

Microevolution of the photosynthetic temperature optimum in relation to the elevational complex gradient¹

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Balsam fir seedlings were grown under uniform conditions from seed collected along an elevational gradient in the White Mountains of New Hampshire. Photosynthetic temperature optimum of the seedlings decreased with increasing elevation of the seed source. The change in temperature optimum with elevation was similar to the adiabatic lapse rate, suggesting a precise adaptation to temperature through natural selection.

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Des plantules de sapin baumier ont été cultivées dans des conditions uniformes à partir de graines récoltées le long d'un gradient altitudinal dans les Montagnes Blanches du New-Hampshire. La température optimale pour la photosynthèse chez les plantules diminue avec l'augmentation de l'altitude de la provenance des graines. Le changement dans la température optimale selon l'altitude est semblable au gradient vertical adiabatique, ce qui suggère une adaptation précise à la température par sélection naturelle.

Introduction

Examples of the evolutionary adaptation of trees to their environment were reported in 1863 (de Vilmorin 1863; review by Langlet 1963). The later extension of uniform environment experiments to herbaceous plants by Turesson (1922, 1925, 1931) showed that genetic divergence among populations was the response adopted by many species in accommodating to spatial variation. The classic investigations by the experimental taxonomy group of the Carnegie Institution revealed ecotype formation within species complexes along transects having an altitudinal component (Clausen *et al.* 1940). However, sampled populations were usually separated by many miles even in the most recent work of the Carnegie Institution. Only a few studies have shown that distinct ecotypes can exist in very close proximity, but the examples are usually associated with edaphic environments of the most extreme kind in which no intermediate habitat exists (Jain and Bradshaw 1966; Squillace and Bingham 1958). A recent report that describes apparently continuous variation over a short distance along an altitudinal gradient deals with phenological and growth responses of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Hermann and Lavender 1968).

Materials

During October 1969, we collected seed of balsam fir (*Abies balsamea* (L.) Mill.) at each of five elevations, 2400, 2800, 3800, 4300, and 4800 ft (731, 853, 1158, 1311, and 1463 m) mean sea level (msl), along the southeast slope of Mt. Moosilauke in the White Mountains of New Hampshire. By using this transect, we managed to restrict the distance between the highest and the lowest subpopulations to only 2 mi (3.2 km). Seed was collected from five to eight widely spaced trees at each elevation but was bulked for the experiment, i.e., mother tree identities were not kept separate.

Balsam fir is an important continuous component of the vegetation from the spruce-hardwood zone below 2500 ft (762 m) to the alpine tundra above 4700 ft (1433 m). The occurrence of balsam fir varies from stands of well-formed dominants of up to 60 ft (18 m) in height at the lower elevations to a stunted *krummholz* at the highest elevations. Based on phenotypic variation, balsam fir in New England changes from purely var. *phanerolepis* at the higher elevations to an intermediate between var. *balsamea* and var. *phanerolepis* at the lower elevations (Myers and Bormann 1963). The morphological characteristics used in the varietal distinction vary clinally across elevational gradients (J. H. Fryer, unpublished data).

After seed germination, seedlings were grown in a greenhouse. After setting a dormant bud, the seedlings were chilled at 2°C for 14 days. Seedlings flushed and elongated when returned to greenhouse conditions. After 6 months of growth, the seedlings were again given a chilling period followed by a return to the greenhouse. Greenhouse conditions averaged 27°C day temperature and 23°C night temperature, and seedlings flushed within 2 to 3 weeks. In this fashion, three seasons of growth were simulated, resulting in seedlings which averaged 6.4 cm in height.

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Methods

Photosynthetic CO₂ uptake was measured 2 months after the termination of the last chilling period. Each sampled elevation was represented by two or three pots, each containing three healthy seedlings, i.e., there were six to nine seedlings for each elevation. All seedlings had set buds at the time of measurement. The seedlings were sealed in a circular, plexiglass cuvette with silicone rubber, and CO₂ uptake was determined for all three seedlings in a pot simultaneously.

Measurements of the CO₂ exchange rate were made with an infrared gas analyzer (IRGA) in a temperature-controlled system. Details of the system were described by Ledig and Clark (1972). Basic measurement was the rate of photosynthetic CO₂ depletion between ca. 330 and 280 ppm by volume CO₂ in a closed system. Air flow rate was kept at 3 liters min⁻¹ which, in the cuvette we used, corresponded to a minimum air speed of 0.4 m h⁻¹ (10 m min⁻¹) over the seedlings. The light source consisted of fluorometric lamps. Radiation flux density was 2000 ft-c at the level of the plant top.

To determine the temperature response of photosynthesis, the rate of CO₂ assimilation was measured at 0°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C. The order of measurement was 20°C, 15°C, 10°C, 5°C, 0°C, 25°C, 30°C, 35°C. Each 5°C temperature change was made over a ½-h interval, allowing gradual acclimation of the plants. Temperature refers to air temperatures in the cuvette.

After CO₂ exchange rates had been determined for each temperature, the needles, stem, and roots were oven-dried at 95°C and weighed. The rate of apparent or net CO₂ exchange was expressed in mg CO₂ h⁻¹ g⁻¹ dry weight of needle tissue.

Results and Discussion

Seedling dry weight generally decreased with elevation of the origin (Table 1). The negative correlation of seedling dry weight with elevation was statistically significant ($r = -0.97$, $P < .01$). The differences in seedling dry weight were not attributable to differences in seed weight; the correlation between seedling dry weight and seed weight at a uniform moisture content was near zero and not statistically significant ($r = -0.13$). The trend toward slower growth at high elevations may indicate that the dwarfism of trees in the *krummholz* has a genetic component.

TABLE 1
Dry weight of balsam fir seedlings by elevation

	Elevation of the seed source, ft				
	2400	2800	3800	4300	4800
Mean seedling dry wt., mg	313.2	334.2	233.5	194.2	181.9

The optimum temperature for CO₂ uptake was taken as the temperature at which the rate of CO₂ uptake was greatest. When the rate was nearly equal at two or more temperatures, the mean temperature was considered as the optimum. Photosynthetic CO₂ uptake of balsam fir seedlings showed a typical unimodal response to temperature, usually with a pronounced optimum at which net CO₂ uptake was most rapid. The temperature optimum for seedlings from 2400 ft (731 m) msl averaged 23°C while the mean for seedlings from 4800 ft (1463 m) msl was 17°C (Fig. 1). Within one elevation, the optimum varied by as much as 5°C between pots and the variability can be seen in Fig. 2.

While there was variation within elevations, a decrease in the temperature optimum with increasing elevation of the seed source was obvious (Fig. 2). Assuming a linear change with elevation, the regression equation is

$$\text{temperature optimum (}^{\circ}\text{C)} = 29.5 - (0.0027 \times \text{elevation (ft)}).$$

That is, there was a 2.7°C change in the temperature optimum for photosynthetic CO₂ uptake with every 1000 ft (305 m) change in elevation. The correlation coefficient ($r = -0.82$) is statistically significant at the .01 level of probability.

Climatic data were not available from Mt. Moosilauke, but a comparison was made with temperature conditions at nearby Pinkham Notch at 2000 ft (610 m) msl and Mt. Washington at 6262 ft (1909 m) msl. According to the mean daily maximum temperatures at these two stations for June, July, and August, there was a 2.4°C change in air temperature per 1000 ft (305 m) change in elevation (U.S. Department of Commerce 1964). By comparison, the dry adiabatic lapse rate is 3°C per 1000 ft (305 m) change in elevation (Taylor 1954). Comparing the rate of change of the temperature optimum with rates of temperature change with elevation is more logical than attempting to find some arbitrary temperature statistic to associate with each elevation. The similarity between the regression coefficient for photosynthetic temperature optima on elevation and the actual change in air temperature with elevation in the White Mountains or the dry adiabatic lapse rate is obvious.

Our results suggest that balsam firs at different elevations are precisely adapted by natural selection to the local temperature regime. If this is so, growth, and concomitantly survival and reproduction, must be closely related to photosynthetic CO_2 uptake. For growth, this is in fact the case. Dry weight (Table 1) of seedlings grown at an average day temperature of 27°C is well correlated with rate of CO_2 uptake at 27°C interpolated from the IRGA measurements ($r = 0.86$, $P < .05$).

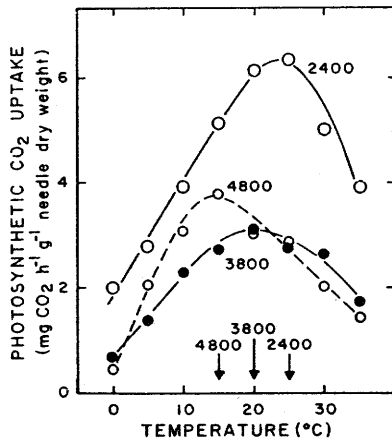


FIG. 1. Net photosynthetic CO_2 uptake of balsam fir seedlings from different elevational origins (2400, 3800, and 4800 ft msl) as a function of temperature. Curves are means for each elevation. Seedlings native to elevations between those shown had intermediate temperature optima (see Fig. 2).

The results must be extrapolated with caution. The temperature optimum is not absolute and will depend on the thermal history and radiant energy received by the plant. It is well known that radiation flux density has an effect on the position of the temperature optimum (e.g., Scott and Menalda 1970) and the present experiment was carried out at only a single light intensity. Also, the sequence in which the plant is exposed to a temperature series, the length of the period during which it is exposed to a particular measurement temperature (especially freezing temperatures as reported in Pharis *et al.* 1970), and the temperature under which it was grown (e.g., Strain and Chase 1966; Mooney and Harrison 1970), in short, its entire thermal history, all affect its rate of CO_2 uptake and the position of the temperature optimum. Though all these factors were controlled in the present experiment, they were not varied to determine their effect on the temperature optimum.

However, the effect of a different thermal history may not be large. Though growth under a different temperature regime may shift the temperature optimum, it should be noted that in the excellent work of Strain and Chase (1966), no more than a 10°C shift and often only a 5°C shift in temperature optimum occurred as a result of a 25°C change in the temperature of acclimation. The purpose of our experiment was to make valid comparisons of different populations under a uniform condition. The investiga-

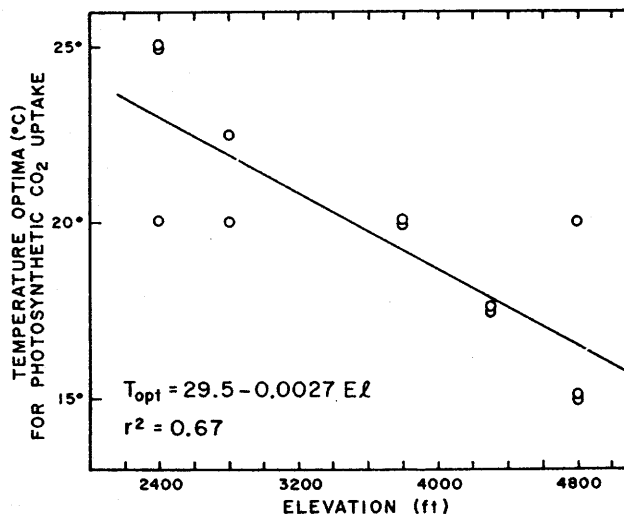


FIG. 2. Relationship of temperature optimum for net photosynthetic CO_2 uptake to elevational origin of balsam fir seedlings. Points represent determinations on individual pots.

tion has demonstrated genetic differences among populations in a potentially important physiological parameter, the temperature optimum for photosynthetic CO₂ uptake, under at least the restricted conditions of the experiment. Furthermore, the observed pattern of temperature optima was sensibly related to expected changes in temperature over the elevational gradient, inspiring confidence in the results.

No previous investigations present an unambiguous picture of the altitudinal pattern of photosynthetic temperature optima within a single species. Because they were designed for other objectives, previous investigations have compared only two extreme populations, have compared different species, or have confounded the elevational complex gradient with geographic gradients (Pisek 1960; Mooney and Billings 1961; Milner and Hiesey 1964; Mooney *et al.* 1964; Tranquillini 1964; Mooney and Shropshire 1967; and Duncan and Hesketh 1968). Some investigators have even felt that phenotypic plasticity through acclimation was sufficient to account for adaptation to the elevational complex gradient, and that constitutive adaptations of the photosynthetic mechanism, particularly to the temperature component of the gradient, were unlikely. To our knowledge, our study is the first to compare CO₂ uptake in plants originating from a continuous population located along a single slope and to conclusively demonstrate a gradual genetic change or cline in the temperature optimum with elevation.

It is of particular interest that subpopulations of an anemophilous species have diverged genetically even though there is a continuous chain of individuals between subpopulations. After all, deposition of conifer pollen in large quantity has been reported at up to 45 mi from forests (Lanner 1966). Either genetic migration is ineffective, perhaps because of phenological differences in flowering among elevations, or selection pressures are high enough to offset migration.

It should not be surprising to find that the elevational complex gradient is as potent a force for genetic divergence in the Appalachians as it is in the higher mountains of the West, where most studies of altitudinal variation have been conducted. In the East, three biomes may be

traversed in a few thousand feet of elevation (Bormann *et al.* 1970). The occurrence of fir in each of these biomes is partially dependent on its genetic capacity to evolve a continuum of races adapted to the temperature component of the elevational complex gradient.

Conclusion

Balsam fir seedlings showed a clinal pattern of variation in their temperature optimum for CO₂ uptake with respect to an elevational complex gradient. Apparently, there was a rather precise adaptation to the temperature component of the gradient. The change in temperature optimum was 2.7°C per 1000 ft while the dry adiabatic lapse rate is 3°C and the observed change estimated from weather stations in the White Mountains is 2.4°C per 1000 ft during the summer. Genetic divergence has occurred despite a lack of physical barriers to gene exchange.

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