, i.e. Tris/HCI (pH 8.0). It was revealed that ten bands, which were discernable in freshly harvested pollen grains, were maintained up to three months of storage while in the fourth month *(Fig. 2, 1—6)* an extra band also made its appearance. There was a further addition of another band in the fifth and sixth months of storage. However, the protein bands from the fresh samples were comparatively sharp as compared to the aged pollen.

The data on viability and germination of pollen grains *(Table 1)* showed that viability started falling significantly

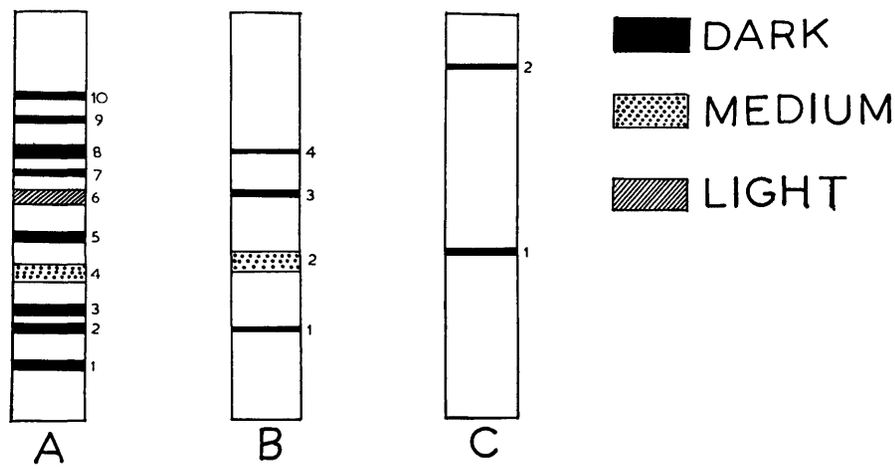

Figure 1. — Zymogram showing protein band pattern in three different buffers (A) Tris/HCl, pH 8.0 (B) β-alanine/acetic acid, pH 4.6 and (C) glycine/acetic acid, pH 3.8.

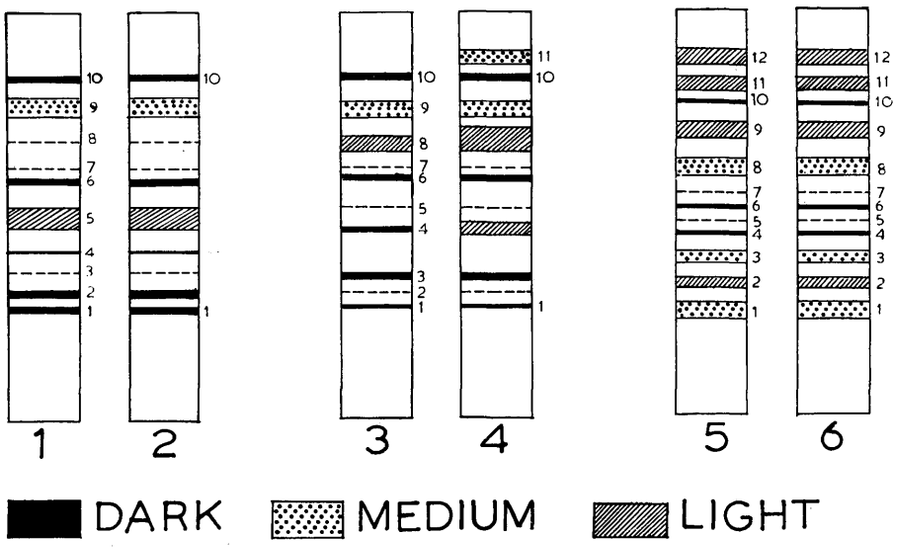

Figure 2. — Zymogram showing protein band pattern after storage (1—6 refers to months after storage).

Table 1. — Effect of storage on viability and germination in *Populus ciliata* pollen grains at 4 ± 1° C (Average of 3 replications)

Storage Period (Month after harvest)	Pollen viability	Agar (1%)	Germination (3) in Sucrose (1%)	Sucrose (5%)	Sucrose (10%)	Sucrose (15%)
Control	85.0 ± 5.3	10.0 ± 2.5	18.1 ± 2.8	22.4 ± 3.5	24.1 ± 3	12.0 ± 3.4
1	80.1 ± 5.6	7.0 ± 2.6	15.3 ± 2.9	20.1 ± 2.1	21.1 ± 2.6	8.0 ± 3.5
2	57.0 ± 4.9	Nil	12.6 ± 3.7	13.3 ± 1.6	15.0 ± 2.1	7.1 ± 3.5
3	50.4 ± 6.7	Nil	7.2 ± 4.5	7.8 ± 1.2	9.1 ± 2.8	6.0 ± 3.6
4	30.1 ± 5.3	Nil	Nil	Nil	Nil	Nil
5	10.7 ± 5.8	Nil	Nil	Nil	Nil	Nil
6	3.0 ± 9.0	Nil	Nil	Nil	Nil	Nil

after 2 months and almost all pollen grains lost viability after 6 months of storage. The pattern of germination was similar and as the pollen aged there was sharp deterioration in its germinability. The loss of viability as well as germinability, along with a simultaneous appearance of new protein bands, apparently showed that this morphogenetic behaviour was accompanied by the appearance of new bands. Reduction in germination capacity, under storage conditions, can therefore be interpreted as an interaction of enzymes and metabolic substances essential for germination (Stanley and Linskens, 1964). Further, pollen on ageing in storage, may develop minor protein components which might obscure the clarity of electrophoretic separation. These factors singly or jointly might be responsible for increasing the number of protein bands. These findings agree with those of Bingham, Krugman and Estermann (1964). They proposed that repeated freezing and thawing during the storage period might contribute to the breakdown of proteins into various components. Mathur (1977) also suggested that pollen grains on storage, under normal conditions, lost their viability due to the auto-oxidation of their metabolites and the formation of toxic compounds.

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