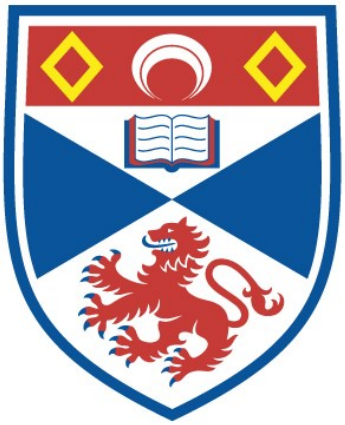
TEMPERATURE ADAPTATIONS IN PERENNIAL GRASSES FROM CLIMATICALLY CONTRASTING HABITATS

Albrecht Schneider

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1980

Thesis Abstract: Temperature adaptations in perennial grasses from climatically contrasting habitats. By Albrecht Schneider

This thesis investigates physiological mechanisms underlying temperature adaptations in perennial grasses. A comparative approach was employed by using pairs of species that have been collected from lowland sites in thermally contrasting habitats up to 2000 km apart on a north-south direction.

The population samples from northern latitudes, i.e. from cooler climates, had higher growth rates at 12°C than southern provenances.

Dark respiration rates between 5 and 25°C did not differ between provenances; but acclimatization at 10°C increased the respiration rate in all northern population samples, whereas some southern population samples reduced their respiration rate as a response to acclimatization at 10°C.

Northern provenances showed a greater activity of carbohydrate formation in light after periods of 48 hours starvation in darkness. This property could be attributed to the higher activity of RuBP-carboxylase in northern genotypes. No evidence was found that the higher activities are due to higher enzyme concentrations in these genotypes. The enzyme exhibited lower energies of activation in northern provenances in the range 5 - 15°C.

Activation energies for succinate dehydrogenase in isolated mitochondria were higher for northern population samples in the range 10 - 25°C.

Higher specific activities were found for malate dehydrogenase from northern plants between 5 and 25°C. In this temperature range the apparent energies of activation were lower for northern plants. The enzyme displayed positive thermal modulation in both northern and southern provenances.

Temperature changes had generally a greater effect on northern provenences.

The significance of these findings is discussed in the light of previous reports in the literature and in relation to a possible advantage in the plant's native habitat.

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A thesis presented for the degree of Ph.D. at the University of St.Andrews, February 1980

by

Albrecht Schneider

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Statement

I, Albrecht Schneider, was admitted as a research student of the University of St.Andrews in April 1977 in accordance with Ordinance General No.12 and the Resolution of the University Court, 1967, No.1. The thesis was completed in February 1980.

Declaration

I hereby declare that this thesis has been composed by myself, and that it is a record of work which has been done by myself. This has not been accepted in any previous application for a degree. Any other sources of information have been specifically acknowledged.

Signed

Certificate

I hereby certify that Albrecht Schneider has been engaged upon research from April 1977 onwards under my supervision to prepare the accompanying thesis for the degree of Doctor of Philosophy.

St.Andrews, February 1980 Signed

Prof.R.M.M.Crawford

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1. GENERAL INTRODUCTION

The generic term adaptation is used in a number of different senses. Went (1958) distinguishes three meanings of adaptation in relation to organisms: a) adaptation as the process of adapting to changing environmental conditions, b) as the state of being adapted through modifications of the organism to fit better to the conditions of the environment, and c) the ability of an organism to cope with the conditions of the environment. There are no fine lines of distinction in these adaptations, and in the present study of the adaptations of higher plants to temperature we shall be dealing with all three possibilities of plants to compensate for thermal conditions in their environment.

The strategies of adaptations of higher plants to the climatic conditions of their habitat can be summarized under three headings: phenology, morphology and metabolism. These forms of adaptation can occur as both spontaneous and reversible responses of plants to climatic conditions (Langridge and McWilliam,1967) and on the genetic level as selection for these conditions (Billings,1974 b).

The genetic variation has been shown in transplant experiments. Plantago maritima is known to show considerable variation in the life form in relation to its habitat. Gregor (1938) collected population samples within Great Britain from inland, island and coastal habitats and transplanted them to an experimental garden. Had the

variation only been of a spontaneous nature, then the differences in life form would have been expected to disappear after growth in one and the same environment. However, differentiation remained and was most marked for growth habit and size characters, thus demonstrating population differentiation of a hereditary nature which has taken place in response to the prevailing environmental conditions. Gregor concluded: "It is customary to regard the species unit as the convenient starting point for evolutionary discussion. Doubtless this practice has been favoured because the species, notwithstanding its numerous interpretations, does on the whole more nearly approximate to an evolutionary entity than do the less well-defined units of lower rank. The species and the variety, however, differ only in degree, and there is actually no valid reason why the latter should not also be employed for the elucidation of evolutionary problems."

The terms "ecotype" and "ecospecies" have been used by botanists and ecologists to indicate genetic difference below the species level. Increasing knowledge in genecology, intra- and interspecific variation and differentiation has made clear thar the evolutionary unit is the population and not the ecotype. This has led to proposals for a more elaborate terminology for the concept of habitat correlated genetically-based variation. Following Quinn (1978), we prefer the terms "populations", "differentiation", "variability among populations", "climatic or ecological races".

Many more transplant studies have been carried out since the early days of population ecology to elucidate genetical adaptation on the phenological (e.g. Hiesey, 1953; McMillan,1956; Langer,1972; Björkman et al.,1974; Clebsch and Billings,1976; Germaneau and Mathon,1976) and metabolic (Pisek,1973; WilliamsIII and Kemp,1976) level. Plants have been shown to adapt to their native habitats by adjustment of their germination, primary productivity and flowering time, in that their individual response to external conditions possesses survival value in the respective environment. The physiological plasticity of individual plants is genetically controlled and differs with the site of origin.

Plant responses to environmental parameters have been studied in the laboratory for many years, and we owe indeed the major part of our knowledge of plant adaptations to the last quarter of the century. For the fundamental ecological research genetically-based, metabolic adaptations to cold, i.e. arctic and alpine environments have been of particular interest. This attraction comes from the nature of these extreme environments with low temperatures and short growing seasons, and by investigating what enables plants to endure extremes of temperature and light intensity and to grow, flower and reproduce under these conditions it is hoped to gain an understanding of general strategies employed in the kingdom of plants to achieve fitness in their habitats. Studying metabolic adaptations to low temperature is moreover one

aspect which may be of practical use as regards the improvement of land use by selecting well-suited plant varieties.

Earlier works on physiological adaptations have been reviewed by Hiesey and Milner (1965). Respiration rate, rate of photosynthesis at different temperatures, light intensities at saturated photosynthesis, light compensation point and carbon dioxide compensation point have been studied in great detail. In many cases it has been possible to relate the physiological response in the laboratory experiment to the climatic conditions at the native site of the plant, and to suggest that interaction of the various factors favours the survival of plants at the site of origin. From many investigations we obtain a rather consistent though not universal picture for adaptations of plants to climatic conditions associated with low temperature. Patterns of population differentiation were found as altitudinal as well as latitudinal clines of variation. Plants of higher altitudes or latitudes possess lower temperature optima for net photosynthesis than plants from warmer climates, while their respiration rate is enhanced at low temperatures. Both properties favour growth in cool habitats.

The more recent literature has added further evidence as to the validity of the concept of metabolic adaptations to low temperature growth. Working with alpine plants from a range of different altitudes, Mooney et al. (1964) observed lower temperature optima for pho-

tosynthesis in plants from higher altitudes. Similar results were reported by Billings et al.(1966) and Slatyer and Ferrar (1977). Eagles (1967) worked with Lolium species from two geographical origins and found higher temperature optima for photosynthesis and lower Q_{10} values for respiration in the southern population than in the northern. Both features appear to adapt the plants to their respective climatic habitat. Pisek et al.(1969) and Moser (1969) compared arctic plants with plants from warmer climates and again found that arctic plants can assimilate at lower temperatures than plants from further south.

Differences in the light saturation of photosynthesis were also found to exist within different provenances of one species. Milner et al.(1959/1960) distinguished races of Mimulus cardinalis from different sites according to the light requirements to saturate photosynthesis. Solanum dulcamara, a species found in an unusually wide range of light habitats, has been suggested to have two distinct physiological races, one adapted to low and one adapted to high light conditions (Gauhl,1976; Clough et al.,1979).

Research into the adaptations of arctic and alpine vegetation has led to the conclusion that alpine populations possess higher phenotypical and metabolic flexibility than their arctic counterparts, thus reflecting the more fluctuating climatic conditions in mountains as opposed to the severe but comparatively constant and re-

gular conditions in the Arctic (Tieszen and Helgager, 1968; Smith and Hadley,1974). The subject was reviewed by Billings (1974 a) who lists 18 features of low temperature adaptation.

The literature on population differentiation in arctic and alpine environments is overwhelming. In studies of this nature separate plant populations have to adjust, on the one hand to varying seasonal temperature changes and on the other, to different diurnal temperature ranges. Comparison of arctic and alpine habitats is further complicated by the fact that both habitats differ in many parameters such as water and nutrient availability, wind speed, light intensity and day length. Moreover, these comparisons introduce a number of unpredictable variables in relation to microclimate which make it difficult to quantify the thermal expectations of the varying plant populations. It is therefore not surprising to find inconsistancies in the published data which make it hard to draw general conclusions. Certain authors obtain the results outlined above, namely that plants from cooler habitats have lower temperature optima for photosynthesis, while others find that photosynthesis is unchanged in its temperature response between different populations (Kemp et al.,1977). Some studies find respiration rate to be the most significant change (Stewart and Bannister, 1974) and yet others report that respiration rates alter only after periods of acclimatization (Billings et al.,1971).

The majority of investigations deals with interspecific rather than intraspecific differences. Such comparisons introduce a further unknown variable. Different species cannot *a priori* be expected to have the same responses to temperature and other factors as their phenological development may not follow the same pattern. But even given far-reaching phenological and morphological similarities, it should not be assumed that the physiological properties must then also be very similar. The tendency in many ecophysiological studies of using the data from a study of one species for comparison with others can lead to a degree of unjustified extrapolation.

Involvement of too many variables leads inevitably to vague conclusions since a number of different causes will be conceivable to explain an observed response. Ideally, to investigate genetical adaptations to one environmental parameter, plants of one and the same species should be chosen from sites that differ only in this parameter.

However, in practice this is clearly not possible. The present study attempts an approximation by avoiding some of the complications involved when comparing arctic with alpine, alpine with lowland plants and plants of one species with other species. The following approach has been made in this study of temperature adaptations.

1. Comparisons are made within species. Ecological races of the same species are well suited for comparative ecophysiological studies since they do not differ in as many structural features as do members of different species.

2. Gramineae were chosen as they provide a single life-form and at the same time a wide geographical distribution.
3. Plant samples were all of lowland origin. This is thought to exclude some of the microclimatic variability associated with alpine habitats.
4. Plants were chosen from areas up to 2000 km apart on a north-south direction. By choosing grasses that are able to colonise the cold areas of the north as well as the warmer areas of the south it is thought to deal with populations, which as a result of their geographical isolation are also genetically isolated and therefoer in many ways different.
5. Comparisons are not only made within one species from different origins, but with a range of different species. In this way it was sought to establish whether or not there is a general pattern of differences between northern and southern populations of these species of the grass family in their response to temperature.
6. No attempt was made to simulate climatic conditions at the site of origin of the plants. Plants of northern and southern provenances were on the contrary cultivated under identical conditions, and all experiments were done with the same conditions for all provenances. This practice is thought to eliminate short-term adaptations of the plants and to reveal only those differences that are genetically based.

It has been pointed out in the foregoing paragraphs that we are well supplied with studies into physiological processes in climatic races such as growth, respiration, photosynthesis, photorespiration and others. However, literature on the molecular basis of variability in populations is scarce. Hochachka and Somero (1973) have, on largely theoretical grounds, suggested a large number of possible mechanisms. This thesis concentrates on biochemical aspects of temperature adaptation. Experiments were designed to elucidate molecular mechanisms by which plants compensate for thermal effects.

Differential growth response of climatic races to different thermal regimes, as known from many examples in the literature, has provided a starting point. The growth experiments described in chapter 3 were done to establish whether there are differences in the growth response to different temperatures in samples from north-south isolated populations.

Subsequently, a number of measurements of the temperature dependence of dark respiration is described (chapter 4). There are conflicting data in the literature as to the significance of latitudinal separation of plants for their respiration rate in relation to temperature. The present experiments were to clarify if there is any pattern in the rate of dark respiration of grasses in relation to thermal conditions at their site of provenance.

The second part of chapter 4 deals with differences

in the potential of climatic races to acclimatize to ambient temperatures. The use of the terms acclimatization and acclimation has been suggested for the case that the pre-history of plant individuals, as far as one certain environmental factor is concerned, can cause tolerance or resistance to changes of this factor (Hoar,1966). Acclimation is the term for laboratory studies, whereas acclimatization is used for effects under natural conditions. A third level of compensation was distinguished and termed adaptation as an indication of phylogenetic evolution towards fitness in a particular environment. There is no consistent use of these terms in the literature, and to avoid confusion, we shall only speak of acclimatization as a specific form of adaptation - in this study adaptation to short-term changes in temperature conditions. To refer to intrinsic differences in population samples from isolated habitats, the term adaptation is prefered.

There is abundant literature on productivity and climate where the net photosynthetic gain is measured, either in the field or in the laboratory simulation of field conditions. Many studies have documented differential photosynthetic response in different climatic races. By contrast, in this investigation it was attempted to follow photosynthetic and productivity properties on the level of carbohydrates (chapter 5). These experiments were designed to determine the biochemical potential for carbohydrate accumulation in different provenances.

Consequently, measurements of ribulose-biphosphate-carboxylase were carried out to decide whether the previously observed differences between population samples could be explained, in part, by differences in the kinetic properties of the key enzyme of photosynthesis (chapter 6). An attempt was then made to determine if differences in the activity of that enzyme are due to different enzyme concentrations or to other reasons.

Chapter 7 follows an initial report in the literature on the adaptive significance of succinate-dehydrogenase. The enzyme is thought to play a key role as regulator of the Krebs cycle and oxydative phosphorylation in animals (May,1976). Its possible modification for the adaptation in plants was investigated.

About another key enzyme, malate-dehydrogenase, a regulator of oxidative decarboxylation, several examinations have been published. Its possible importance for adaptive strategies has been subject of conflicting results. Assays to determine kinetic properties of malate-dehydrogenase in grasses from climatically contrasting regions were here carried out to decide whether there is any consistent pattern of temperature response in that enzyme related to the temperature conditions at the original site (chapter 8).

The question:To what extent does variability between populations coincide with their fitness in the respective environment ?, lends itself to speculation.

In this field, where ecology and genetics are overlapping, too many factors are of importance to render possible statements about fitness from single observations. Moreover, there is the complication of extrapolating from laboratory experiments with few variables to the behaviour in nature with numerous variables, and, even more difficult, the extrapolation from in vitro to in vivo. It cannot be proven that one property measured in the laboratory contributes to fitness unless there is evidence that this property enhances survival and reproduction in a particular environment. However, if a common pattern of response can be found in a range of plant varieties, then it will not be assumed that this response is merely accidental. It is furthermore usually thought that in vitro measurements are meaningful in so far they reflect not differences between genotypes in absolute terms but do reflect the basic patterns of response. These complications will be borne in mind when in the final chapter the observed population differences will be discussed in relation to their relevance as a possible advantage of the plants at their native sites.

2. PLANT MATERIAL, CULTIVATION AND CLIMATOLOGY

There are considerable differences in the patterns of geographical distributions of higher plants. Endemic species are confined to limited areas, while other species are able to colonize wide ranges of latitude, longitude and altitude. The habitats of endemic plants are uniform for one particular species, whereas habitats of widely distributed plants differ in many climatic and edaphic features. It is in the latter type of plants that spatial separation has led to ecological differentiation which can be correlated with habitat differences (Heslop-Harrison, 1964). This selection for differentiated populations makes wide-range distribution plants an ideal object for the study of adaptation to a particular environment.

For the present investigation of temperature adaptations in perennial grasses, species have therefore been chosen with a wide geographical amplitude. It was not possible to work with the same population samples throughout the period of this research in all cases. Detailed lists of the species and provenances used in the experiments will be given in the corresponding chapters. A collection of individuals of the following species was used in most of the experiments: Festuca arundinacea Schreb., Lolium perenne L., Poa pratensis L., Holcus lanatus L., Deschampsia caespitosa Beauv. and Dactylis glomerata L. With the exception of Deschampsia caespitosa, which occurs in temperate and arctic regions, these species are distributed throughout Europe,

NW-Africa and temperate Asia (Hubbard,1976).

In order to have pairs of species which can be discriminated by intraspecific differences, plants were chosen for each species from two sites with a large latitudinal difference. For reasons discussed in the introduction, samples were all from lowland sites with no altitude exceeding 400m, the only exception being the Festuca arundinacea plants from Morocco which were collected at 1500m above sea level. Table 2-1 (page 15) lists for the species named above the provenances, the nearest meteorological stations in the publications of the Meteorological Office (1958 and 1967), the temperatures for the hottest month and the frost free period.

Habitats from contrasting latitudes differ not only in the seasonal pattern of their temperature regime, but also in their radiation flux (table 2-2).

Table 2-2. Mean daily heat sum received on clear days in various latitudes of the northern hemisphere (from Fairbridge,1967)

Latitude (degrees)	0	15	30	45	60	75
Heat sum ($cal/cm^2/day$)	510	510	470	380	300	220

Provided sufficient moisture is available, an increase in radiation flux leads at all temperatures to enhanced plant productivity (Lockwood,1974). However, radiation measurements are scarce, and it has therefore become customary to relate phenological features such as dormancy, growing season, flowering etc. simply to tem-

Table 2-1. Species, provenances, July mean temperatures and frost free periods at the site of origin of plants used in this study. Meteorological data from Meteorological Office (1958 and 1967). The data are mean values from at least 30 years of observation between 1915 and 1955. The only exception are the data given for Rome which are based on measurements during 9 years. July mean temperatures have been computed as 0.5 · (average daily maximum + average daily minimum) for July. As frost free period the number of months has been given in which the average monthly minimum temperature does not fall below 0°C. All temperatures are converted to °C.

Species	Provenances	Meteor.Station	July mean (°C)	Frost free period (months)
Festuca arundinacea	England	Oxford	16.9	5-6
	Morocco	Marrakech	28.9	12
Lolium perenne	Trondheim, Norway	Trondheim	14.7	4-5
	Lazio, Italy	Rome	24.4	8-9
Poa pratensis	St.Andrews, Scotland	Edinburgh	14.7	6-7
	Lazio, Italy	Rome	24.4	8-9
Holcus lanatus and Dactylis glomerata	Orkney, Scotland	Wick	12.5	4-5
	Lazio, Italy	Rome	24.4	8-9
Deschampsia caespitosa	Iceland	Akureyri	11.1	3-4
	Caenlochan, Scotland	Perth	15.3	4-5

perature. A mean daily air temperature of 6 °C has frequently been considered as the temperature threshold for plant growth, and on this basis it is possible to calculate the length of the growing season at different sites. These vary from 200-365 days for England to only about 125 days in Central Finland (Lockwood,1974). Long days can however compensate to some extent for the short growing seasons in northern latitudes.

Many plants used in this study are derived from maritime climates. This is particularly the case for those plants collected in England, Scotland and the Orkney Islands. It could be argued that these populations contrast poorly with their counterparts from warmer climates in Southern Europe because the accumulated temperature in the buffered, maritime regions of Northern Europe may not be very much different from that in the Mediterranean. However, fig.2-1 illustrates that there is a good linear relationship between the July mean temperature and the latitude of geographic origin. From the slope of the line a latitudinal temperature increment for the July mean can be calculated of about 5.5°C per 10 degrees decrease in latitude.

The statement of July mean temperatures is not particularly meaningful for temperate grasses in Southern Europe where plant growth ceases during the long summer dry period. A similar relationship as in fig. 2-1 can however be seen when the frost free period is plotted versus latitude (fig. 2-2).

The contrasting thermal conditions at four loca-

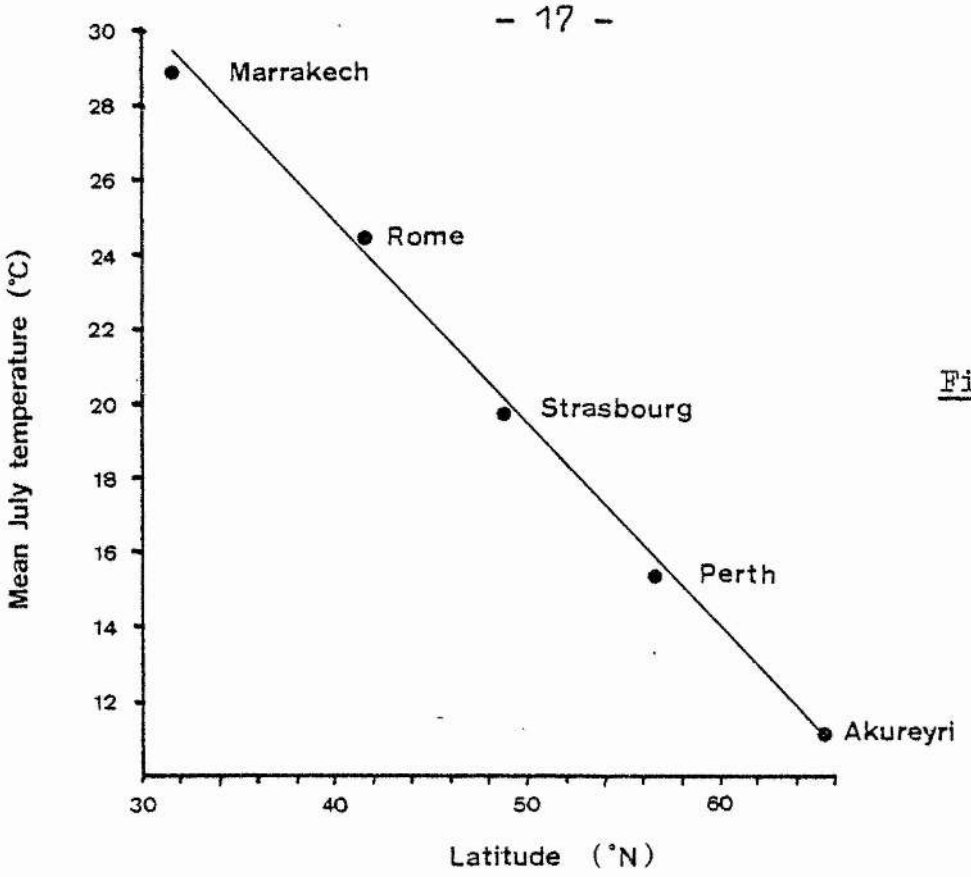
Mean July temperature (°C)
30
28
26
24
22
20
18
16
14
12
Marrakech
Rome
Strasbourg
Perth
Akureyri
30
40
50
60
Latitude (°N)

Fig. 2-1

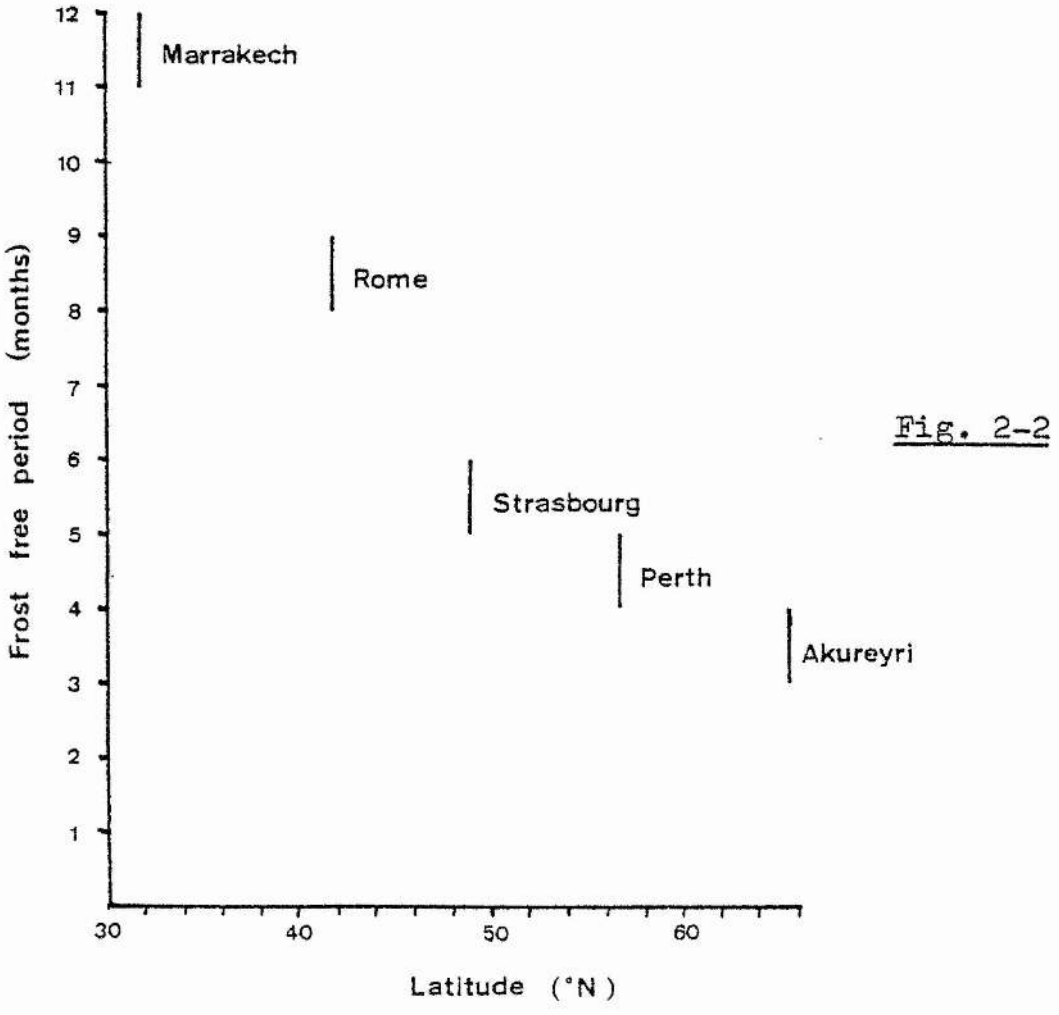
Frost free period (months)
12
11
10
9
8
7
6
5
4
3
2
1
Marrakech
Rome
Strasbourg
Perth
Akureyri
30
40
50
60
Latitude (°N)

Fig. 2-2

tions within the range of original sites used in this thesis are shown in fig. 2-3. Temperature amplitudes are smallest at the maritime site. Absolute maximum and minimum temperatures were 35°C and -16°C for Oxford but 35°C and -30°C for Trondheim.

In investigations into population differentiation, some authors prefer cloned material to ensure genetic uniformity. For the work reported here, clone material has not been used because it is not possible to select clones which are representative for the whole population. Plants were collected in the wild from within a homogenous area. Other plants were grown from seeds which were either collected in the wild or obtained from various Botanic Gardens. In every case, mature plants were pooled and planted in 25 cm diameter plastic pots with Levington compost as substrate. They were watered every two or three days with tap water and, once a month, with modified Hoagland's solution (Johnson et al., 1957).

The pots were kept in the glasshouse in natural daylight with additional illumination from high pressure mercury lamps to give a constant photoperiod of 16 hours day / 8 hours night. The temperature in the glasshouse lay between 18° and 22°C in summer and was maintained at approximately 18°C during the colder season. The light intensity in height of the plants was above 5000 lux. All plants have been growing under these conditions for at least three months before they were used for experiments.

Perennial grasses with a wide geographical distri-

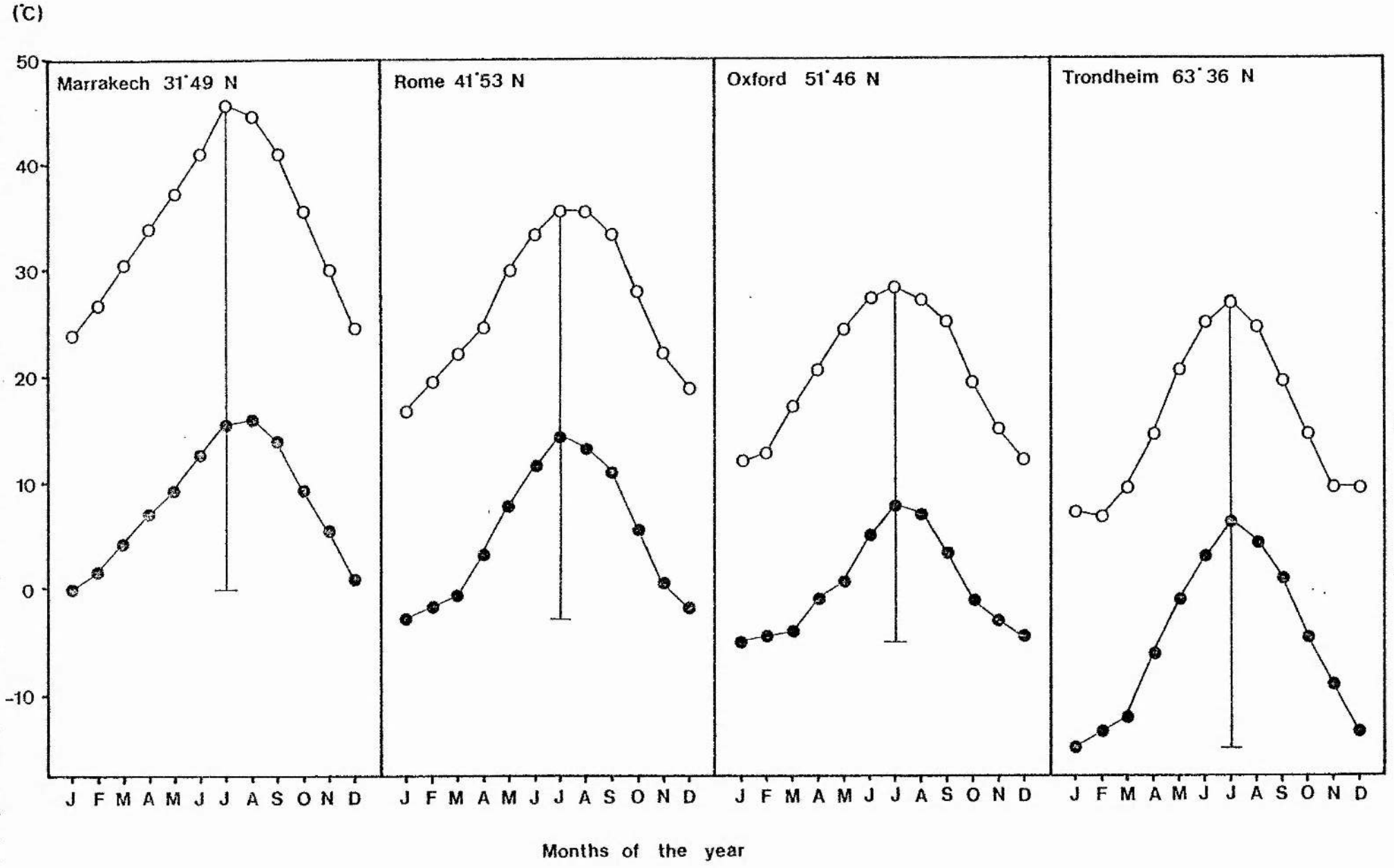

Fig. 2-3. Average monthly mean minimum (●—●) and mean maximum (○—○) temperatures at 4 sites

bution exist in numerous strains and climatic races (Hubbard,1976). Population samples used here were however morphologically very similar. The only noticeable difference in the physical appearance of species from contrasting latitudes was in the depth of their greenness in the leaves. Especially in Deschampsia caespitosa and Holcus lanatus the colour was slightly lighter in collections from warmer areas.

3. GROWTH RESPONSE IN DIVERSE POPULATIONS AT TWO DIFFERENT TEMPERATURES

3.1. Introduction

The existence of differences in the phenological development of plants in relation to climatic conditions is well established. Seeds of some grass species require diurnal temperature changes for germination, while seeds of other species germinate readily at constant temperature (Mayer and Poljakoff-Mayber,1975). Seed germination in late autumn and early winter and inhibition from germination in late spring by high temperatures, long photoperiods and low moisture has been described as an adaptation to the Mediterranean climate (Cruden,1974). Conversely, varieties restricted to cold montane or subalpine areas are winter dormant and germinate in spring (Tyler et al.,1978). Seeds of alpine populations germinate rapidly under a lowland temperature regime, whereas only few species of subalpine origin can germinate in a simulated alpine regime (Smith,1975). Under field conditions, the differential requirements of temperature, day length and moisture result in different times for growth and flowering for different ecological races and species. Clary (1975), working with populations of the grass species Sitanion hystrix, found that flowering was early for plants from warm and dry habitats but late for plants from moderate and humid climates.

Similarly, climate-related differences in verna-

lization requirements have been reported for grasses from contrasting latitudes. Tropical grasses were found to have no vernalization requirement, but in species from northerly latitudes flowering was initiated only after a cold treatment (Langer,1972). Similar findings were described by Hodgkinson and Quinn (1978) for the Australian grass Danthonia caespitosa from a latitudinal range of 11 degrees. Only the northernmost populations and hence the populations closest to the equator did not respond to vernalization.

For the same grass species, growth rates were found to be highest at low temperatures in populations from the north where summer temperatures are very high (Hodgkinson and Quinn, 1976). In 10 populations from different climates of Poa annua a similar response pattern was found (Calder,1973). Populations from Mediterranean climates showed lower growth rates at high temperatures than populations from cooler regions.

Differences in phenological features were interpreted as selection for plants adapted to their climate (Hiesey and Milner,1965). In this study measurements were undertaken to determine the growth response at two different temperatures. These experiments were to establish whether or not the plants responded in a pattern similar to that reported in the literature, and to add information on the level of plant growth to contrast with researches into metabolic adaptations presented in subsequent chapters.

3.2. Materials and Methods

Experiments were carried out with climatic races of *Lolium perenne*, *Dactylis glomerata* and *Holcus lanatus*. They were collected in the wild as seeds from northern and southern latitudes as indicated in table 3-1.

Table 3-1. Seed collection sites

Species	Provenance N		Provenance S	
Lolium perenne	Wales, U.K.	53°N	Lazio, Italy	42°N
Dactylis glomerata	Vaerøy, Norway	68°N	Lazio, Italy	42°N
Holcus lanatus	Fife, U.K.	56°N	Lazio, Italy	42°N

The seeds were kept in a refrigerator at approximately +5°C for one week to ensure sufficient germination. They were planted in plastic trays with Levington compost which was watered adequately with tap water to keep it moist. The trays were kept in the glasshouse at 18-20°C in natural daylight with supplemantary illumination from mercury lamps to give 16 hours daylength.

After about three weeks growth under these conditions, seedlings were selected of similar height and weight in the two-leaf-stage. Fife seedlings were used from each origin. These were weighed and then fixed in the central whole of polystyrene tiles which were placed onto glass cylinders. The glass cylinders (10 cm in height and 9.5 cm in diameter) were filled with half strength modified Hoagland's solution (Johnson *et al.*, 1957) which was renewed weekly. To prevent inhibition of root growth by light, the cylinders had a cover of

black polythene foil. The arrangement was such that the roots of the seedlings dipped into the nutrient solution.

The glass cylinders were then transferred into growth cabinets (Fisons Series II Growth Cabinets Model 140 G2) in which the temperature was maintained at constant 12±1°C and 20±1°C respectively. Hence, each cabinet contained fife replicates of each species and each site of origin in unaereated water culture. Each cabinet was lit with 9 white fluorescent tubes which gave a light intensity in plant height of about 14000 lux. The photoperiod was 14 h days/10 h nights. The nutrient solution in the glass cylinders was topped up daily with tap water to replace evaporation losses.

The seedlings were surface dried and then weighed, first after one week, then at intervals of three or four days. The measurements were terminated after three weeks. The plants were then divided into shoot and root which were weighed individually to determine the shoot/root ratio for each treatment and each climatic provenance.

3.3. Results

The mean fresh weights during the three weeks of the experiment for Lolium perenne, Dactylis glomerata and Holcus lanatus are shown in figs. 3-1,3-2 and 3-3 respectively. In Lolium perenne, the plants from the southern population show higher biomass production than the plants from the north at both temperatures 12°C and

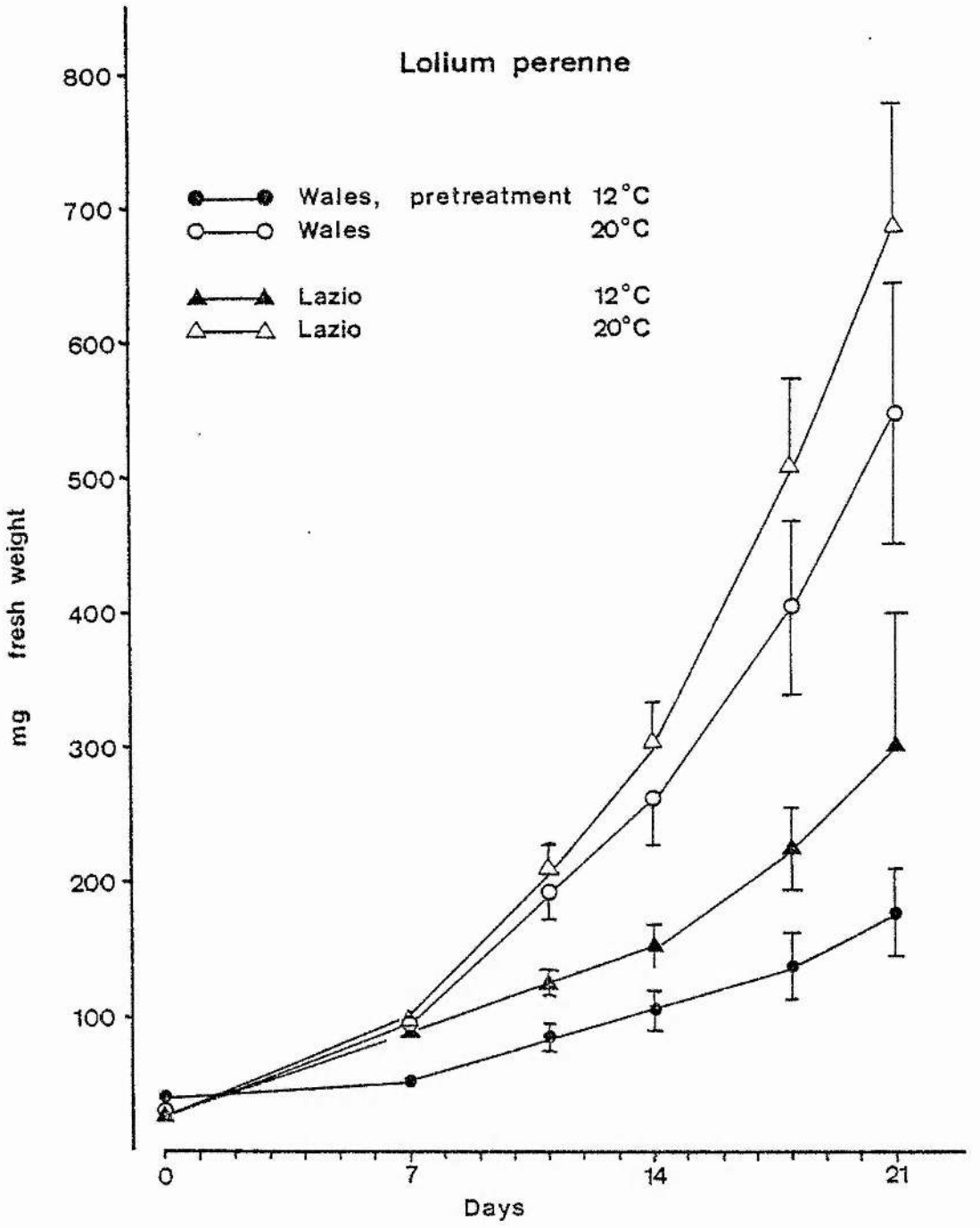

Fig 3-1. Growth of two climatic provenances of Lolium perenne at 12°C and 20°C.

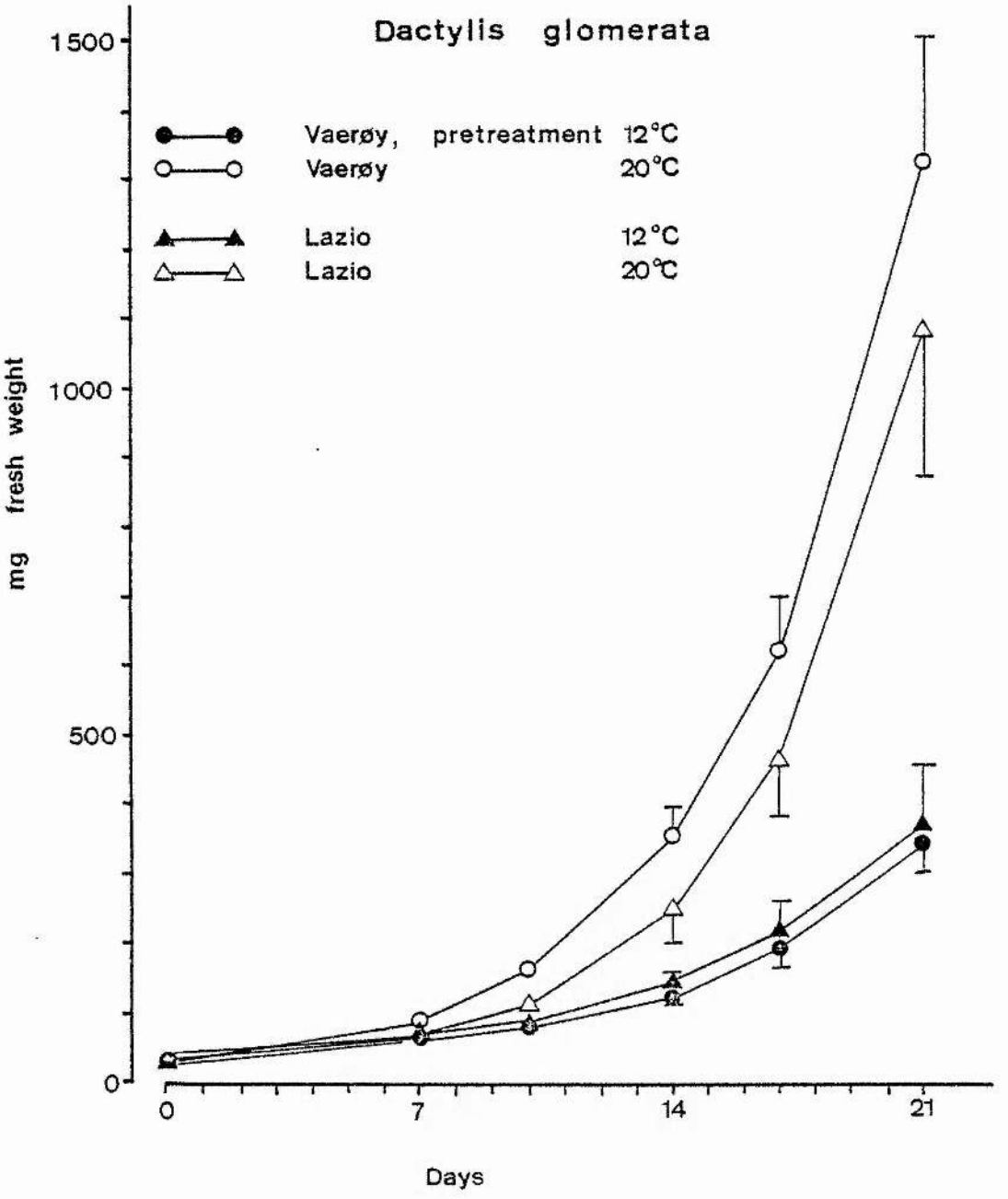

Fig 3-2. Growth of two climatic provenances of Dactylis glomerata at 12°C and 20°C.

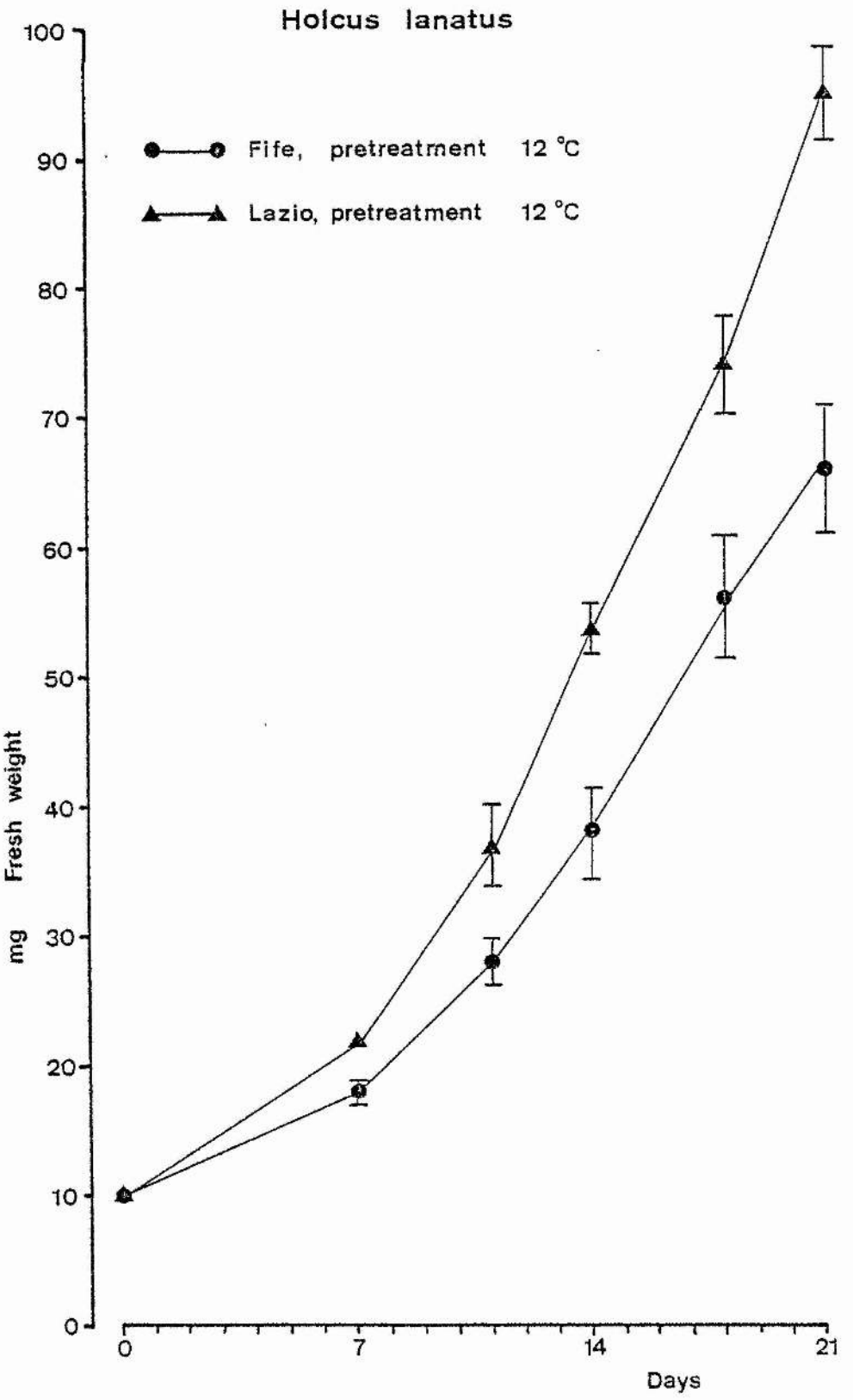

Fig 3-3. Growth of two climatic provenances of Holcus lanatus at 12°C.

20°C. In Dactylis glomerata, the plants from the south have higher productivity only at the lower temperature. Due to a lack of plant material, the experiment with Holcus lanatus could only be carried out in the low temperature cabinet. Like with the other two species, productivity is higher for the southern population at 12°C. The mean fresh weights recorded in this experiment and the total fresh weight gain in each species are given in tables 3-2, 3-3 and 3-4. The t-test shows that the higher growth rate of the southern population samples is statistically significant on the p=5% level for Lolium perenne and Holcus lanatus. Variability in the Dactylis glomerata samples has prevented the intraspecific differences from being statistically significant.

To give an estimate of the temperature influence on the rate of fresh weight gain, the ratio of total fresh weight increase at 20°C and 12°C was calculated from the values in tables 3-2 to 3-4 for each interval. These values could of course only be calculated for Lolium perenne and Dactylis glomerata and are shown in table 3-5. For both species, the values for the northern plants are constantly higher than for the plants from the south. This indicates, that the growth rate increases relatively more in the plants from the north when the temperature is raised from 12°C to 20°C.

Plants from a latitudinal gradient, thus acclimatized at different temperatures, are known to differ in their capacity for phosphate absorption. A negative cor-

Table 3-2. Mean fresh weights (FW) of Lolium perenne seedlings growing under controlled conditions

Days	Wales, U.K. FW in mg ± S.E.	Wales, U.K. total FW increase	Lazio, Italy FW in mg ± S.E.	Lazio, Italy total FW increase	
0	42 ± 2.2	-	32 ± 2.5	-	
7	52 ± 3.9	10	84 ± 5.3	52	
11	83 ± 9.3	41	124 ± 9.0	92	
14	103 ± 15.4	61	152 ± 14.6	120	
18	137 ± 25.2	95	224 ± 29.5	192	12°C
21	175 ± 34.4	133	301 ± 96.1	269	
0	31 ± 4.3	-	32 ± 3.4	-	
7	94 ± 11.9	63	101 ± 7.7	69	
11	192 ± 21.3	161	210 ± 16.4	178	
14	262 ± 34.0	231	304 ± 29.8	272	20°C
18	405 ± 65.4	374	510 ± 69.3	478	
21	548 ± 103.1	517	688 ± 96.6	656	

Table 3-3. Mean fresh weights (FW) of Dactylis glomerata seedlings growing under controlled conditions

Days	Vaerøy, Norway FW in mg ± S.E.	Vaerøy, Norway total FW increase	Lazio, Italy FW in mg ± S.E.	Lazio, Italy total FW increase	
0	36 ± 3.0	-	42 ± 6.4	-	
7	59 ± 8.0	23	68 ± 10.5	26	
10	79 ± 12.5	43	86 ± 11.3	44	
14	128 ± 22.0	92	147 ± 25.2	105	12°C
17	199 ± 32.5	163	220 ± 42.4	178	
21	354 ± 47.4	318	375 ± 87.3	333	
0	33 ± 2.3	-	31 ± 2.7	-	
7	90 ± 6.0	57	66 ± 8.0	35	
10	165 ± 13.0	132	115 ± 20.2	84	
14	361 ± 40.1	328	255 ± 52.6	224	20°C
17	625 ± 75.0	592	470 ± 85.0	439	
21	1331 ± 180.8	1298	1089 ± 209.4	1085	

Table 3-4. Mean fresh weights (FW) of Holcus lanatus seedlings growing under controlled conditions

Days	Fife, U.K. FW in mg ± S.E.	Fife, U.K. total FW increase	Lazio, Italy FW in mg ± S.E.	Lazio, Italy total FW increase	
0	10 ± 0.5	-	10 ± 0.9	-	
7	18 ± 1.0	8	22 ± 0.0	13	
11	28 ± 1.8	18	37 ± 3.2	28	12°C
14	38 ± 3.5	28	54 ± 1.7	45	
18	56 ± 4.8	46	74 ± 3.8	65	
21	66 ± 5.1	56	95 ± 3.8	86	

Table 3-5. Temperature influence on the fresh weight gain, expressed as the ratio of total fresh weight increase at 20°C and 12°C ($\frac{\text{tot. FW increase } 20°}{\text{tot. FW increase } 12°}$).

Days	Lolium perenne Wales, U.K.	Lolium perenne Lazio, Italy	Days	Dactylis glomerata Vaerøy, Norway	Dactylis glomerata Lazio, Italy
7	6.3	1.3	7	2.5	1.3
11	3.9	1.9	10	3.1	1.9
14	3.8	2.3	14	3.6	2.1
18	3.9	2.5	17	3.6	2.5
21	3.9	2.4	21	4.1	3.2

Table 3-6. Mean shoot/root ratios (± S.E.) after 3 weeks of growth under controlled conditions

	Lolium perenne Wales	Lolium perenne Lazio	Dactylis glomerata Vaerøy	Dactylis glomerata Lazio	Holcus lanatus Fife	Holcus lanatus Lazio
12°C	4.8±0.5	2.4±0.2	1.2±0.1	2.3±0.4	1.1±0.1	1.2±0.2
20°C	3.1±0.3	2.5±0.2	1.4±0.2	1.9±0.2	-	-

relation was found for marsh plants between their rate of phosphate absorption and the mean July temperature at their site of origin (Chapin,1974). On the other hand, the nutrient supply influences the growth ratio between different plant organs (Rogan and Smith,1975), and low nutrient supply was found to reduce the frost tolerance in grasses (Larsen,1978). It was therefore of interest to compare shoot/root ratios for the different provenances in this experiment at the two temperatures. For this purpose after three weeks of growth the weights of shoot and root were measured separately for each individual. The mean values for the fife replicates are indicated in table 3-6 (page 30).

No pattern was however found related to the geographic origin of the population samples. In Lolium perenne the fresh weight shoot/root ratio was consistently higher in the plants from the south, whereas in Dactylis glomerata and Holcus lanatus it was the northern plants which developed relatively more roots. Temperature had considerable influence on the shoot/root ratio in the northern plants of Lolium perenne but no influence on the southern ones. With Dactylis glomerata on the other hand, the temperature effect was greater on the Mediterranean plants than on the plants from Norway.

3.4. Discussion

Although the species showed considerable differences in their absolute fresh weight increment, two con-

sistent patterns were found in relative terms related to the geographic origin. First, all southern plants exhibited higher growth rates at 12°C as compared to 20°C; second, the temperature effect on the biomass productivity was greater in the northern populations than in the southern.

These experiments did not simulate natural conditions. All plants were grown at constant temperature and constant photoperiod. Furthermore, the water cultures used were not aereated, and it is known that aeration of the cultures improves plant growth. Still, Troughton (1972) has demonstrated that growth rates can be compared also in unaerated water culture with essentially the same results as in aerated culture solution. It is likely that the results found here do reflect adaptational differences. The greater growth rate of the plants from the Mediterranean at 12°C is in accordance with reports in the literature where similar results have been found for a range of Festucoid grasses (Cooper and Tainton,1968).

These findings do not permit any statement about the final plant weight or, in more agricultural terms, about the herbage yield. The optimum temperatures for growth do not differ greatly for northern and southern populations of temperate grasses and lie between 20°C and 25°C (Cooper and Tainton,1968). Southern populations will grow at temperatures closer to this optimum than populations from the north. Plant productivity may nevertheless be higher under lower tempera-

ture regimes because longer growing seasons can more than compensate for the reduced growth rate at lower temperatures (Rüegg and Nösberger, 1977).

The differences in growth response to temperature are interpreted as ecological adaptations to the native habitat. Low temperature germination (Thompson, 1970) and growth (Robson, 1965; Cooper and Tainton, 1968) is related to the cold season growing activity in the Mediterranean habitat and the necessity to reproduce before the summer months which may be extremely arid. The ability to germinate and grow at moderate or high temperatures would exclude species and ecological races characteristic of northern zones in a Mediterranean climate. The low temperature germination and growth found in Mediterranean races would suggest some chances for their establishment in northern climates.

Attempts have indeed been made to exploit the cold season activity of Mediterranean grasses for the improvement of pasture growth in the British Isles. From mid October till mid March, grasses scarcely grow in northern Europe and pasture growth is therefore highly seasonal. Mediterranean species were cultivated in Britain, which were capable of growth at temperatures below the 6°C-threshold and were thought to extend the seasonality of growth (Alberda, 1966) (see fig. 3-4). This strategy of land improvement had however to be abandoned. It turned out that cold season growing activity in the Mediterranean populations is

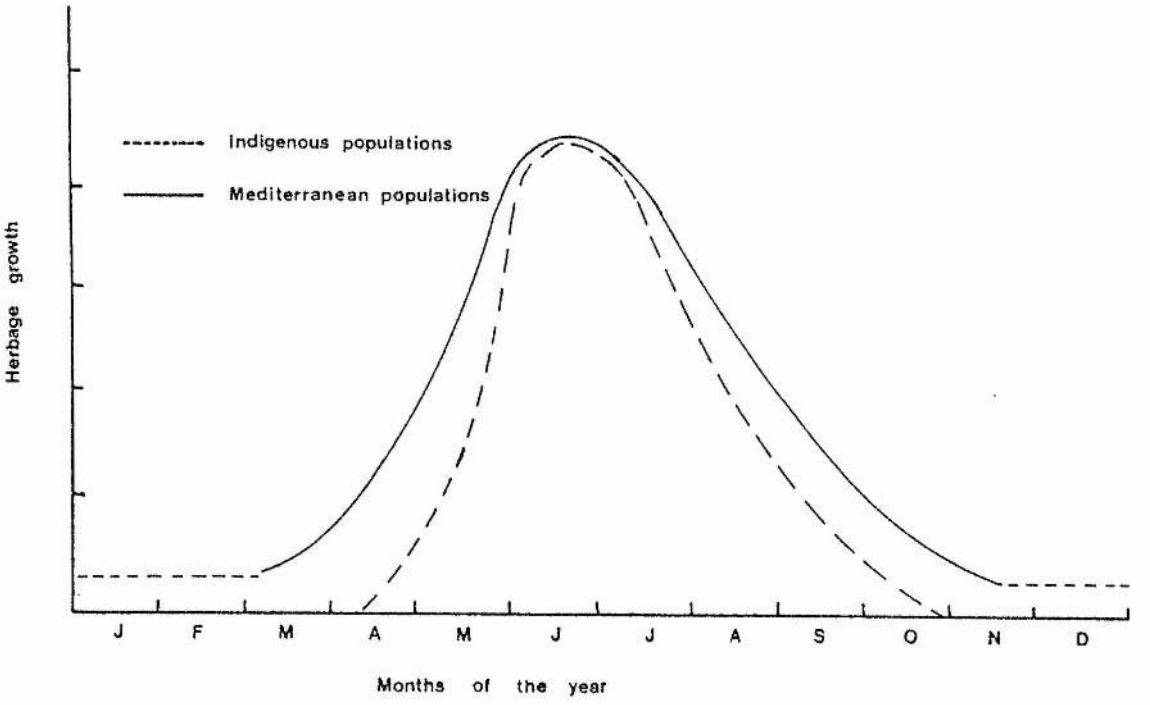

Fig.3-4. Schematic representation of the growth of indigenous and Mediterranean grasses in Britain. (After Ollerenshaw et al.,1978)

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assoziated with susceptibility to frost (Cooper,1963), whereas northern material becomes winter dormant but is frost hardy (Cooper,1964).

4. DARK RESPIRATION

4.1. Dark respiration in grasses grown under identical temperature conditions

4.1.1. Introduction

Attempts have been made to explain the differences between varieties in the effect of climatic conditions on vegetative growth. Chatterjee (1961), working with climatic races of Festuca arundinacea from Britain and N-Africa, has related differences in winter production to several morphological and physiological characteristics. He concludes, that winter growth in the N-African plants is due to an inherently greater physiological efficiency under winter conditions. Cooper (1963) suggested that variation in the rates of photosynthesis and respiration may account, in part, for the differences in vegetative growth.

The energy or chemical products of respiration are needed for many aspects of growth, e.g. nutrient uptake, cell division and cell expansion. Evidence has accumulated during the past three decades that plants from higher altitudes or latitudes frequently have higher respiratory activity than plants from lower altitudes or latitudes (Björkman and Holmgren,1961; Stewart and Bannister,1974; Ollerenshaw et al.,1976). This is thought to compensate for the lower temperatures experienced at higher altitudes or latitudes. It can be seen from fig. 4-1 (page 40) that plants adapted to high latitudes by higher respiration rates achieve the same respiration

rate at lower temperatures than plants from lower altitudes.

Comparative respiratory rates of ecological races of one and the same species are however available for relatively few plants. Measurements have therefore been undertaken to determine the rate of dark respiration with a range of species derived from populations from contrasting climatic regions. The respiration rate of roots which serve as perennating organs and places for carbohydrate storage may not be compared with that of deciduous leaves. Respiration rates of leaves and, with some of the plants, roots were therefore measured separately in a temperature range of respiratory activity.

4.1.2. Materials and Methods

The plants used to measure dark respiration in leaves and roots are indicated in table 4-1 (page 37). No specific pretreatment had been given to them previously. They have been growing under identical conditions in the glasshouse as described in chapter 2. Dark respiration was determined manometrically, using a Gilson Differential Respirometer (Model GRP 14: Gilson Medical Electronics, Wisconsin). For leaves and roots, ca. 0.5 g fresh weight was placed in each Warburg flask. With leaves, each Warburg flask contained 2 ml of 0.1 M dipotassium hydrogenphosphate - citric acid buffer at pH 5.4. Roots instead were moistened in this buffer and then transferred into Warburg flasks. Since respiration rates depend on the age of roots and

Table 4-1. Species and provenances used for the measurement of dark respiration rates in leaves and roots

Species	Resp. rate of leaves Provenances		Resp. rate of roots Provenances	
Anthoxanthum odoratum	Shetland, U.K.	60°N	Shetland, U.K.	60°N
	Peloponese, Greece	37°N	Peloponese, Greece	37°N
Dactylis glomerata	Orkney Is., U.K.	59°N		
	Lazio, Italy	42°N		
Festuca arundinacea	Sweden	61°N	England	52°N
	England	52°N	Portugal	38°N
	Morocco	32°N	Morocco	32°N
Festuca rubra	Lofoten Is., Norway	68°N	Lofoten Is. Norway	68°N
	Perth, Scotland	57°N	Perth, Scotland	57°N
Holcus lanatus	Orkney Is., U.K.	59°N	Orkney Is., U.K.	59°N
	Lazio, Italy	42°N	Corfu, Greece	39°N
Lolium perenne	Orkney Is., U.K.	59°N		
	Lazio, Italy	42°N		
Phleum phleoides	England	52°N	England	52°N
	Crete	35°N	Crete	35°N
Poa pratensis	Fife, Scotland	56°N		
	Lazio, Italy	42°N		
Poa trivialis	England	51°N		
	Peloponese, Greece	37°N		

aerial parts (Mogensen, 1977) and do even show diurnal fluctuations (Hansen, 1977), plants were uniformly harvested at 9.00 h in the morning. Only fully expanded leaves and thin root fibres were used. Leaves had to be cut into pieces of approximately 1 cm length; roots were used as intact systems. All Warburg flasks were covered with aluminium foil to exclude light, and im-

mersed in a water bath set at the lowest of six experimental temperatures (4°C or 5°C). At this temperature the samples were equilibrated for 1 hour before the measurements started.

After equilibration, readings of gas pressure changes were taken for between 45 and 60 minutes at each experimental temperature, depending on the respiration rate. After each set of readings the temperature of the water bath was raised and the respiration rate was allowed to stabilize for 40 minutes, before the next readings were taken. A shaking rate of 100 Hertz was used in all experiments. This frequency was found to be sufficient to ensure free gas exchange not impeded by diffusive resistance.

At the end of each experiment, the plant material from each Warburg flask was oven dried at 95°C for 12 hours to determine its dry weight. The results were converted to normal conditions (25°C, 760 mm Hg) by multiplying the values for gas exchange (in $\mu l \cdot h^{-1}$) with a correction factor k after the following formula (Umbreit, 1957):

$$k = \frac{298\ (P_b - 3 - P_w)}{(t+273) \cdot 760}$$

where P_b is the atmospheric pressure in mm Hg

P_w is the vapour pressure of water at the experimental temperature t

t is the temperature of the water bath

In order to be able to express respiration rates

both in terms of oxygen uptake and CO_2 output, carbon dioxide evolution was measured by the difference method, using flasks with and without 0.3 ml of 5 % potassium hydroxide solution absorbed on filter paper in the central well. However, no fundamental differences were found to exist between the two measures. Nearly all RQ-values were between 0.8 and 0.9, independent of species and origin. Oxygen uptake rates showed less fluctuations than the gas exchange rate resulting from oxygen uptake and carbon dioxide release. For these reasons, only oxygen uptake rates (Q_{O_2}) are presented here and expressed as ml per g dry weight and hour. All values are means of at least three replicates.

4.1.3. Results and Discussion

Rates of oxygen uptake at 6 different temperatures are shown in figs. 4-1 to 4-9 for leaves and in 4-10 to 4-14 for roots. Only the plants from two populations of Festuca rubra differ significantly in their respiration rate, equally for leaves and roots (figs. 4-1 and 4-10 respectively). In all other examples, respiration rates of leaves are equal or very similar. In roots there is a greater difference between climatic races. However, it can be seen that the respiration rate of roots is generally lower than that of leaves. The measuring error is therefore higher. Moreover, there appears to be no pattern as to the climate at the site of origin. The samples of Festuca rubra (fig. 4-10) and Phleum phleoides (fig. 4-14) respire faster in genotypes from the

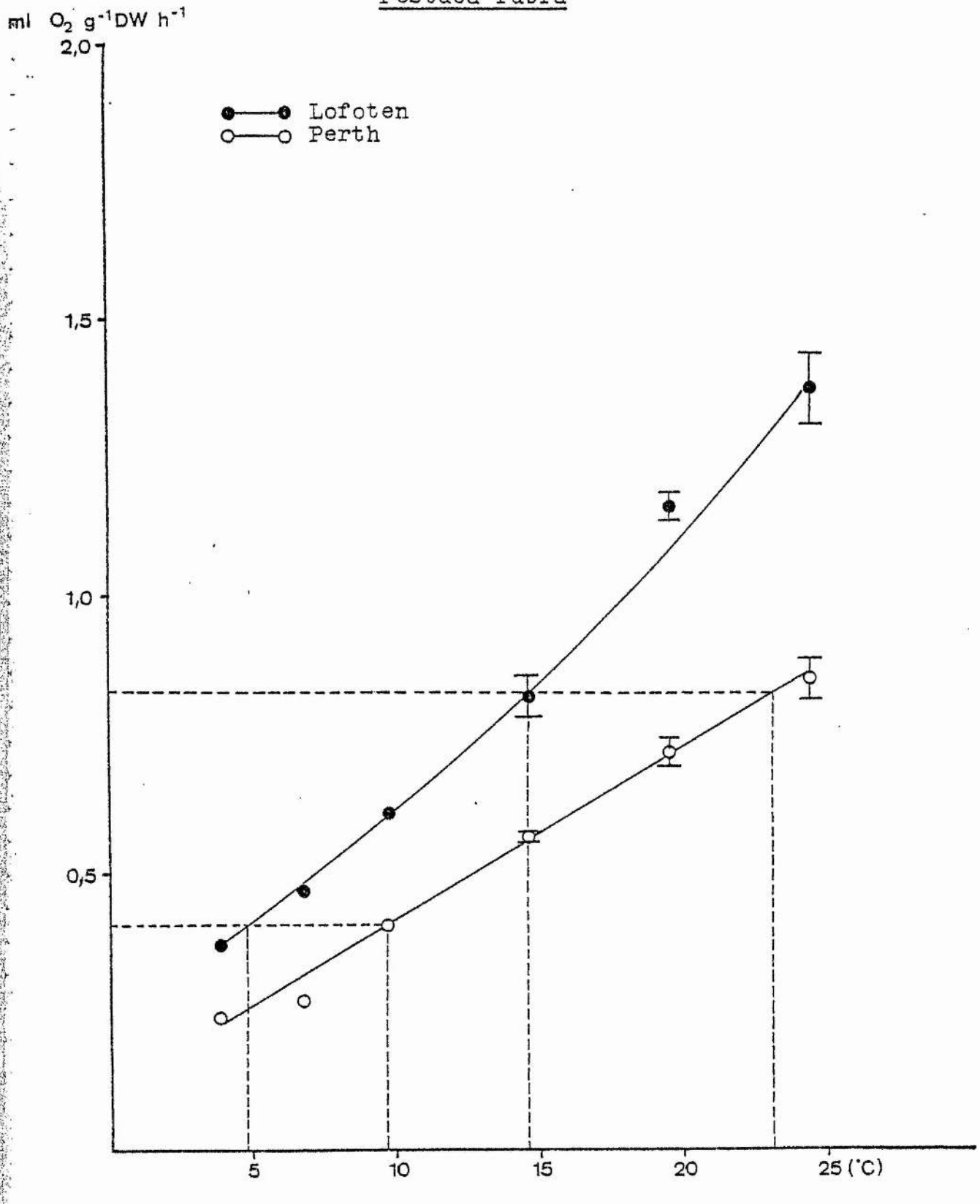


Fig.4-1. Dark respiration rates of leaves of two Festuca rubra population samples

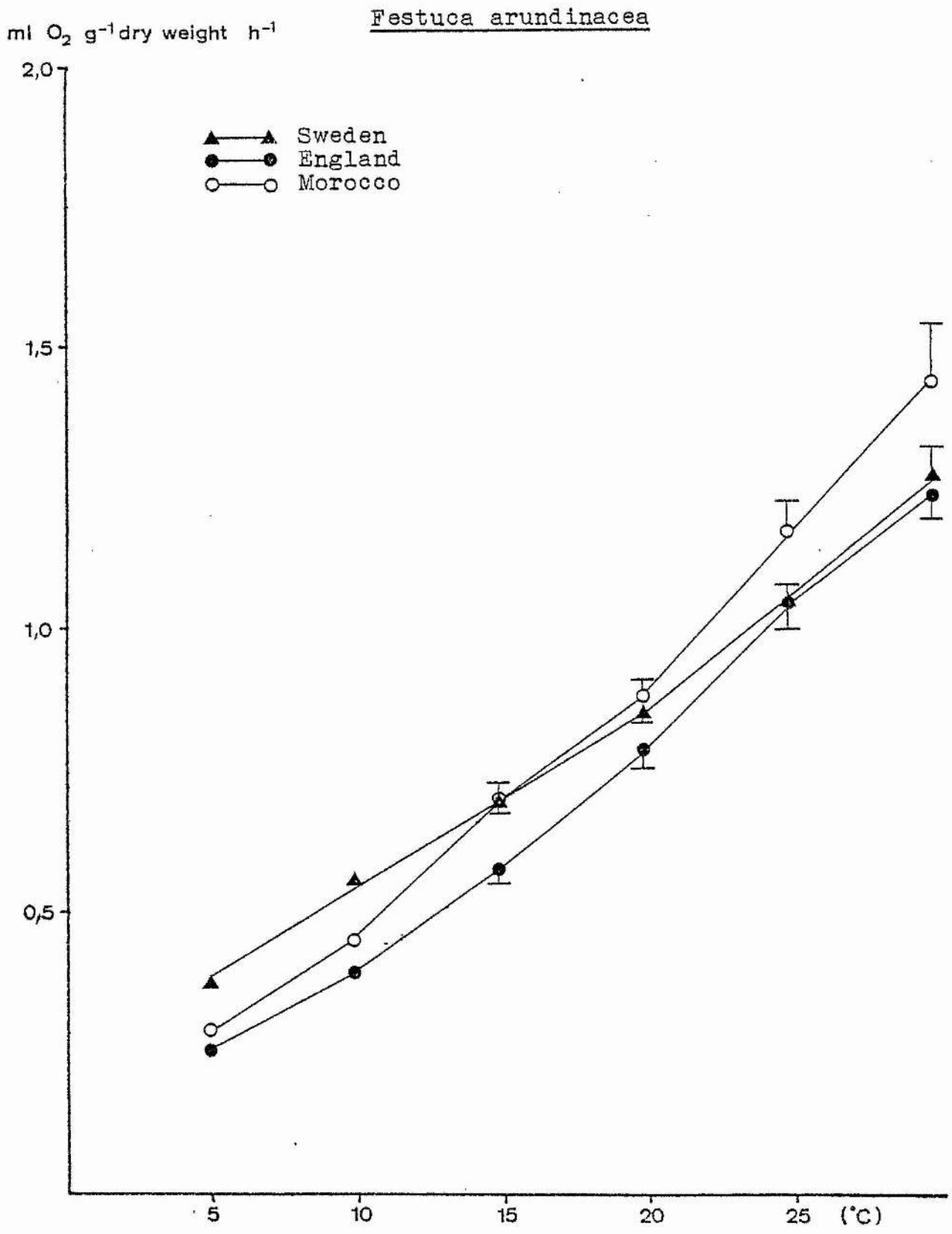


Fig.4-2. Dark respiration rates of leaves of three Festuca arundinacea population samples

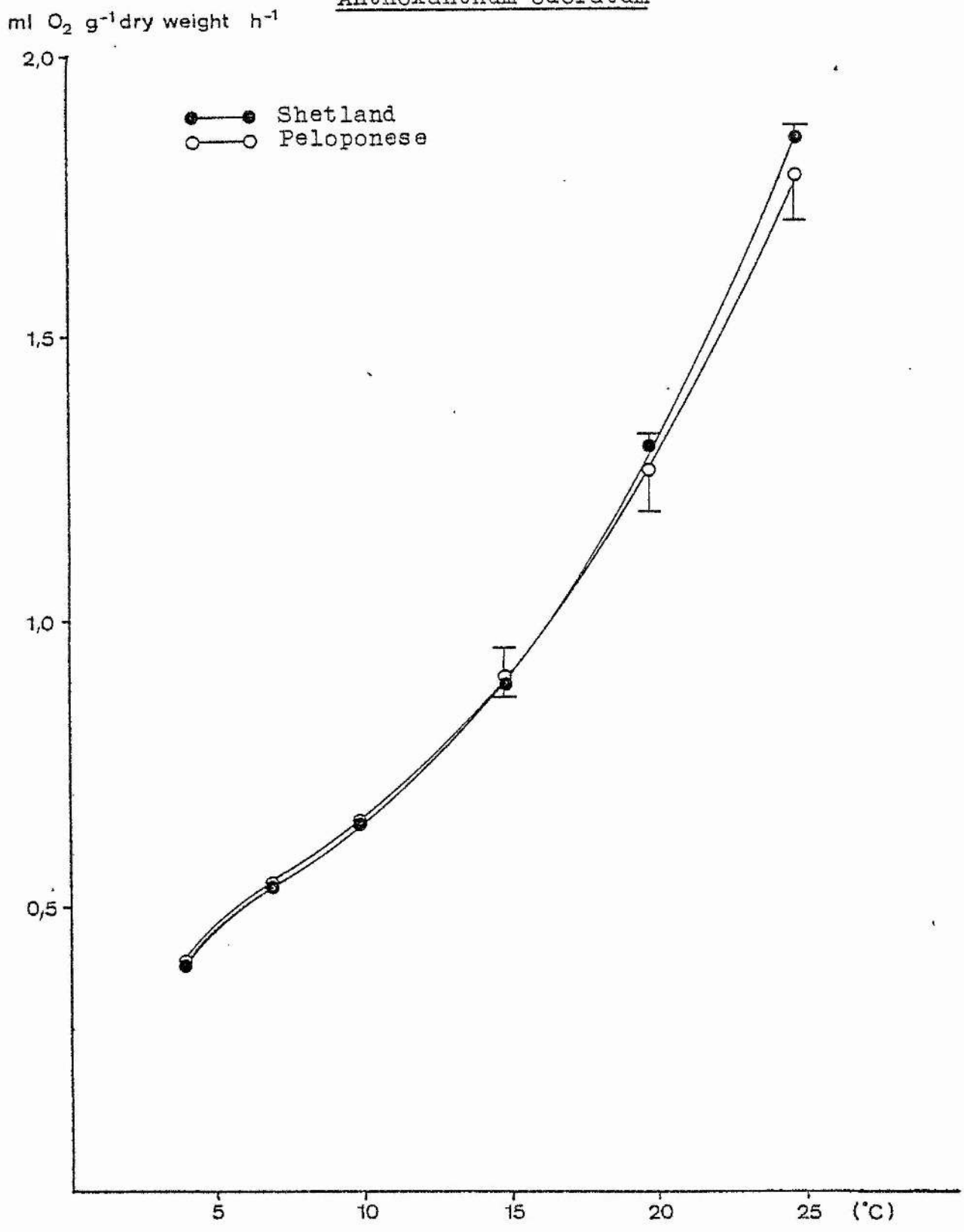


Fig.4-3. Dark respiration rates of leaves of two Anthoxanthum odoratum population samples

Dactylis glomerata

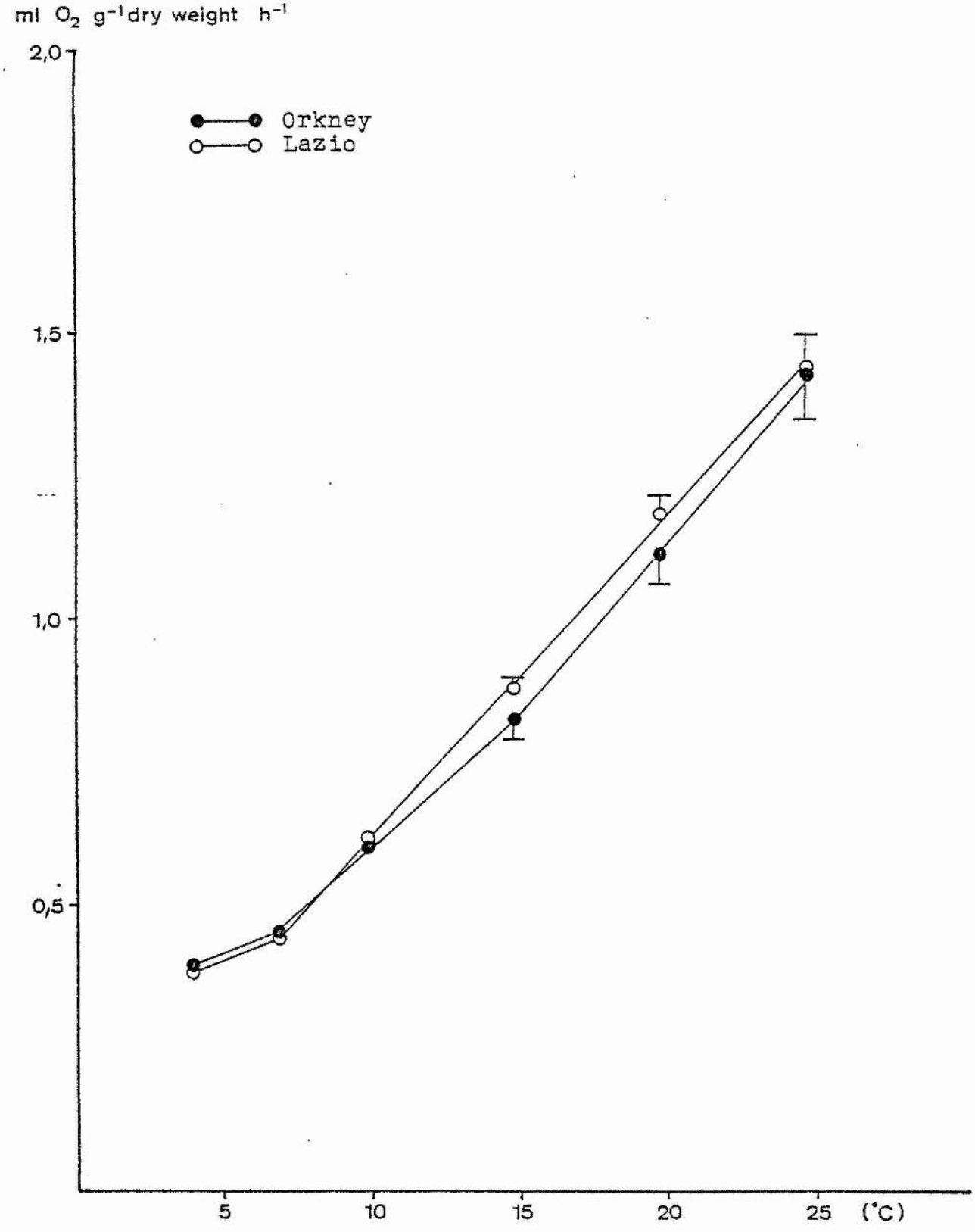


Fig.4-4. Dark respiration rates of leaves of two Dactylis glomerata population samples

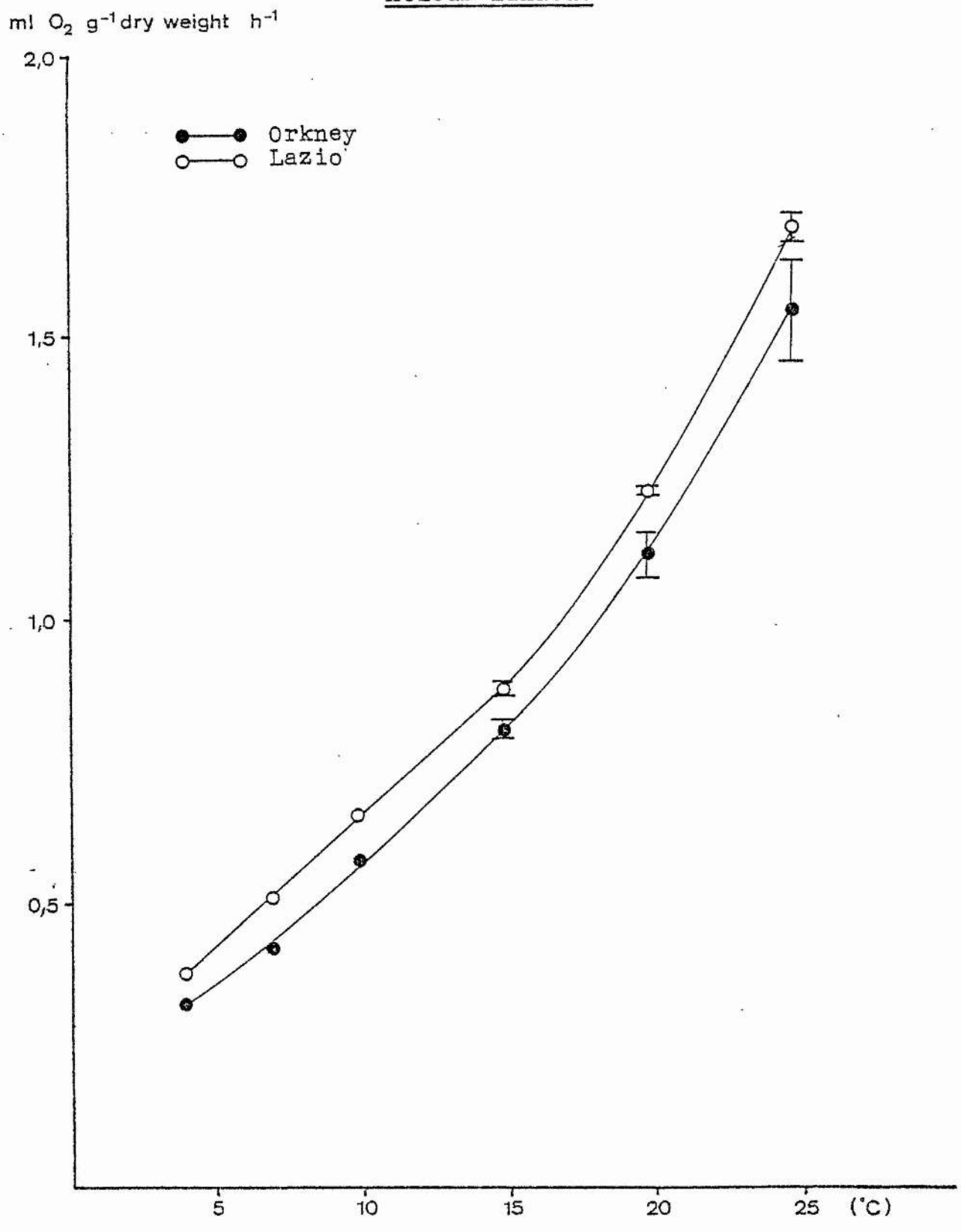


Fig.4-5. Dark respiration rates of leaves of two Holcus lanatus population samples

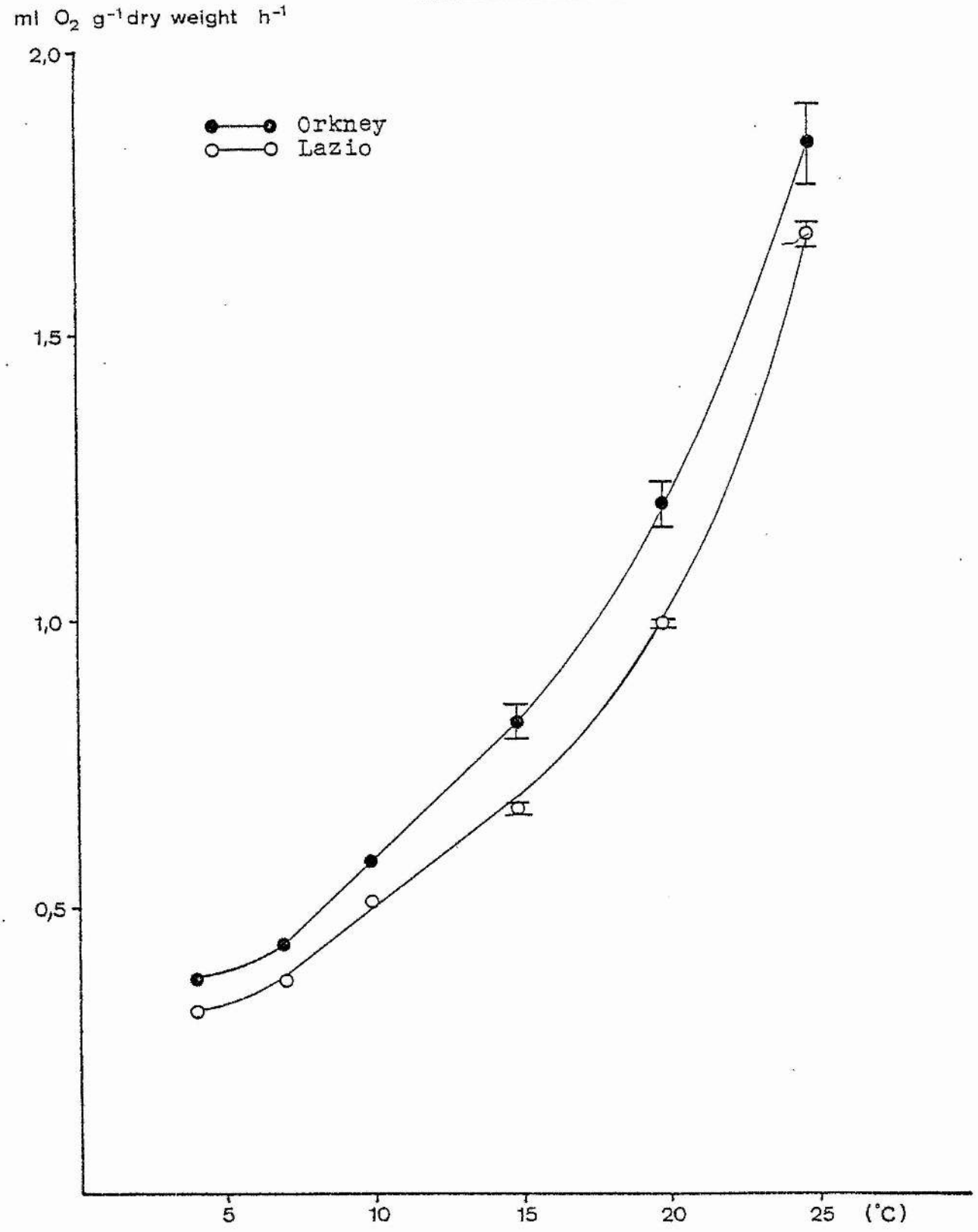


Fig.4-6. Dark respiration rates of leaves of two Lolium perenne population samples

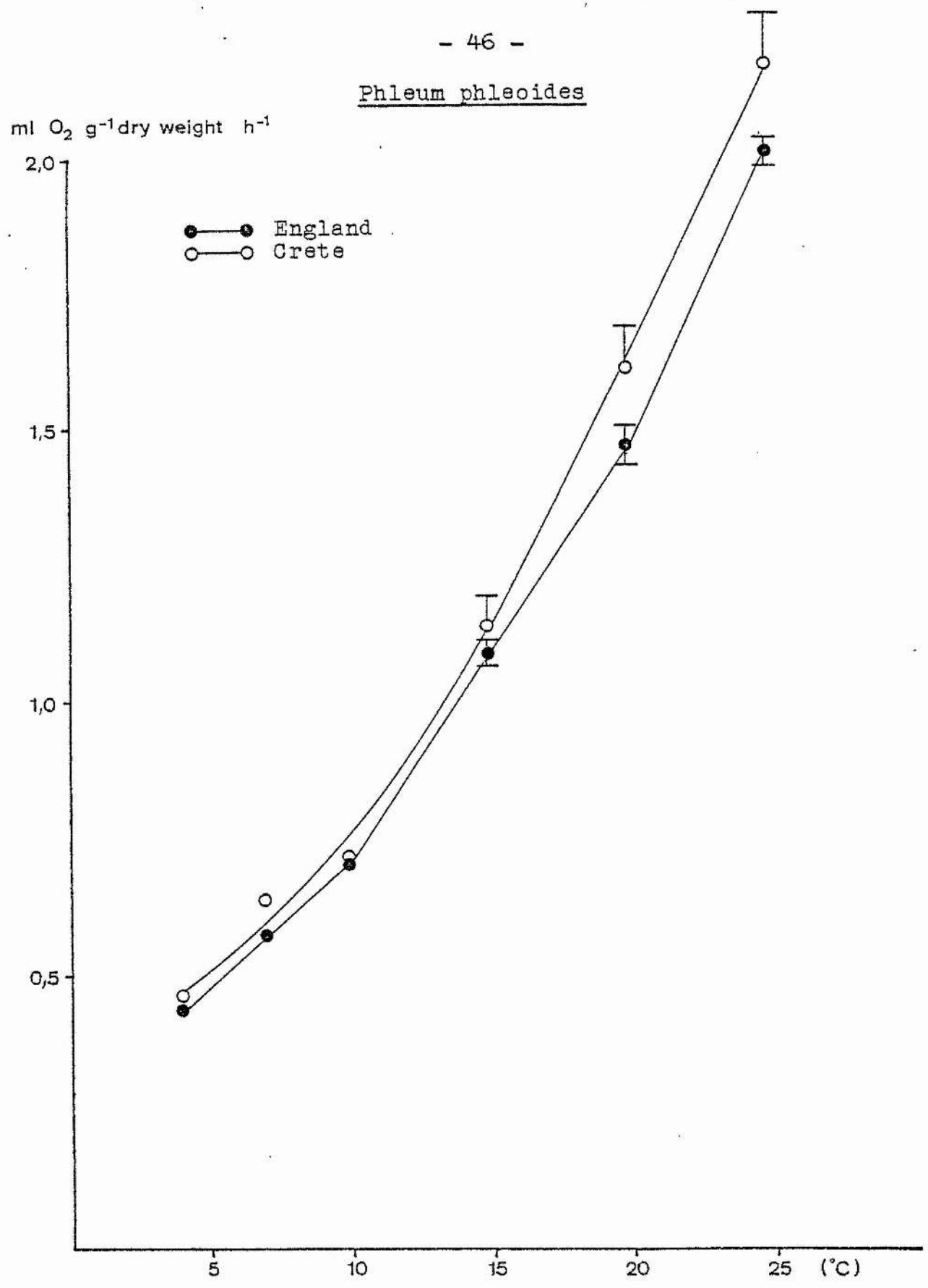


Fig.4-7. Dark respiration rates of leaves of two Phleum phleoides population samples

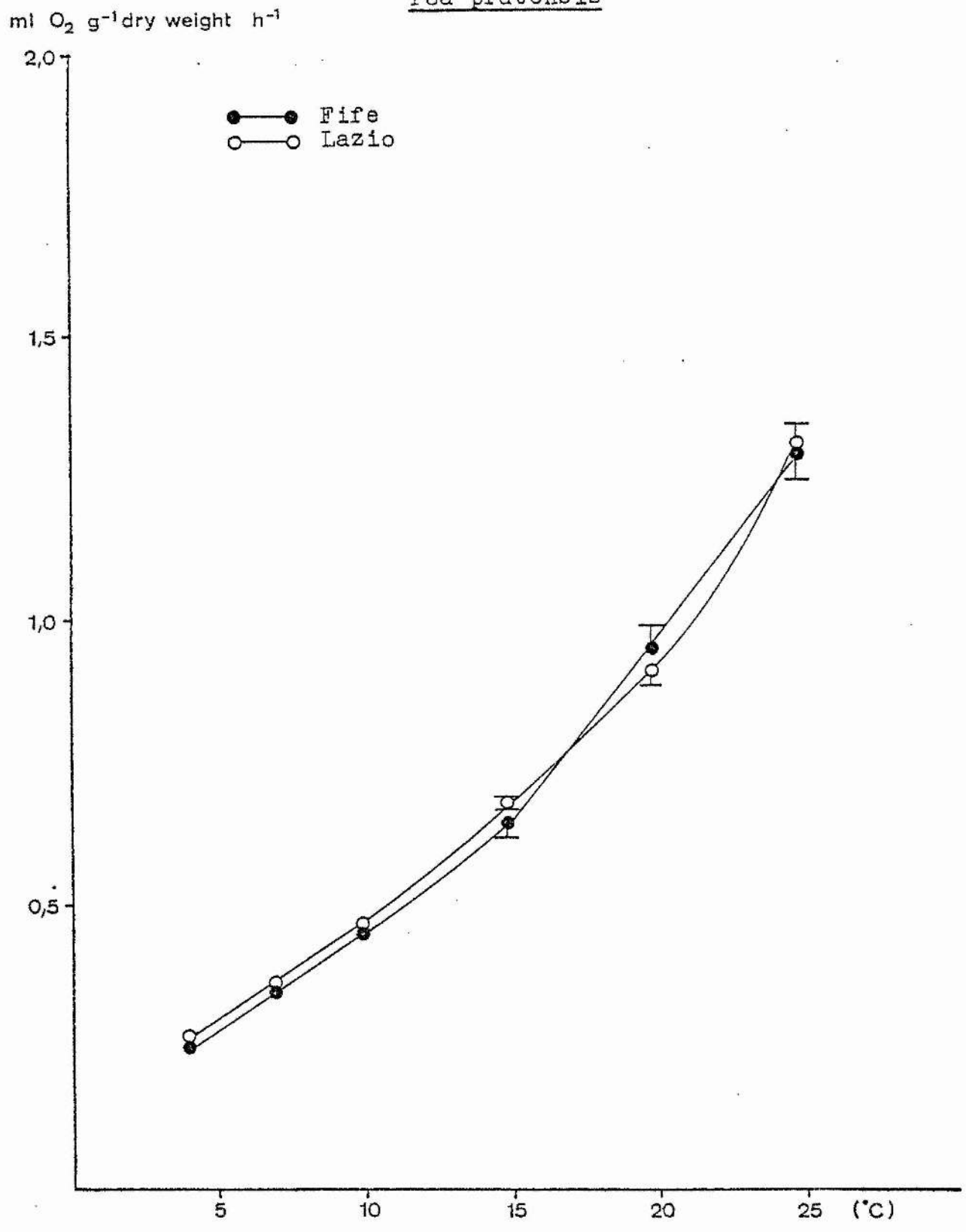


Fig.4-8. Dark respiration rates of leaves of two Poa pratensis population samples

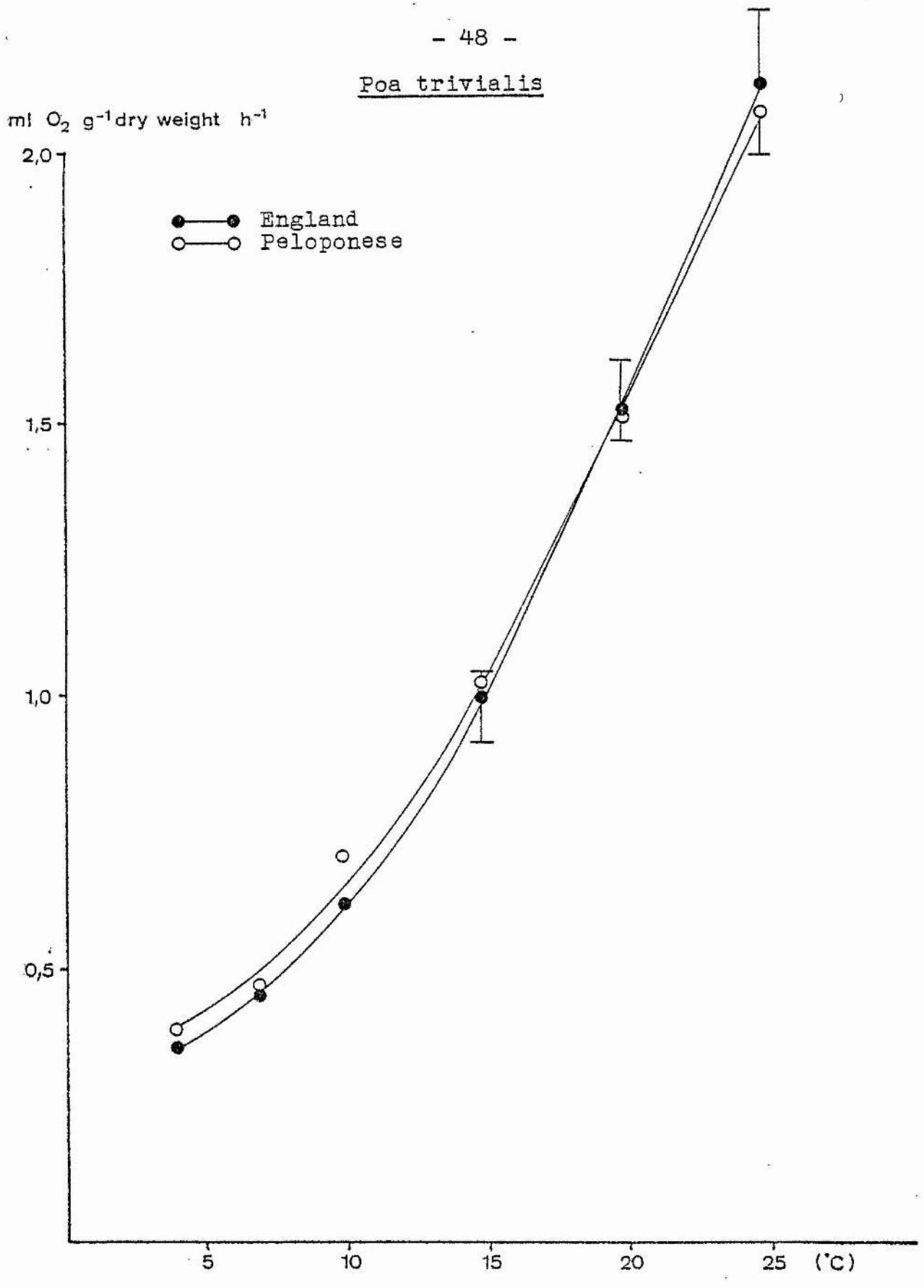


Fig.4-9. Dark respiration rates of leaves of two Poa trivialis population samples

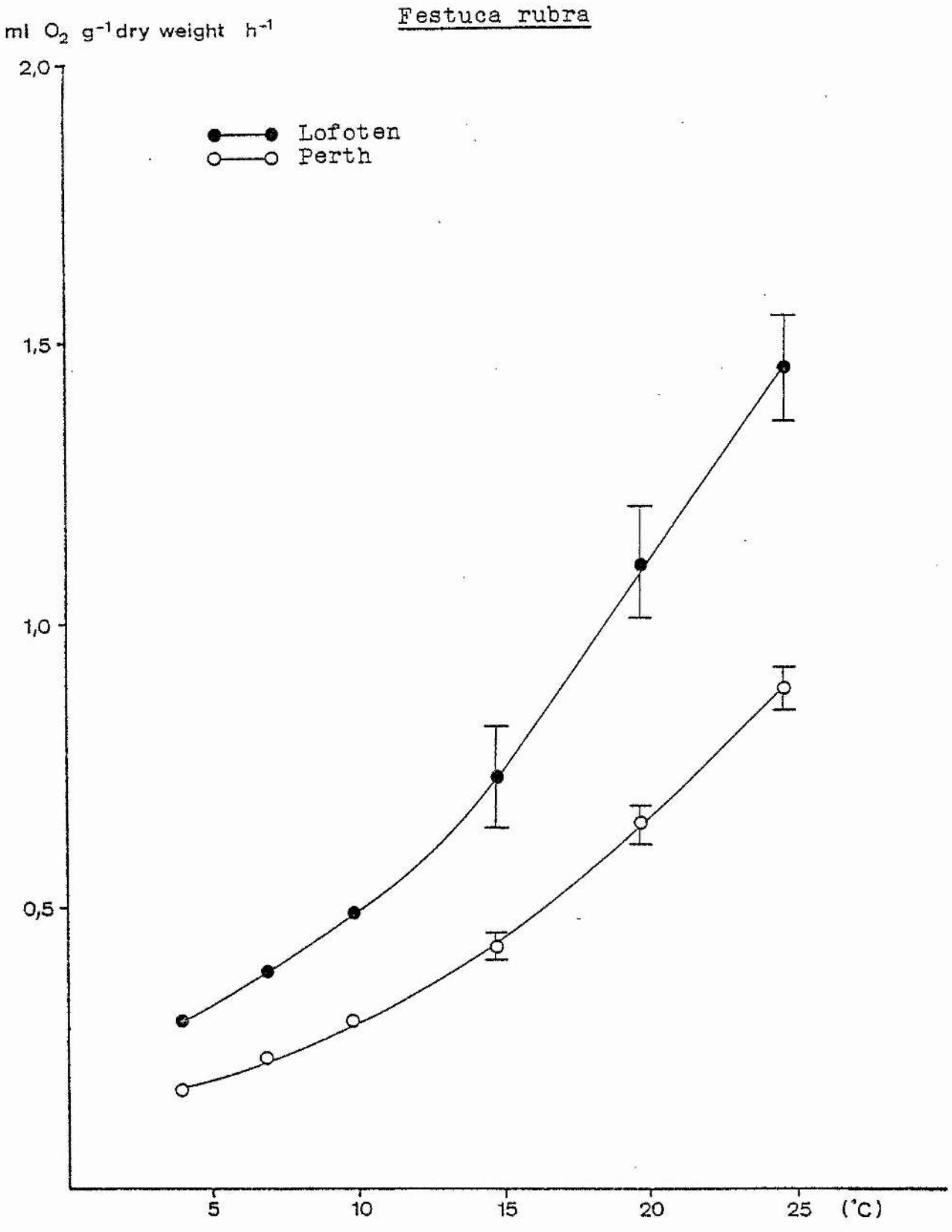


Fig.4-10. Dark respiration rates of roots of two Festuca rubra population samples

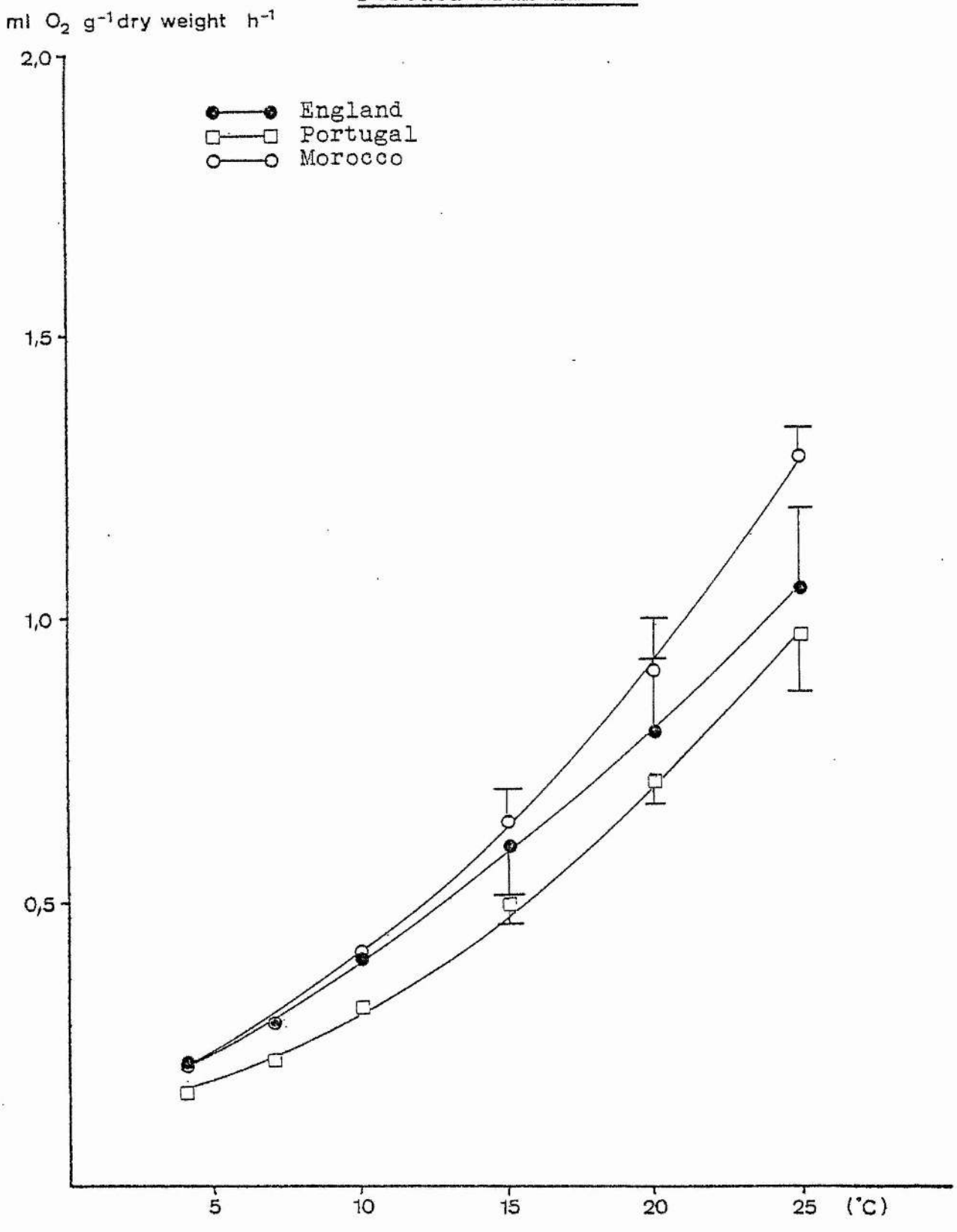


Fig.4-11. Dark respiration rates of roots of three Festuca arundinacea population samples

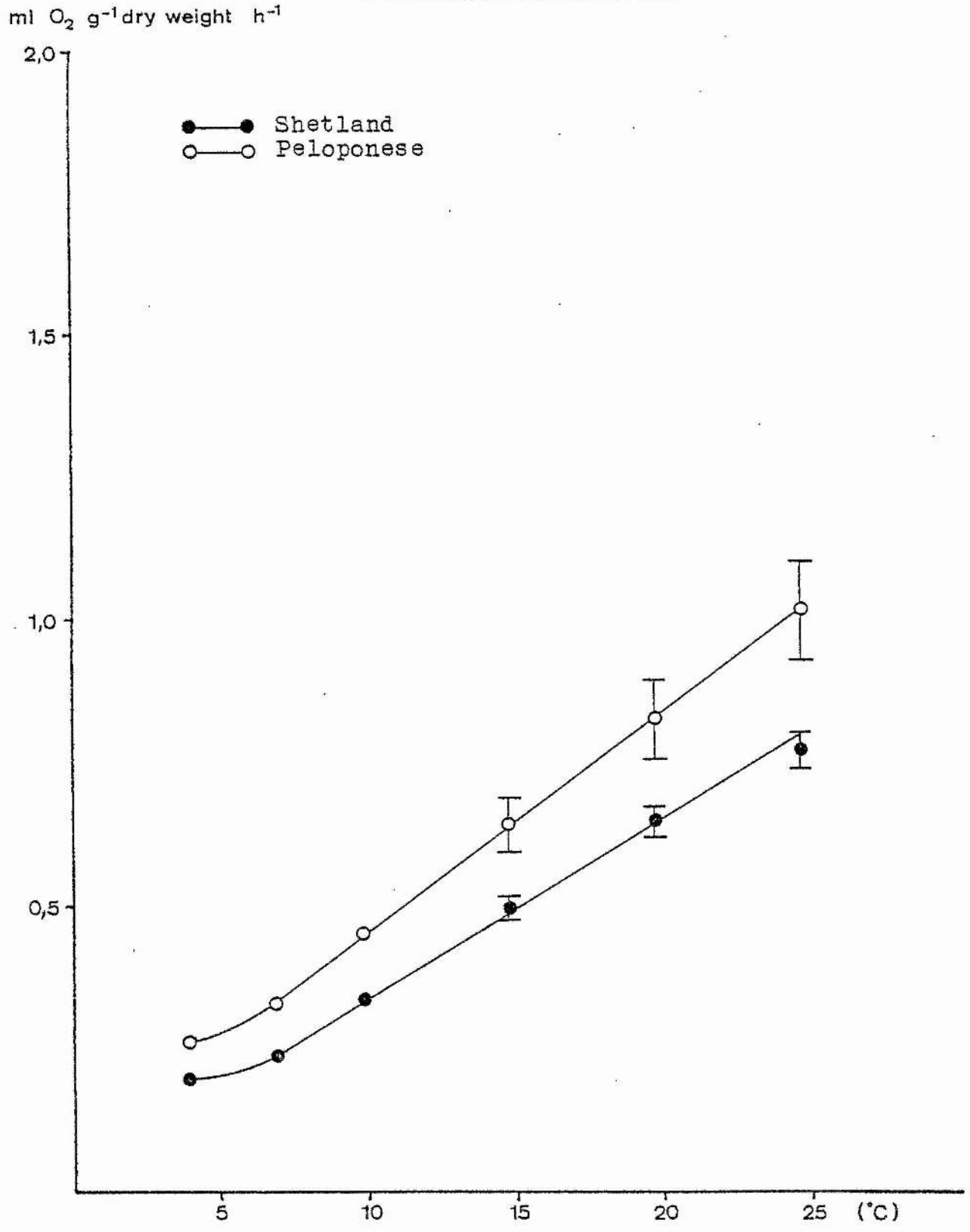


Fig.4-12. Dark respiration rates of roots of two Anthoxanthum odoratum population samples

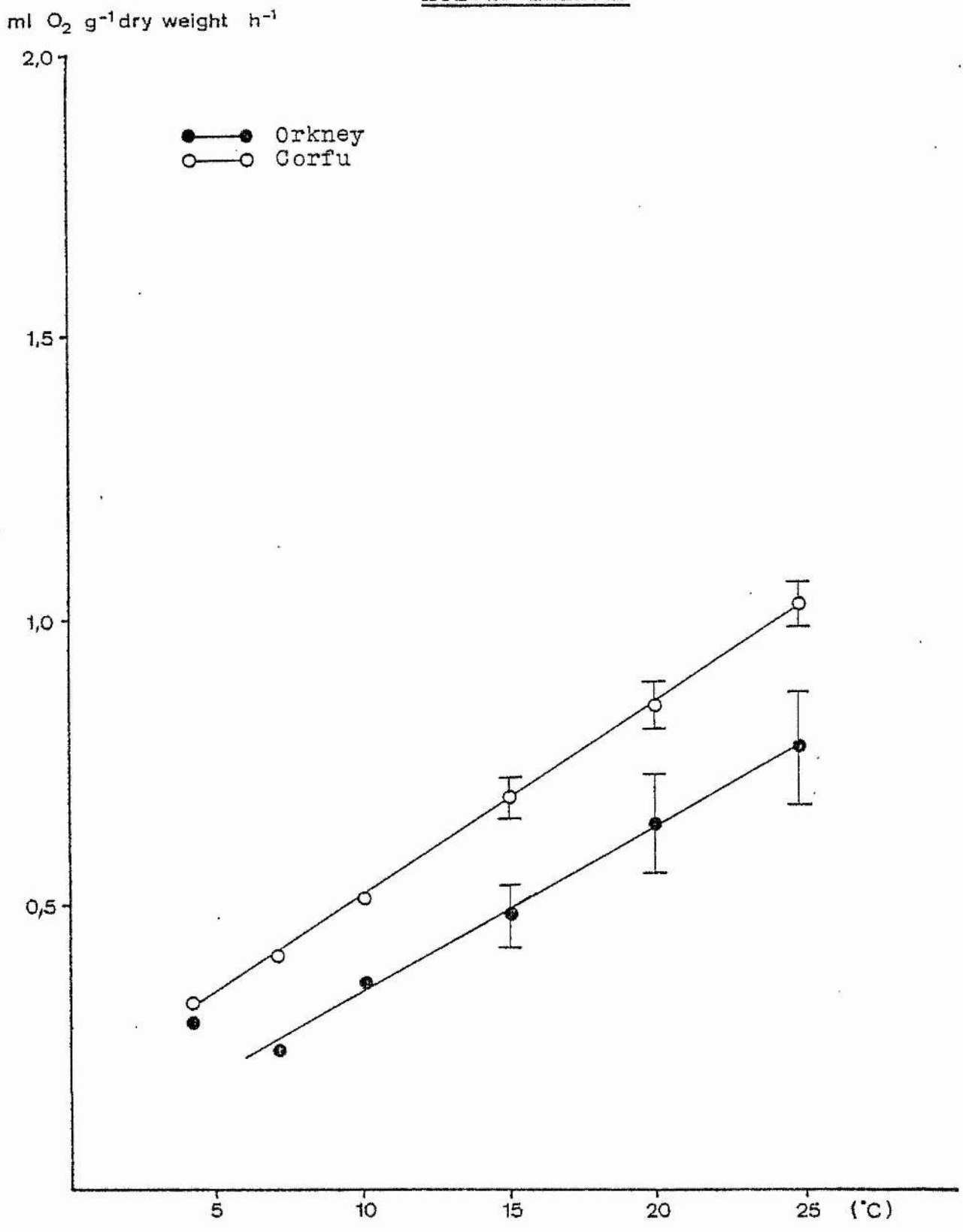


Fig.4-13. Dark respiration rates of roots of two Holcus lanatus population samples

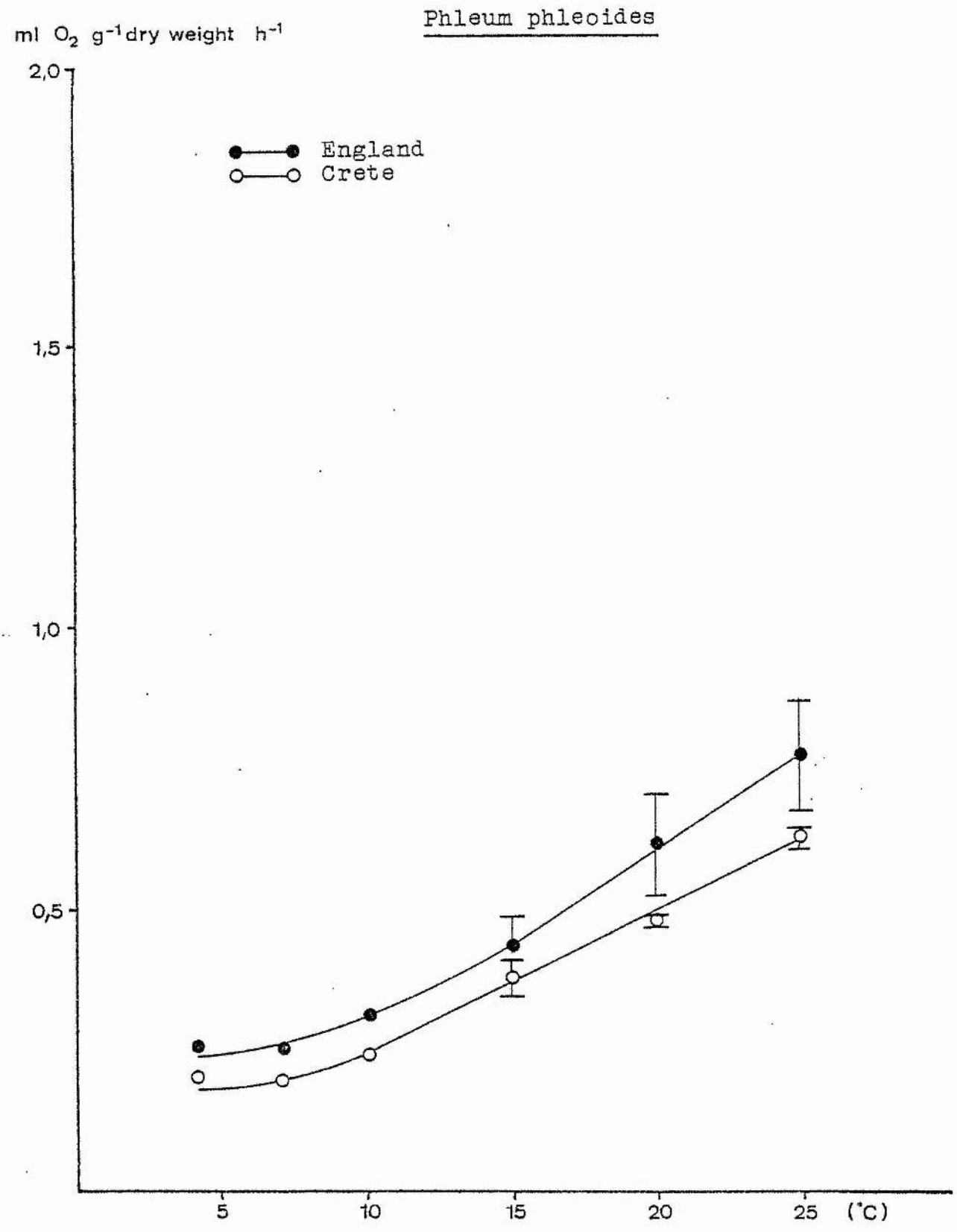


Fig.4-14. Dark respiration rates of roots of two Phleum phleoides population samples

northern population; with Anthoxanthum odoratum and Holcus lanatus (figs. 4-12, 4-13) it is the Mediterranean population sample which has the higher rate at all six temperatures. It is worth noting that, again irrespective of northern or southern origin, there seems to be a good homeostasis of respiration, particularly in the temperature range between 15°C and 25°C. This results in almost straight lines for the rate-temperature-curves, rather than in an exponential relationship as would be expected assuming a temperature coefficient of approximately 2. Most of the Q_{10} values for the range 15°-25°C were between 1.5 and 1.9, but there was no relation to the origin of the plants. In the ranges 5°-15°C and 10°-20°C Q_{10} values were generally higher (mostly between 2.0 and 2.7), but again showed no consistent distinction between climatic races.

These findings suggest that on the level of respiratory activity latitudinal isolation did not result in selection for differing rates in the grass species examined. It is interesting that the results of respiration measurements available in the literature are conflicting as to the significance of respiration rates for adaptive processes such as low temperature metabolism. As pointed out in the introduction (4.1.1.), some authors find that respiration rates increase with altitude or latitude. On the other hand, an investigation of 17 latitudinal races of Oxyria digyna failed to reveal any genetic control of dark respiration (Billings et al.,1971). Similarly, no statistically

significant differences in respiratory rates were found for three species of flowering plants and two lichens from a latitudinal gradient (Scholander and Kanwisher,1959); and Kemp et al.(1977), working with three altitudinally diverse populations of Taraxacum officinale, did not discover differences in dark respiration in the range of 10°-30°C, although the same populations did show differences on the enzyme level. Stewart and Bannister (1974), in contrast, found that only altitude influenced significantly the rate of dark respiration in Vaccinium species.

It is difficult to explain why there should be selection for higher respiration rates in cold climates in some plants and not in others. Perhaps the great diversity in experimental designs to measure respiration is one contributing factor for the diversity of the results. Some authors attempt to determine respiration rates in the field or in a laboratory simulation of field conditions, while others compare plants cultivated under identical conditions. Some use manometric techniques, others prefer infra-red spectrophotometry. There is the additional complication that respiration rates vary with season,daytime, rate of photosynthesis (Hansen,1977) and that cutting plant material causes enhanced or fluctuating respiration rates due to wound respiration (Billings et al.,1977). More standardized techniques are required to make measurements more comparable.

4.2. Dark respiration in grasses acclimatized at two different temperatures

4.2.1. Introduction

The lack of differentiation in diverse populations in their immediate response of respiration to temperature does not preclude genetically-based differences in their capacity to acclimatize to different temperature regimes. If plants could increase their respiration rate when cultivated at lower temperatures, this could be regarded as a means of maintaining growth in low temperature environments. There are in fact some reports of such reactions to be found in the literature. In their study of latitudinal races of Oxyria digyna cited above, Billings et al. (1971) observed that cold adaptation enhanced dark respiration rates in all populations. Roots and rhizomes of arctic grasses have been found to respire faster at low soil temperatures than roots in other ecosystems. This was described as an adaptation to the arctic summer with its short growing season (Billings et al.,1977). Similarly, in a study with Festuca arundinacea grown at 10° and 25°C respectively, dark respiration of leaves increased up to 35°C in all plants but was greater in those grown at 10°C (Nelson et al.,1975).

These studies compare acclimatization response either between different species or between different pretreatments of one species. The object of the experiments described here was to subject pairs of climatic races of a range of species to different pretreatments and to look

for a common pattern of response. The different characteristics of germination and growth of climatic races in relation to climate have been pointed out in chapter 3. If plants can adjust their respiratory rate in relation to the temperature at which they were grown, then it may be expected that climatic races differ in the extent of their adjustment to an artificially imposed temperature regime or, in the range of temperatures at which such adjustment can take place.

4.2.2. Materials and Methods

Plants were from the stock collection of climatic races that have been cultivated in the glasshouse as described in chapter 2. Ramets were removed from the original pots and planted in 16 cm diameter plastic pots in Levington compost. The species used are indicated in table 4-2 (page 58). Two pots were prepared for each population sample and transfered to growth cabinets (Fisons Series II Growth Cabinets Model 140 G2) set to a low (10°C) and a high (16°, 18°or 20°C) constant temperature respectively (see table 4-2). The growth cabinets were illuminated by 9 white fluorescent tubes, resulting in a light intensity of approximately 14000 lux. The photoperiod was 16 hour days / 8 hour nights. The humidity could not be regulated in the cabinets, but the plant pots were watered regularly to keep the substrate moist.

It is likely that switches in metabolic rates such as respiration must be partly due to changes in catalytic properties. Given a half life time for enzymes in the or-

Table 4-2. Plant species, provenances and experimental details in the measurement of dark respiration after acclimatization at two different temperatures

Species	Provenances	Latitude	Plant organ used	Acclimatization temperature	Acclimatization time
Festuca arundinacea	Morocco	32°N	roots+leaves	10°/20°	3 months
Holcus lanatus	Alsace, France	49°N	leaves	10°/16°	2 weeks
	Corfu, Greece	39°N	leaves	10°/16°	2 weeks
Anthoxanthum odoratum	Shetland, Scotland	60°N	leaves	10°/16°	2 weeks
	Peloponese, Greece	37°N	leaves	10°/16°	2 weeks
Holcus lanatus	Orkney Is.,Scotland	59°N	leaves	10°/18°	1 month
	Lazio, Italy	42°N	leaves	10°/18°	1 month
Dactylis glomerata	Orkney Is.,Scotland	59°N	leaves	10°/18°	1 month
	Lazio, Italy	42°N	leaves	10°/18°	1 month
Lolium perenne	Yorkshire, England	54°N	leaves	10°/18°	1 month
	Lazio, Italy	42°N	leaves	10°/18°	1 month
Poa pratensis	Fife, Scotland	56°N	leaves	10°/18°	1 month
	Lazio, Italy	42°N	leaves	10°/18°	1 month

der of magnitude of days or weeks (Alexandrov,1977) and allowing for a delay of altered enzyme forms to become manifest, an acclimatization time of 1 month was eventually considered sufficient to produce effects. This duration of growth at two temperatures was chosen after two preliminary experiments with 3 months and 2 weeks acclimatization time (table 4-2).

After acclimatization leaves and - for the first experiment in this series - roots were harvested and their dark respiration rate determined in the Gilson respirometer at 4°,7°,10°,15°,20° and 25°C as described in section 4.1.2. Only oxygen uptake rates were measured. As in the previous experiments, all values are means of at least three replicates.

4.2.3. Results and Discussion

A pilot experiment was carried out with Festuca arundinacea plants from Morocco. These plants had been subjected to constant 10° and 20°C in the growth cabinets for three months before the dark respiration was measured in roots and leaves. The results are plotted in fig. 4-15 (page 60). Acclimatization at the lower temperature has clearly enhanced the dark respiration in both roots and leaves at all experimental temperatures. The amount of enhancement depends on the temperature at which respiration is measured, the greatest enhancement being observed at the highest temperature. Thus, the same pattern of response has resulted as in the measurements by Billings et al.(1971) and Nelson

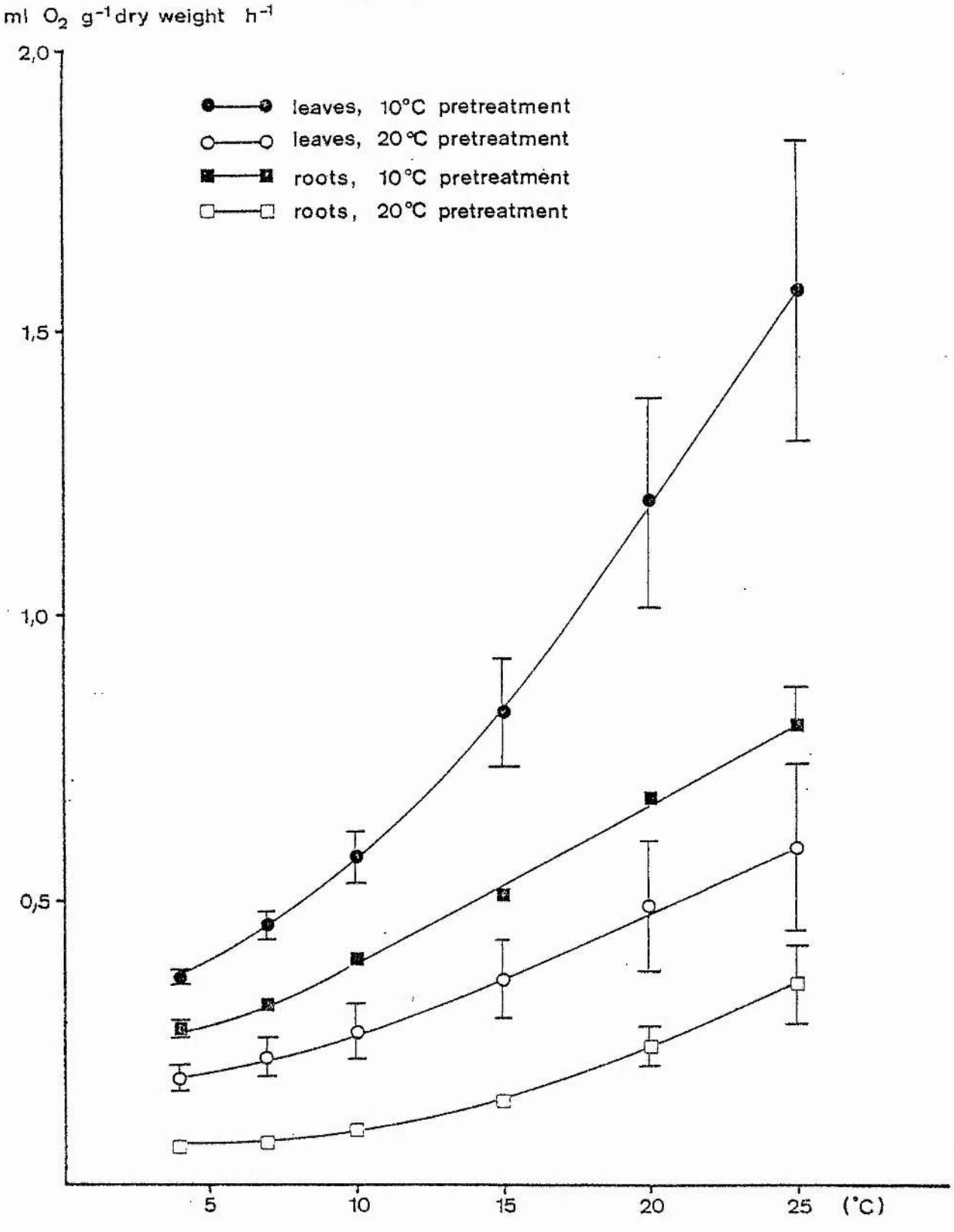


Fig. 4-15. Dark respiration rates of leaves and roots of Festuca arundinacea plants from Morocco after acclimatization at 10° and 20°

et al.(1975).

After acclimatization response had been established, subsequent experiments were carried out with different population samples of Holcus lanatus and Anthoxanthum odoratum at 16°/10°C and Holcus lanatus, Dactylis glomerata, Lolium perenne and Poa pratensis at 18°/10°C. All southern plants came from the Mediterranean. Only leaves were used for the measurements as roots were expected to react similarly. This assumption seemed reasonable after the above pilot experiment. Furthermore, the marked differences in the dark respiration rate of Festuca rubra leaves (section 4.1.3.) recured in the roots of these genotypes; and studies with Lolium multiflorum (Hansen,1977) have demonstrated the dependence of root respiration on the activity of the photosynthetic organs.

These measurements yielded the result that, depending on the origin, some plants responded according to the Festuca arundinacea plants in the experiment above, while others did not. This can be clearly seen when the dark respiration rate in the plants cultivated at 10°C is taken as 100 % at all six temperatures and the rate for the plants cultivated at the higher temperature is expressed as per cent change in respiration rate, thus:

$$\frac{Q0_2 \text{ cultivated at } 18^\circ \text{or } 16^\circ - Q0_2 \text{ cultivated at } 10^\circ}{Q0_2 \text{ cultivated at } 10^\circ} \times 100$$

This representation of results is given in figs. 4-16 and 4-17. Negative values in these graphs indicate that

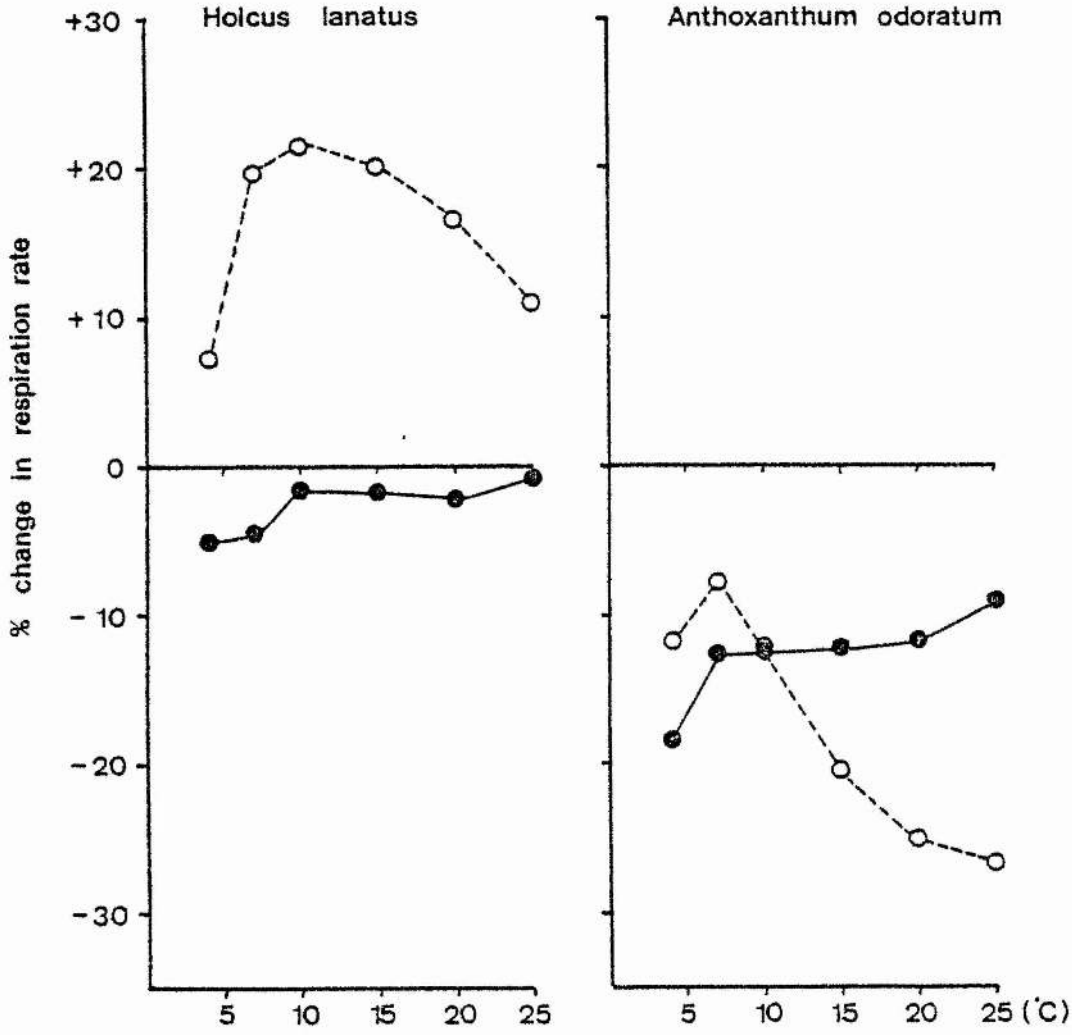


Fig. 4-16. Per cent change in the rate of dark respiration of northern (●——●) and southern (○---○) population samples as a result of acclimatization (see text page 61) at 10° and 16°C

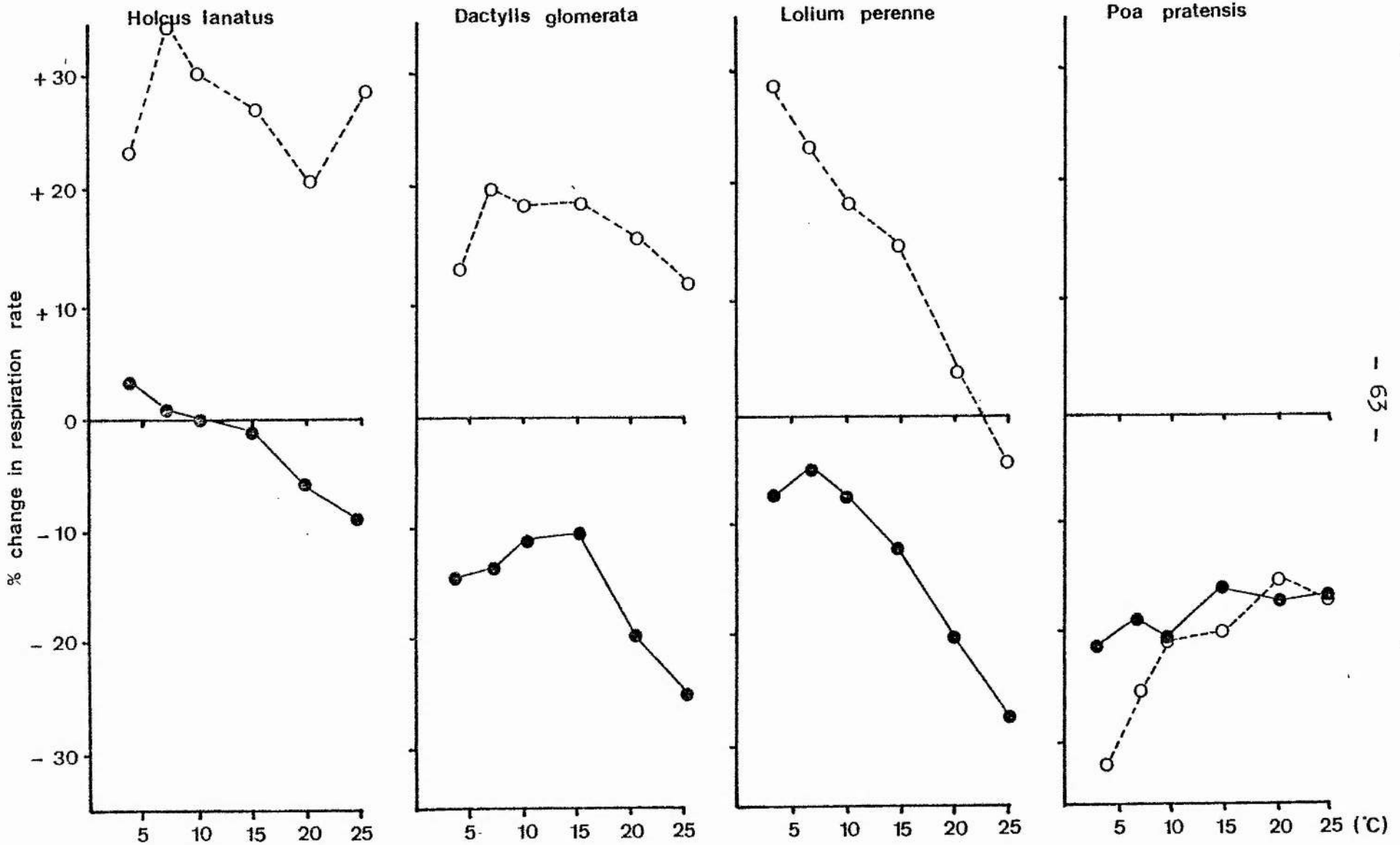


Fig. 4-17. Per cent change in the rate of dark respiration of northern (●—●) and southern (○—○) population samples as a result of acclimatization at 10° and 18°C (see text page 61)

acclimatization at the higher temperature has depressed the respiration rate. This pattern of response, similar to Festuca arundinacea in the previous example, was found in all plants of northern origin, but only with Anthoxanthum odoratum and Poa pratensis did also the southern plants respond in the same manner. In case of Holcus lanatus, Dactylis glomerata and Lolium perenne, pre-cultivation at 10°C has decreased the respiration rate in the southern population but increased in the northern population. It is remarkable that essentially the same response was found in both pairs of climatic races of Holcus lanatus, the one from Alsace and Corfu (fig. 4-16), the other from Orkney and Lazio (fig. 4-17).

It has long been known that temperature is an essential factor in triggering the conversion of carbohydrate reserves to sugars. In many plants carbohydrates are dissolved in late autumn and winter when temperatures drop below 10°C (Larcher, 1973). The increase in the content of sugars, particularly oligosaccharides, is often correlated with an increase in the resistance to chilling and frost. It has been shown for the roots of pea and maize, that the maintenance of respiration and growth at low temperatures is dependent on adequate sugar supplies to the root tip (Crawford and Huxter, 1977). It thus appears reasonable to assume that growth at low temperatures increases the respiration rate via the conversion of storage carbohydrates to soluble sugars.

The increase in respiration after exposure to low temperatures in northern and southern populations of

Festuca arundinacea, Anthoxanthum odoratum and Poa pratensis can be interpreted to mean a support of growth at low temperatures or the ability to restrict respiration and thus carbohydrate losses at high temperatures. Such a property would act as a temperature compensating mechanism against carbohydrate loss at high temperatures. Why then does this mechanism not occur in four of the Mediterranean samples examined? One possible explanation would be that in Mediterranean grasses which do not naturally grow under high temperatures because of the summer drought there is no need for a mechanism to prevent carbohydrate depletion at high temperatures . In the Mediterranean, there may therefore not have been a selective force for plants that do possess a mechanism compensating for high temperatures. On the other hand, to decrease the respiratory rate at low temperatures may well be a factor contributing to the frost susceptibility of Mediterranean grasses noted in chapter 3. It is of interest in this context to cite the work of Treharne and Eagles (1970) with climatic races of Dactylis glomerata from Norway and Portugal. The authors cultivated these plants at 5°,15° and 25°C. They did not determine dark respiration rates but measured photosynthesis and found a response pattern very similar to that obtained for the respiration of the Holcus, Dactylis and Lolium species in our experiments. Cultivation at 25°C has depressed the photosynthetic rate in the Norwegian plants but increased in the plants from Portugal.

Great care is needed to explain these population

differences in relation to meteorological factors associated with the geographical regions of their range. Like most of the germination studies mentioned in the previous chapter, our results for acclimatization were obtained under conditions of constant temperatures unlike those occurring naturally. Further work is needed to elucidate the adaptive response to temperature in populations from contrasting latitudes in order to establish the extent of variation with respect to dark respiration.

In conclusion, this investigation with northern and southern populations from a related group of species casts further doubt on the significance of intrinsic variation in the rate of dark respiration. No ecoclinal genetical differentiation was found in the dark respiration rate of leaves and roots in populations from contrasting climates when the plants had been cultivated at room temperature. Population differences were however evident in the acclimatization response. The ecological significance of respiration may thus lie in the ability of plants to respond adequately to temperature changes. The reduction in respiration rates upon exposure to higher temperatures in all northern genotypes examined may play a decisive role in maintaining a positive carbon balance and hence adapting the plants to their habitats.

5. REPLENISHMENT OF CARBOHYDRATES AFTER DARK STARVATION

5.1. Introduction

The previous chapter described an increase in the rate of dark respiration for northern grass genotypes and a decrease in some species of southern origin as a response to growth at low temperature. Chapter 3 dealt with findings that plants from Mediterranean climates have higher growth rates at low temperatures than the same species from northern populations. Higher growth rates must be expected to be accompanied by higher respiration rates, for respiration provides energy and chemical products required for active growth. Consequently, modification of respiratory rates cannot account generally for the observed differences of the growth response to temperature in populations from contrasting climates.

In the long run, higher growth rates would equally be expected to be associated with higher assimilation rates. If this were not the case, plants could fall into a negative carbon balance and deplete their carbohydrate reserves. Investigations of ecological adaptations of higher plants to growth in low temperature environments have therefore long been concerned with both aspects of a plant's energy balance, respiration and photosynthesis.

The relation between growth rate and net assimilation rate has been confirmed in studies with Bromus inermis cultivars (Tan et al., 1978), where the correlation between both parameters was found to be highly signifi-

cant. West (1973) cultivated plants of the tropical grass Pennisetum typhoides at low and high temperatures in diurnal rhythm in such a way, that one lot received the low temperature at daytime and another lot at night. He found higher fresh weights and higher starch contents in the plants from the latter treatment and concluded, that temperature influences growth via its effect on photosynthesis. Despite reports where photosynthetic rates were not found to differ in populations from contrasting altitudes (McNaughton et al.,1974; Kemp et al., 1977), intraspecific and temperature related variations in photosynthesis are now well established. Plants from cooler habitats were found to possess lower temperature optima for photosynthesis than plants from warmer areas (Billings et al.,1966; Williams III and Kemp,1976; Mächler and Nösberger,1977), no matter matter if the populations were separated altitudinally or latitudinally. Differences in the amount of photorespiration were also suggested (Mächler et al.,1977).

For grass species in particular, the same relation was found. Ecological races of Lolium perenne, when grown under identical conditions, differed in their rate of net photosynthesis by as much as 50 % (Wilson and Cooper, 1967). In measurements with Algerian and Danish populations of the same species, the Algerian population had the higher temperature optimum for photosynthesis (Eagles,1967). Essentially the same results were obtained for north-south separated populations of Dactylis glomerata (Treharne and Eagles,1970) and Festuca rubra

(Ollerenshaw et al.,1976). These findings were interpreted in terms of adaptation to temperatures prevailing at the native site of the populations.

In contrast to most contemporary studies on photosynthesis and habitat adaptation, this investigation tries to detect ecospecific differences on the carbohydrate level. Nonstructural carbohydrates are essential for growth and for survival and production of new tissue during periods when carbohydrate utilization exceeds the supply from photosynthesis. If the diverse productivities of Mediterranean and Continental grasses cannot be attributed to corresponding differences of their respiration response, are there differences in the effectivity to produce carbohydrates between northern and southern populations?

The approach via carbohydrate synthesis was thought to exclude some of the experimental difficulties associated with the measurement of photosynthetic rates in the field or in the laboratory simulation of field conditions. The experiments were designed to establish whether northern and southern populations differ in the rate at which they can accumulate carbohydrates and in what way these rates are affected by temperature. No attempt was made to simulate natural conditions. Instead the populations are compared for their ability to replenish their carbohydrate supplies after being starved in darkness.

5.2. Materials and Methods

The species listed in table 5-1 were collected in the field. All southern plants were from the Lazio region in Italy. At each collection site, a number of plants were taken, pooled and planted in 25 cm diameter pots in earth. These pots were then transfered to growth rooms at constant 18°C, where they were kept for 4-6 weeks,

Table 5-1. Sites of origin of the populations of grass species used in the carbohydrate accumulation studies

Species	Provenances	Latitude
Dactylis glomerata	Orkney Is.,Scotland	59° N
	Lazio, Italy	42° N
Lolium perenne	Orkney Is.,Scotland	59° N
	Lazio, Italy	42° N
Festuca rubra	Lofoten Is.,Norway	68° N
	Lazio, Italy	42° N
Holcus lanatus	Orkney Is.,Scotland	59° N
	Lazio, Italy	42° N
Poa pratensis	Fife, Scotland	56° N
	Lazio, Italy	42° N

illuminated with two white fluorescent tubes at approximately 4000 lux and a photoperiod of 14 h days/ 10 h nights. Before each carbohydrate accumulation experiment, the plants were placed in total darkness at 18°C so that their carbohydrate content would be minimised. In preliminary measurements the carbohydrate content of leaves was found to be markedly reduced after dark starvation for one day, but to decrease further when the starvation period was prolonged. A dark starvation peri-

od of 48 hours was eventually chosen for the experiments as longer periods did not further reduce the amount of carbohydrates present in leaves.

It has been shown that the rate of assimilation depends on the age of the leaves (Woledge,1973). Only fully developed leaves were therefore used. After the dark starvation period, they were detatched, washed and placed on moist blotting paper and transfered to controlled temperature chambers at 12° and 20°C, where they were illuminated by white fluorescent tubes at ca. 8000 lux. Detatched leaves were used in order to prevent carbohydrate translocation out of the leaf. The leaves were illuminated for three hours and samples were taken at 20- 45 minute intervals. The samples were oven dried for one hour at 100°C. To heat-dry plant material leads inevitably to carbohydrate losses. In a preliminary assay the carbohydrate content in 0.5 g fresh weight of leaves was compared in fresh and oven dried material. The content of nonstructural carbohydrates was approximately 15 % lower in the samples dried for one hour at 100°C. This procedure was nevertheless found to keep carbohydrate losses low in comparison to other drying times or temperatures (Smith,1969).

In studying grasses, carbohydrate analyses have to take into account the fact that fructosans are the main storage products in species of temperate origin (Pollock and Ruggles,1976). Among the other nonstructural carbohydrates, glucose, fructose, sucrose, maltose and starch are of importance (Smith,1972), while other sugars are

present only in minor quantities and can be neglected. The carbohydrate analyses were carried out with 0.05 - 0.1 g oven dried material. This was ground with approximately 9 ml of 1.25 % hydrochloric acid and hydrolysed for 30 minutes at 100°C in a water bath. The resulting suspensions were then adjusted to pH 4-5 with 4 N NaOH and made up to 10 ml with distilled water. The suspensions were then centrifuged in a table centrifuge. The supernatants were used to assay nonstructural carbohydrates in a procedure modified after Boehringer (1977/78).

Starch hydrolysis was completed by incubating 0.1 or 0.2 ml of supernatant with 0.2 ml citrate buffer (50 mmol/l; pH 4.6) and 0.005 ml of amyloglucosidase for 15 minutes at 55°-60°C in glass cuvettes. To each cuvette were then added: 1.0 ml triethanolamine buffer (0.3 mol/l; pH 7.5; $MgSO_4$ 4 mmol/l); 0.1 ml NADP (12 mmol/l); 0.1 ml ATP (15 mmol/l); 1.4 ml H_2O. The absorption was measured spectrophotometrically at 340 nm (E_1). 0.005 ml of a mixture of hexokinase and glucose-6-phosphate-dehydrogenase (2 mg HK/ml; 1 mg G6P-DH/ml) was then added and absorption E_2 measured after 15 minutes incubation at room temperature. E_3 was subsequently measured, following incubation with 0.005 ml phosphoglucose-isomerase (2 mg PGI/ml) for 15 minutes at room temperature. Thus, after hydrolysis to glucose and fructose, the hexoses were measured in sequence enzymatically. The glucose fraction in the hydrolysates was calculated from the absorption difference E_2-E_1, its fructose fraction

from E_3-E_2. All enzymes were from Boehringer Mannheim. Distilled water was used in preliminary measurements in place of the supernatant as a reaction blank. Only negligible absorption differences were however found and all samples were therefore measured against distilled water only. The leaf content in nonstructural carbohydrates was expressed as mg equivalents of glucose · g^{-1} dry weight. Each value is the mean of duplicate measurements which agreed within 5 %.

5.3. Results

The values for nonstructural carbohydrates as well as of the glucose and fructose portions in the five species tested are listed in tables 5-2 to 5-6. Regression lines were fitted through the points obtained (table 5-7) and are plotted in figs. 5-1 and 5-2. It can be seen from tables 5-3 and 5-4 that in the case of the northern genotypes of Lolium perenne and Festuca rubra the fructose content fluctuated with the duration of illumination without showing any form of progressive increase in amount. For these two species therefore the accumulation of carbohydrate is based solely on the glucose content which did show a steady increase during the period of illumination.

The graphs show that there is a distinct difference in the activity of assimilation between the northern and southern genotypes. This can be seen more clearly when the total increase in carbohydrate during the 3 hour experiments is calculated from the fitted regression lines, using the data tabulated in table 5-7. This increase is

Table 5-2. Accumulation of carbohydrates in northern (N) and southern (S) provenances of Dactylis glomerata at 12° and 20°C (see text). Units for content are in mg equivalents of glucose per g dry weight; time in minutes.

	Total	Glucose	Fructose	% Glucose	% Fructose	Time
N	4.1	3.3	0.8	80.5	19.5	0
(12°)	7.0	5.0	2.0	71.4	28.6	30
	10.8	7.4	3.4	68.5	31.5	60
	16.6	10.9	5.7	65.7	34.3	90
	20.7	12.8	7.9	61.8	38.2	120
	28.5	18.3	10.2	64.2	35.8	150
	41.2	26.0	15.2	63.1	36.9	180
N	3.2	2.8	0.4	87.5	12.5	0
(20°)	5.9	4.5	1.4	76.3	23.7	30
	18.2	10.0	8.2	54.9	45.1	60
	24.0	15.8	8.2	65.8	34.2	90
	37.3	23.1	14.2	61.9	38.1	120
	43.2	28.2	15.0	65.3	34.7	150
	44.1	29.0	15.1	65.8	34.2	180
S	6.4	4.7	1.7	73.4	26.6	0
(12°)	7.1	5.1	2.0	71.8	28.2	30
	10.8	7.2	3.6	66.7	33.3	60
	15.1	9.6	5.5	63.6	36.4	90
	17.5	11.1	6.4	63.4	36.6	120
	22.2	13.9	8.3	62.6	37.4	150
	21.0	12.7	8.3	60.5	39.5	180
S	3.7	3.1	0.6	83.8	16.2	0
(20°)	6.7	4.8	1.9	71.6	28.4	20
	8.8	6.5	2.3	73.9	26.1	40
	11.7	7.5	4.2	64.1	35.9	60
	14.2	8.9	5.3	62.7	37.3	90
	18.9	11.4	7.5	60.3	39.7	135
	32.3	19.9	12.4	61.6	38.4	180

Table 5-3. Accumulation of carbohydrates in northern (N) and southern (S) provenances of Lolium perenne at 12° and 20° (see text). Units for content are in mg equivalents of glucose per g dry weight; time in minutes.

	Total	Glucose	Fructose	% Glucose	% Fructose	Time
N (12°)	27.9	7.7	20.2	27.6	72.4	0
	56.2	9.6	46.6	17.1	82.9	30
	28.4	9.3	19.1	32.7	67.3	60
	54.0	13.7	40.3	25.4	74.6	90
	32.8	15.7	17.1	47.9	52.1	120
	38.0	21.2	16.8	55.8	44.2	150
	62.1	31.7	30.4	51.0	49.0	180
N (20°)	53.2	9.9	43.3	18.6	81.4	0
	77.7	13.9	63.8	17.9	82.1	30
	54.4	13.8	40.6	25.4	74.6	60
	51.0	18.4	32.6	36.1	63.9	100
	92.1	28.2	63.9	30.6	69.4	120
	62.0	27.6	34.4	44.5	55.5	150
	123.2	43.0	80.2	34.9	65.1	180
S (12°)	5.5	4.4	1.1	80.0	20.0	0
	6.6	4.5	2.1	68.2	31.8	30
	9.6	6.5	3.1	67.7	32.3	60
	7.9	5.0	2.9	63.3	36.7	90
	14.1	8.7	5.4	61.7	38.3	120
	13.1	7.8	5.3	59.5	40.5	150
	18.6	10.9	7.7	58.6	41.4	180
S (20°)	10.8	8.2	2.6	75.9	24.1	0
	10.6	7.2	3.4	67.9	32.1	20
	13.4	8.9	4.5	66.4	33.6	40
	14.4	9.0	5.4	62.5	37.5	60
	18.7	12.0	6.7	64.2	35.8	90
	22.3	13.6	8.7	61.0	39.0	135
	29.7	16.7	13.0	56.2	43.8	180

Table 5-4. Accumulation of carbohydrates in northern (N) and southern (S) provenances of Festuca rubra at 12° and 20° (see text). Units for content are in mg equivalents of glucose per g dry weight; time in minutes.

	Total	Glucose	Fructose	% Glucose	% Fructose	Time
N (12°)	19.8	15.0	4.8	75.6	24.4	0
	44.7	20.1	24.6	45.0	55.0	30
	56.4	21.5	34.9	38.1	61.9	60
	46.4	23.1	23.3	49.8	50.2	90
	38.5	24.1	14.4	62.6	37.4	120
	58.9	29.0	29.9	49.2	50.8	150
	50.0	30.8	19.2	61.6	38.4	180
N (20°)	67.5	26.4	41.1	39.1	60.9	0
	46.8	24.1	22.7	51.5	48.5	30
	54.0	26.7	27.3	49.4	50.6	60
	48.5	27.3	21.2	56.3	43.7	90
	61.5	36.2	25.3	58.9	41.1	120
	65.8	37.0	28.8	56.2	43.8	150
	72.7	39.8	32.9	54.7	45.3	180
S (12°)	6.4	4.8	1.6	75.0	25.0	0
	15.2	7.5	7.7	49.3	50.7	20
	10.3	6.8	3.5	66.0	34.0	40
	27.4	10.4	17.0	38.0	62.0	60
	17.6	10.2	7.4	58.0	42.0	90
	24.0	14.5	9.5	60.4	39.6	135
	28.9	15.4	13.5	53.3	46.7	180
S (20°)	38.1	14.8	23.3	38.8	61.2	0
	23.3	10.5	12.8	45.1	54.9	30
	23.5	12.5	11.0	53.2	46.8	60
	24.5	14.6	9.9	59.6	40.4	90
	32.9	15.8	17.1	48.0	52.0	120
	32.1	18.9	13.2	58.8	41.2	150
	39.9	23.4	16.5	58.6	41.4	180

Table 5-5. Accumulation of carbohydrates in northern (N) and southern (S) provenances of *Holcus lanatus* at 12° and 20°C (see text). Units for content are in mg equivalents of glucose per g dry weight; time in minutes.

	Total	Glucose	Fructose	% Glucose	% Fructose	Time
N (12°)	23.2	11.9	11.3	51.3	48.7	0
	14.1	8.3	5.8	58.9	41.1	30
	34.1	20.3	13.8	59.5	40.5	60
	43.6	23.4	20.2	53.7	46.3	90
	53.3	29.4	23.9	55.2	44.8	120
	56.5	32.8	23.7	58.1	41.9	150
	80.5	45.2	35.3	56.1	43.9	180
N (20°)	16.5	9.2	7.3	55.8	44.2	0
	32.9	16.8	16.1	51.1	48.9	30
	31.1	17.3	13.8	55.6	44.4	60
	46.3	25.2	21.1	54.4	45.6	100
	61.9	33.2	28.7	53.6	46.6	120
	75.4	35.2	40.2	46.7	53.3	150
	79.9	42.6	37.3	53.3	46.7	180
S (12°)	9.7	9.2	0.5	95.0	5.0	0
	10.9	9.5	1.4	87.1	12.9	30
	16.7	13.0	3.7	77.8	22.2	60
	16.9	12.6	4.3	74.6	25.4	90
	21.3	14.8	6.5	69.5	30.5	120
	25.6	17.5	8.1	68.3	31.7	150
	29.9	19.8	10.1	66.2	33.8	180
S (20°)	9.5	8.8	0.7	92.6	7.4	0
	14.0	11.7	2.3	83.6	16.4	20
	18.7	14.7	4.0	78.6	21.4	40
	20.7	15.3	5.4	73.9	26.1	60
	23.8	16.8	7.0	70.6	29.4	90
	27.1	17.8	9.3	65.7	34.3	135
	29.3	19.5	9.8	66.6	33.4	180

Table 5-6. Accumulation of carbohydrates in northern (N) and southern (S) provenances of Poa pratensis at 12° and 20°C (see text). Units for content are in mg equivalents of glucose per g dry weight; time in minutes

	Total	Glucose	Fructose	% Glucose	% Fructose	Time
N (12°)	18.1	10.3	7.8	56.9	43.1	0
	15.9	8.9	7.0	56.0	44.0	30
	33.9	14.9	19.0	44.0	56.0	60
	22.2	13.0	9.2	58.6	41.4	90
	32.3	9.7	22.6	30.0	70.0	120
	40.2	22.8	17.4	56.7	43.3	150
	46.4	26.6	19.8	57.3	42.7	180
N (20°)	25.2	12.8	12.4	50.8	49.2	0
	29.1	12.7	16.4	43.6	56.4	30
	25.4	13.0	12.4	51.2	48.8	60
	32.2	17.7	14.5	55.0	45.0	90
	40.2	20.3	19.9	50.5	49.5	120
	47.0	24.0	23.0	51.1	48.9	150
	48.7	26.4	22.3	54.2	45.8	180
S (12°)	23.4	11.8	11.6	50.4	49.6	0
	25.1	13.6	11.5	54.2	45.8	20
	24.6	13.6	11.0	55.3	44.7	40
	23.3	12.8	10.5	54.9	45.1	60
	31.0	15.4	15.6	49.7	50.3	90
	31.7	16.8	14.9	53.0	47.0	135
	42.0	23.0	19.0	54.8	45.2	180
S (20°)	14.3	8.5	5.8	59.4	40.6	0
	15.6	9.5	6.1	60.9	39.1	20
	15.3	9.6	5.7	62.7	37.3	40
	21.0	12.8	8.2	61.0	39.0	60
	29.4	17.2	12.2	58.5	41.5	90
	41.0	23.2	17.8	56.6	43.4	135
	46.5	25.4	21.1	54.6	45.4	180

Table 5-7. Linear regression to fit straight lines for the increase in carbohydrate content. The table lists the coefficients of determination r^2; the carbohydrate content at t=0 min and t=180 min calculated from the regression lines (in mg/g dry weight); the slopes of the regression lines (in mg/g dry weight · min). The calculation for Festuca rubra and Lolium perenne is based on the glucose content only (see tables 5-3 and 5-4).

Species and provenances		r^2	t=0	t=180	slope
Dactylis glomerata	N 20°	0.9642	1.9	48.3	0.2576
	N 12°	0.9416	0.8	36.0	0.1955
	S 20°	0.9488	2.9	28.9	0.1446
	S 12°	0.9506	5.7	22.9	0.0961
Lolium perenne	N 20°	0.8671	6.9	36.8	0.1659
	N 12°	0.8528	4.7	26.4	0.1210
	S 20°	0.9468	6.9	16.2	0.0518
	S 12°	0.7991	3.8	9.9	0.0337
Festuca rubra	N 20°	0.8434	23.0	39.2	0.0899
	N 12°	0.9559	16.1	30.6	0.0807
	S 20°	0.6916	10.9	20.7	0.0546
	S 12°	0.9345	5.5	16.1	0.0591
Holcus lanatus	N 20°	0.9570	16.1	81.2	0.3619
	N 12°	0.9066	14.1	73.2	0.3285
	S 20°	0.9164	12.6	31.4	0.1047
	S 12°	0.9718	8.6	28.9	0.1126
Poa pratensis	N 20°	0.8900	22.4	48.4	0.1442
	N 12°	0.7819	15.7	44.0	0.1570
	S 20°	0.9633	11.1	47.2	0.2005
	S 12°	0.8514	21.4	39.0	0.0974

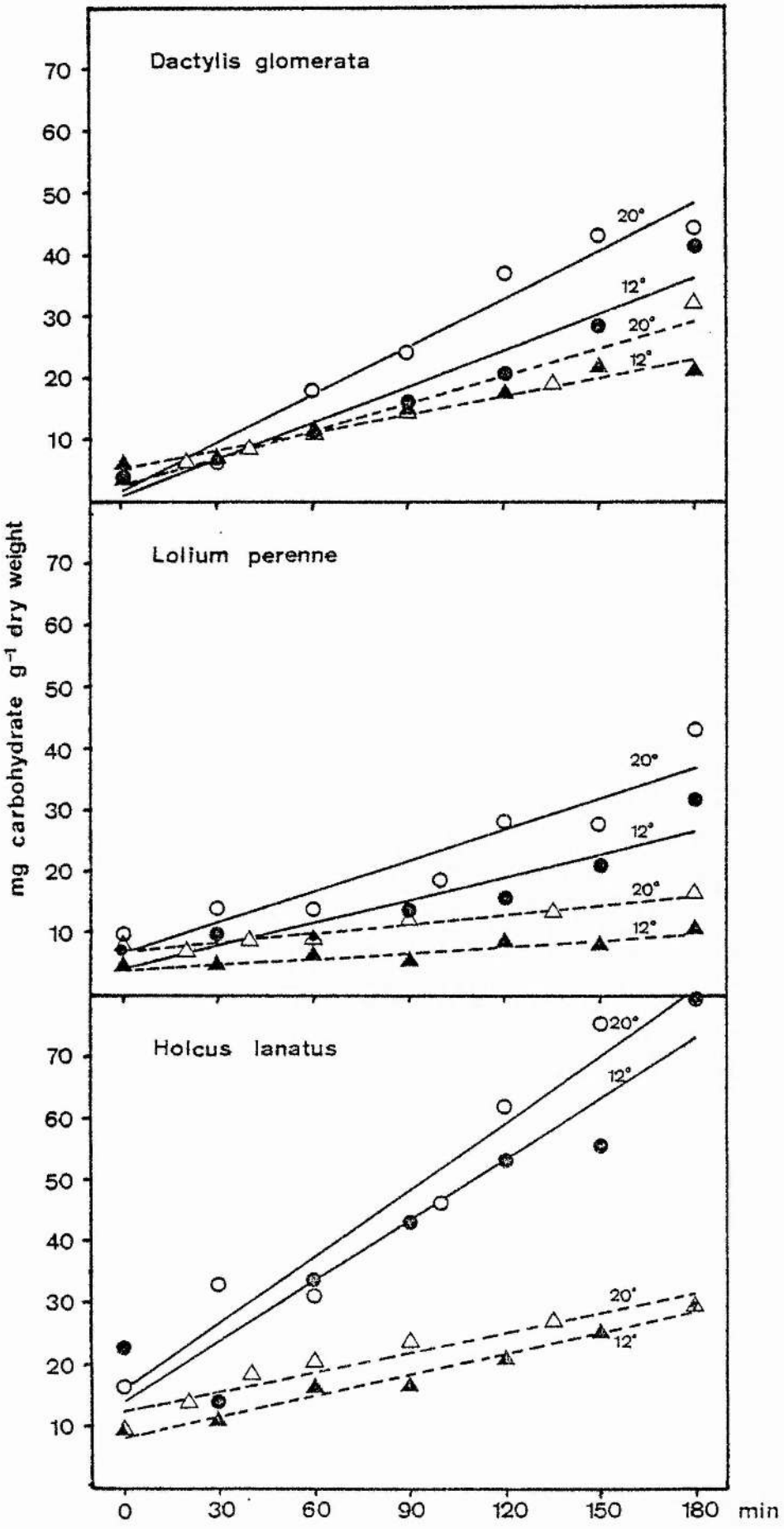


Fig. 5-1. Time course of increase of nonstructural carbohydrates in northern (circles) and southern (triangles) genotypes assayed at 12° (solid marks) and 20°C (open marks)

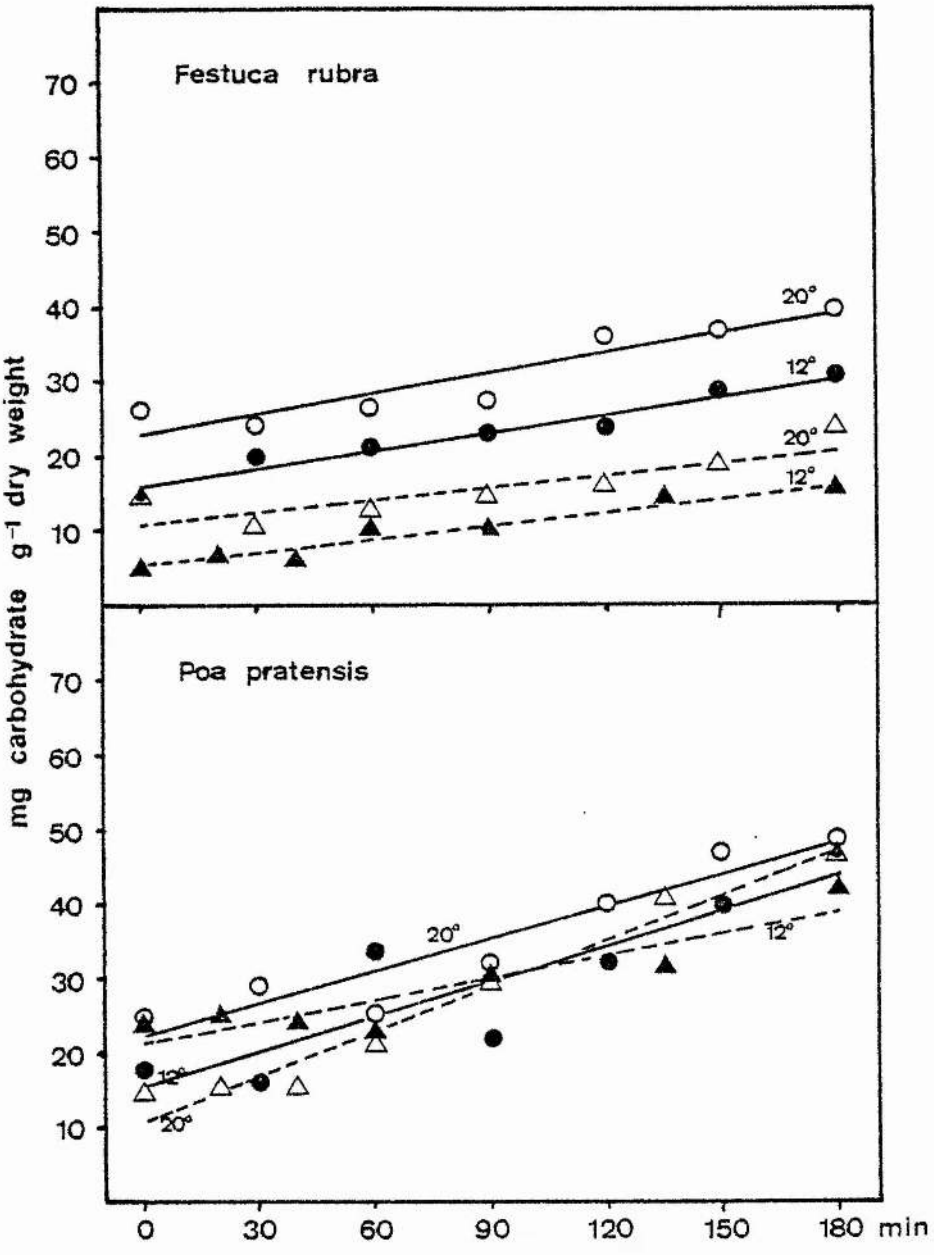
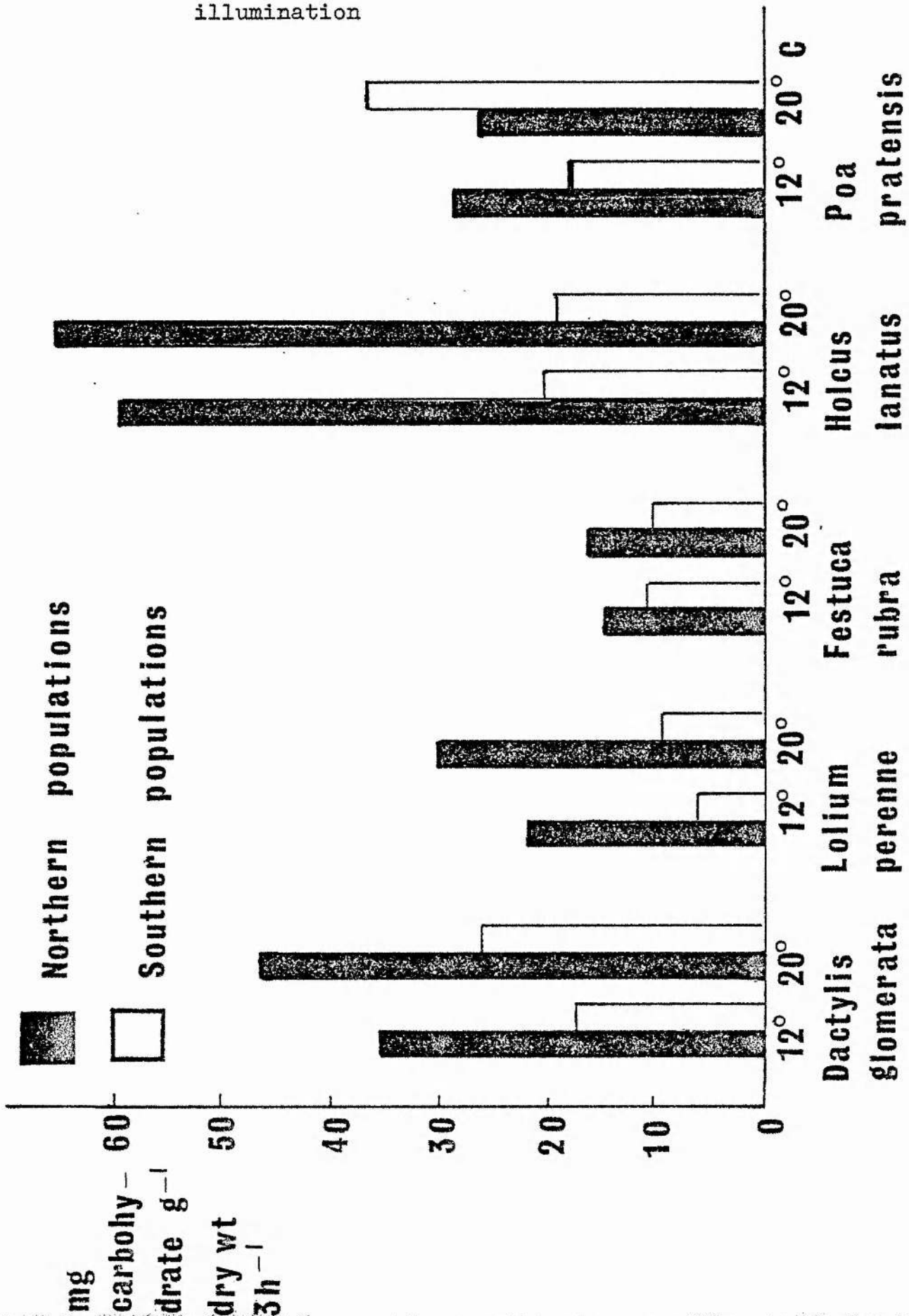


Fig. 5-2. Time course of increase of nonstructural carbohydrates in northern (circles) and southern (triangles) genotypes assayed at 12°C (solid marks) and 20°C (open marks).

Fig. 5-3. Total carbohydrate accumulation during 3 hours illumination



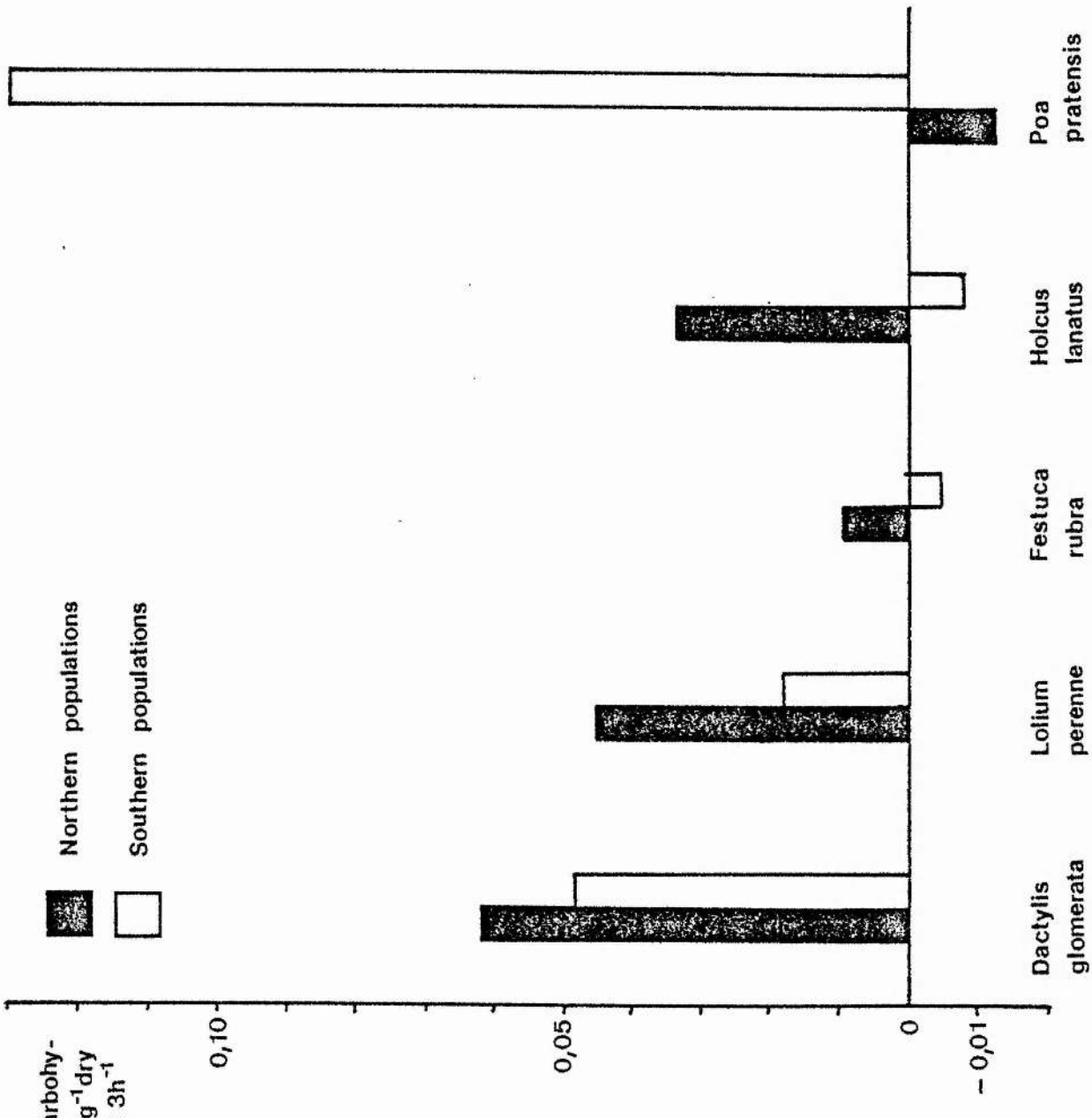


Fig. 5-4. Temperature influence on the velocity of carbohydrate accumulation

illustrated in fig.5-3 (page 82). The northern genotypes accumulate more carbohydrate per g dry weight than the southern genotypes. Only the northern plants of Poa pratensis (Scotland) do not match the pattern. This may be due to the fact that Poa pratensis is a plant with southern distribution. Going up north in Britain, Poa pratensis is gradually replaced by Poa trivialis. Poa pratensis is not native to Orkney (Bullard,1975), but has been introduced as seed. Thus it is likely that the Poa pratensis sample from Fife was an agricultural variety of southern origin.

To give an estimate of the temperature influence on the velocity of carbohydrate accumulation, the data from the linear regression in table 5-7 (page 79) have been used to calculate the differences in slope between the lines obtained for 20°C and 12°C. These are plotted in fig. 5-4 (page 83). With the exception of Poa pratensis again, higher temperature causes a greater increase in accumulated carbohydrate in the northern than in the southern population samples. In other words, the temperature effect on carbohydrate accumulation is greater in the northern plants than in the southern.

5.4. Discussion

A distinction in this study from most contemporary investigations on plant productivity in relation to environmental parameters is the technique of artificially starving the plants by placing them in total darkness for 48 hours before each assay. This pre-treatment was

designed to ensure that the plants achieve maximum biochemical potential for carbohydrate synthesis. This method revealed clearly that northern grass species can outstrip their southern counterparts in their activity of carbohydrate synthesis.

In contrast to the studies on photosynthetic activity cited in the introduction to this chapter, the present experiments did not identify optimum temperatures for assimilation. However, as plants are unlikely to experience optimum temperatures for *in vivo* biochemical reactions at least in northern populations, the estimation of optimum temperatures is of little relevance as long as reaction rates are unknown at temperatures prevailing in the plant's habitat. The similarity of optimum temperatures for growth in northern and southern populations but their dissimilar growth rates at lower temperatures discussed in section 3.4. may illustrate the complication. Our experiments with grasses suggest that the northern populations are more efficient accumulators of photosynthate over the temperature range of from 12° to 20°C and that their photosynthetic efficiency is not just confined therefore to low temperatures. Although these experiments could be criticized for not being carried out under natural or simulated ecological conditions, they nevertheless demonstrate an important ecological property, namely, that after periods of starvation - and every night is such a period, then it is the northern populations that can accomplish this accumulation more rapidly than the southern populations. Further, an in-

crease in temperature within the range from 12° to 20°C will allow the northern population to magnify this advantage.

How can these findings be related to the results of the acclimatization experiments described in the previous chapter? Northern plants of Holcus lanatus, Dactylis glomerata and Lolium perenne were there found to lower their respiration rate when grown at 18°C rather than at 10°C (fig. 4-17, page 63). For the same species and Festuca rubra, a temperature increase from 12° to 20°C was more beneficial for the carbohydrate supply in northern than in southern plants (fig. 5-4, page 83). Both properties could act synergistically as a temperature compensating mechanism against carbohydrate loss at higher temperatures.

Why the plants from northern latitudes display a higher potential for carbohydrate synthesis, but at the same time lower growth rates at low temperatures than plants from the Mediterranean (chapter 3), cannot be explained from these experiments. It would however appear as one possibility that their enhanced respiration rates after acclimatization to low temperatures (section 4.2.3.) compensates their higher efficiency for carbohydrate synthesis. If some speculation will be permitted, it can be calculated from the absolute values of dark respiration in the acclimatization experiment, that after growth at 10°C the respiration rates were higher in most northern than in the southern plants. If the respiration rates measured at 10°C are assumed as 100 % in

the southern plants, then the percentages for the northern ones are as follows: 100 % for Holcus lanatus, 112 % for Dactylis glomerata, 108 % for Lolium perenne. These figures can be compared with the velocities of carbohydrate accumulation, i.e. with the slopes of the regression lines in fig. 5-1, belonging to a temperature of 12°C (see table 5-7, page 79). We find then for the same species, that at 12°C the speed of carbohydrate accumulation in the northern population is 200-400 % of that in the southern population. This suggests that, even at lower temperatures (10° or 12°C), the northern plants are more efficient carbohydrate accumulators. There is however another environmental parameter which could compensate for the higher efficiency of carbohydrate synthesis in northern plants, and that is light intensity. The light intensity of 8000 lux which was used for both northern and southern species is well in the order of magnitude of light intensities experienced in the field. Higher carbohydrate accumulation in northern samples at 12° and 20°C may be interpreted as both an adaptation to growth under lower temperatures and lower light intensities. In northern latitudes, both parameters act synergistically to reduce the amount of carbohydrates formed during periods of photosynthetic activity.

6. RIBULOSE-1,5-BIPHOSPHATE-CARBOXYLASE

6.1. Ribulose-1,5-biphosphate carboxylase activity in grasses from contrasting latitudes

6.1.1. Introduction

Ribulose-1,5-biphosphate carboxylase (EC 4.1.1.39) catalyses the reaction of CO_2 with ribulose-1,5-biphosphate and is the enzyme responsible for the bulk of CO_2 fixation in all C_3-plants. It has been suggested that the temperature response of the photosynthetic apparatus may be directly related to the temperature sensitivity of the major carboxylating enzymes (Treharne and Cooper, 1969; Björkman and Badger, 1976/77). Given that northern grasses were found in the previous experiments (chapter 5) to exhibit higher activities of carbohydrate accumulation, it was reasonable to ask whether this property could be attributed to higher activities of the key photosynthetic enzyme. Measurements were therefore done to determine the specific activities of ribulose-1,5-biphosphate carboxylase (RuBPCarboxylase) in plants from northern and southern populations.

6.1.2. Materials and Methods

The plants in table 6-1 (page 89) were collected from populations in the wild and cultivated in the glasshouse as described in chapter 2. Leaf age plays a great role for the activity of RuBPCarboxylase (Kannagara and Woolhouse, 1968; Dowton and Slatyer, 1971). To reduce this source of variability, only fully developed leaves were

used for the enzyme assays.

Table 6-1. Plants used to study the RuBPCarboxylase relations in grasses from contrasting climates

Species	Provenances	Latitude
Dactylis glomerata	Orkney Is.,Scotland	59°N
	Lazio, Italy	42°N
Lolium perenne	Trondheim, Norway	64°N
	Lazio, Italy	42°N
Holcus lanatus	Orkney Is.,Scotland	59°N
	Lazio, Italy	42°N
Festuca arundinacea	England	52°N
	Morocco	32°N
Deschampsia caespitosa	Iceland	65°N
	Angus, Scotland	56°N
Poa pratensis	Fife, Scotland	56°N
	Lazio, Italy	42°N

Preparation of crude extracts and enzyme assays

Extraction and assay methods were similar to those used by Björkman (1968). 60 mg fresh leaves were collected from the middle section of 4 to 6 different leaves of each species. This was ground with 3 ml extraction medium in an ice-chilled mortar. The extraction medium consisted of 0.04 M Tris; 0.01 M $MgCl_2 \cdot 6\ H_2O$; 0.25 mM EDTA; reduced glutathione was added shortly before each extraction to bring the final volume to a concentration of 5.0 mM. The medium was adjusted to pH 7.7 with HCl at room temperature. The suspension of ground plant material in extraction buffer was centrifuged for 2 minutes in a Beckman Microfuge B and the supernatant used

as crude extract for the determinations of RuBPCarboxylase activity.

These were carried out with the sodium salt of ribulose biphosphate (obtained from Calbiochem-Behring Corp.) and $NaH^{14}CO_3$ (from Amersham) as substrates. The assays were carried out at 5°,10°,15°,20° and 25°C. 0.15 ml of extraction medium, 0.05 ml of radioactive bicarbonate solution (0.1 µMole; 2.5 µCi) and 0.1 ml RuBP (approx. 0.1 µMole) were pipetted into small test tubes and preincubated in a water bath maintained at the desired temperature for 10 minutes. The reaction was then started by adding 0.1 ml of crude extract to each test tube and allowed to proceed for 10 minutes. This long incubation time is necessary for several reasons. First, maximum rates of RuBPCarboxylase activity are often reached only after 3-4 minutes lag phase (Racker,1962). Secondly, RuBPCarboxylase is a "lazy" enzyme with a turnover number of only 1300 mole HCO_3^- fixed per mole enzyme and minute at pH 7.9 and 30°C (Zelitch,1971). Thirdly, the enzyme uses CO_2 and not HCO_3^- as substrate (Cooper et al.,1969), and the equilibration between CO_2 and HCO_3^- is a slow reaction in absence of carbonic anhydrase. After 10 minutes the reaction was stopped by 0.1 ml of 6 M acetic acid which contained 1 ml Teepol per 50 ml as a detergent.

0.1 ml aliquots were then spread out on one-inch aluminium planchets and dried on hotplates for 1 hour at ca. 70°C. The activity of the samples was then measured as counts per 10 minutes on a Tracerlab Multi

Matic counter. All samples were run in triplicate which agreed within 14 %.

The mean background activity of empty planchets from a great number of measurements was 382 counts per 10 minutes. Reaction blanks were run at 20°C, using exactly the same procedure as above, but replacing the crude extract by 0.1 ml of distilled water. This resulted in an average activity of 411 counts per 10 minutes. Both these values are so low that they could be neglected in calculations.

It was noticed, however, that the enzyme activity was rather unstable in the crude extracts, although they were kept at 0°C in ice. Using a crude extract of Dactylis glomerata from Strasbourg, France, a standard curve for the loss of RuBPCarboxylase activity was therefore prepared by incubating samples in 30 minute intervals over a 4 hour period. As a result, activity decreased linearly (r^2= 0.952) by 25 % during 4 hours. Less than 2 hours were needed to measure RuBPCarboxylase activities at 5 temperatures. Therefore the corrections applied never exceeded 12 %. All values obtained were corrected for initial activities after the standard curve.

The protein content in the crude extracts was estimated with the Folin method (Plummer,1971) and found to lie between 1.17 and 1.66 mg/ml.

6.1.3. Results and Discussion

No matter whether the activities were expressed on a protein basis as specific activities (upper graphs in

figs. 6-1 to 6-6) or on a fresh weight basis (lower graphs), higher activities were found in the plants from northern populations without exception. In measurements of RuBPCarbocylase activity in particular, reference to total protein content is a reasonable measure for two reasons: RuBPCarboxylase constitutes a large proportion of the cell's total protein content (Zelitch,1971) and is highly specific (Racker,1962). These results strongly suggest that differences between northern and southern populations of the grass species tested in RuBPCarboxylase activity account, at least in part, for the differences in their potential for carbohydrate accumulation. They also parallel reports which demonstrate a close relationship between the activity of RuBPCarboxylase and photosynthetic rates. Wareing et al.(1968) have related the increased photosynthetic activity of bean plants after partial defoliation to higher activities of the carboxylating enzyme. Björkman (1968) showed a correlation between the activity of RuBPCarboxylase and the rate of photosynthesis at light saturation in sun- and shade-adapted plants. Studies of population differentiation in the activity of RuBPCarboxylase have so far been concerned with arctic and alpine or else with tropical and temperate plants. The present results show that it is possible to detect population differences which are common to a range of species of temperate origin.

Many former studies on the activity of RuBPCarboxylase in higher plants have been conducted to deter-

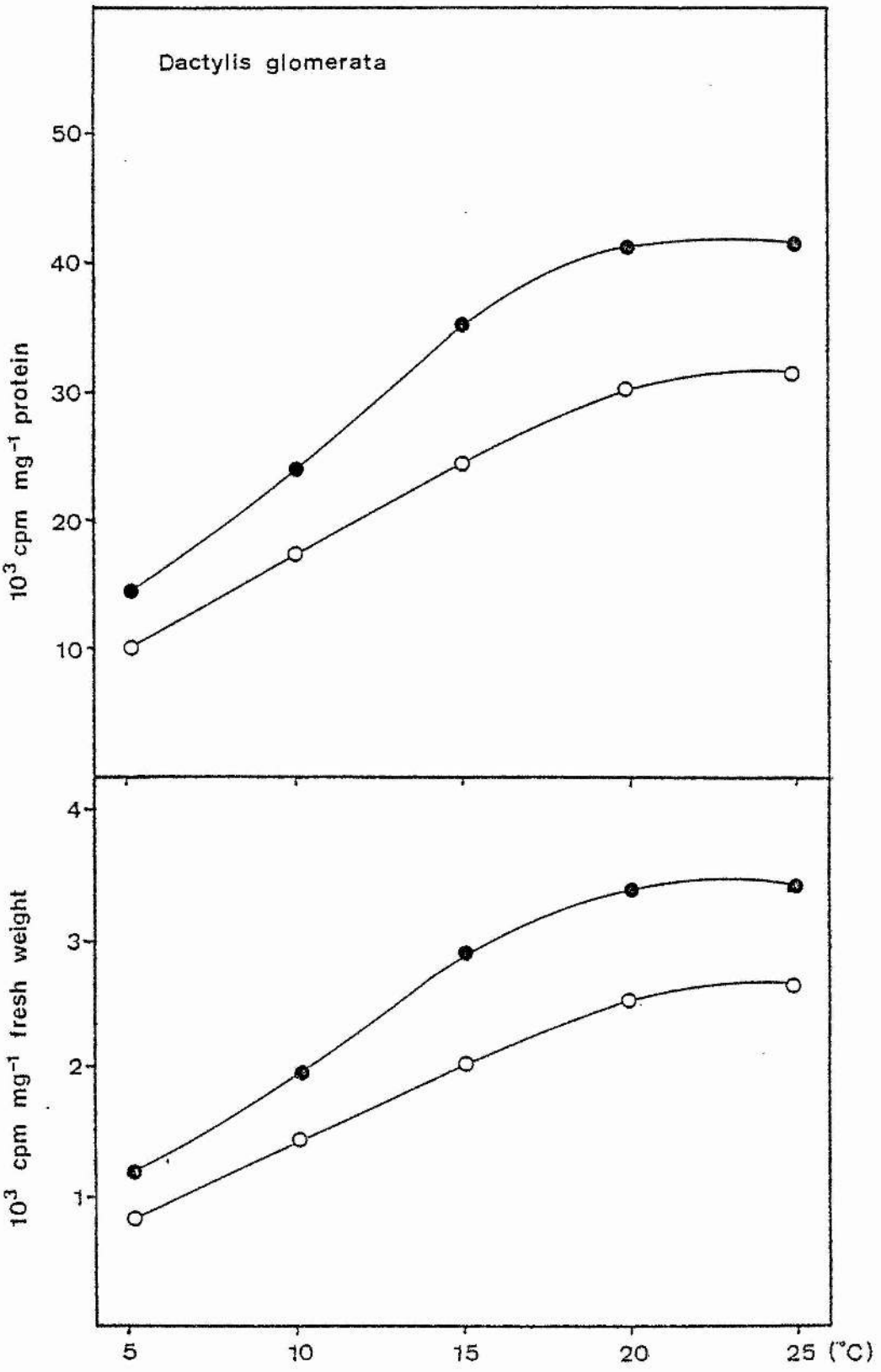


Fig.6-1. RuBPCarboxylase activity in northern (●——●) and southern (○——○) provenances.

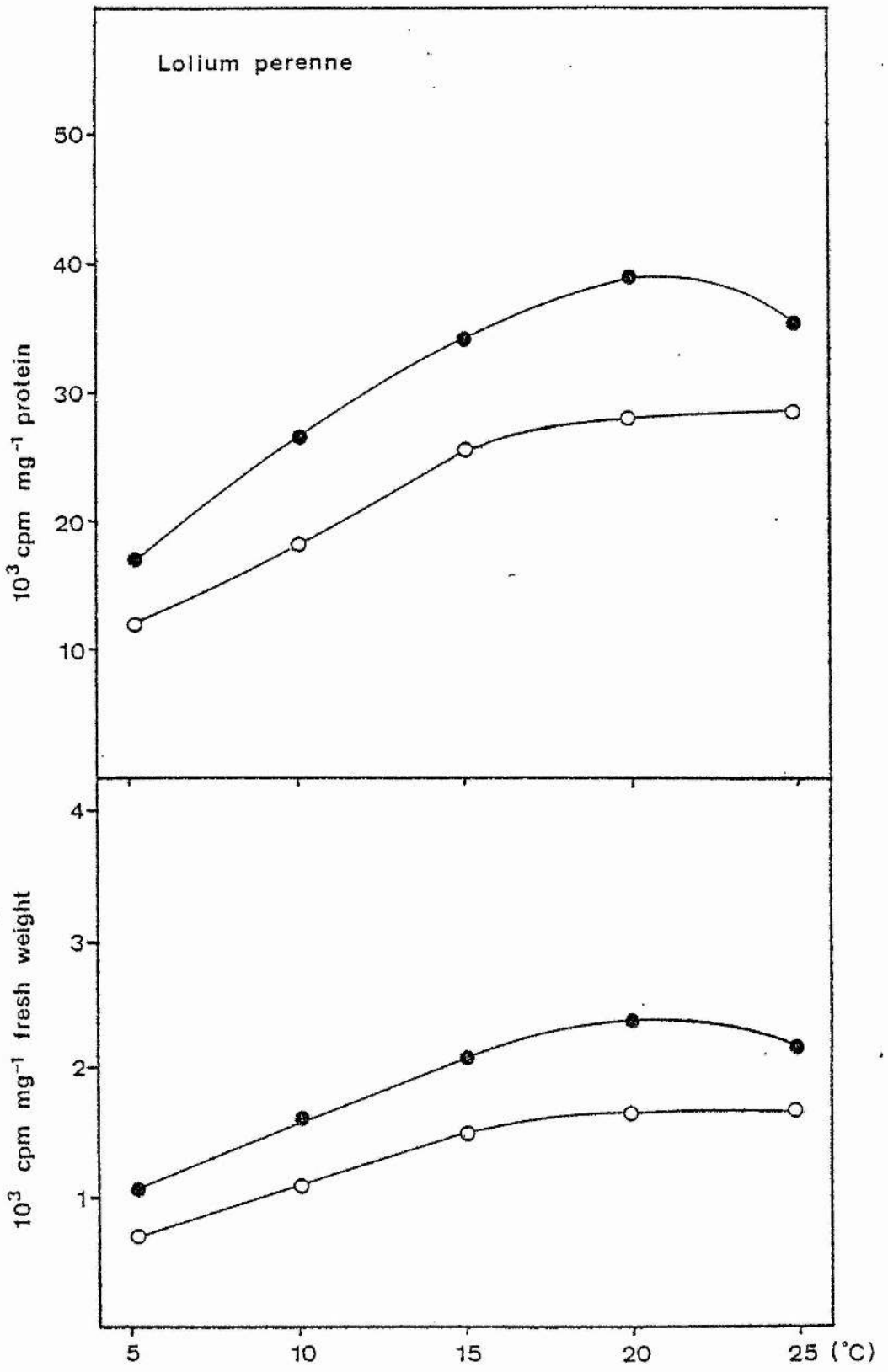


Fig. 6-2. RuBPCarboxylase activity in northern (●——●) and southern provenances (○——○)

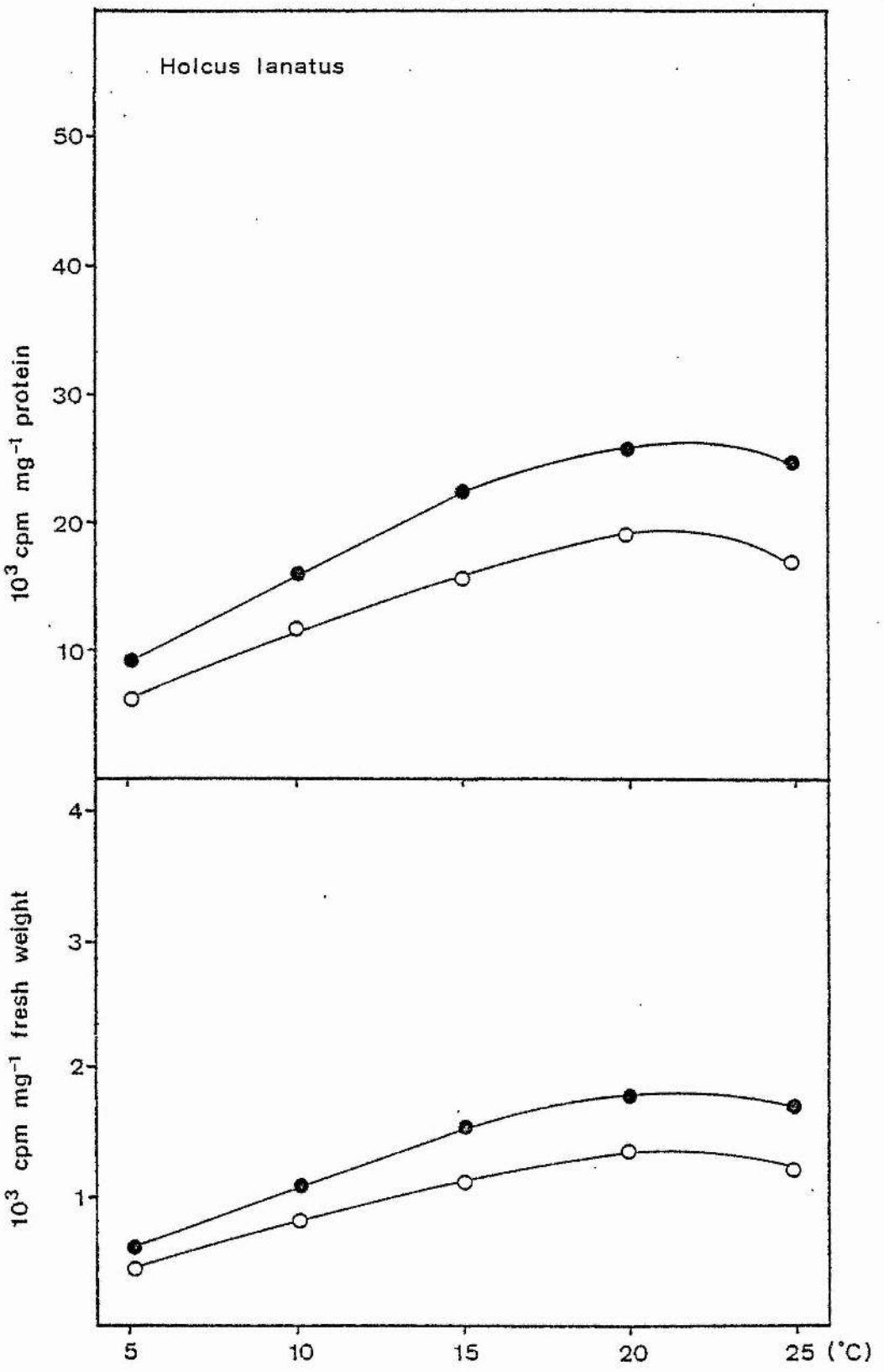


Fig. 6-3. RuBPCarboxylase activity in northern (●——●) and southern (o——o) provenances

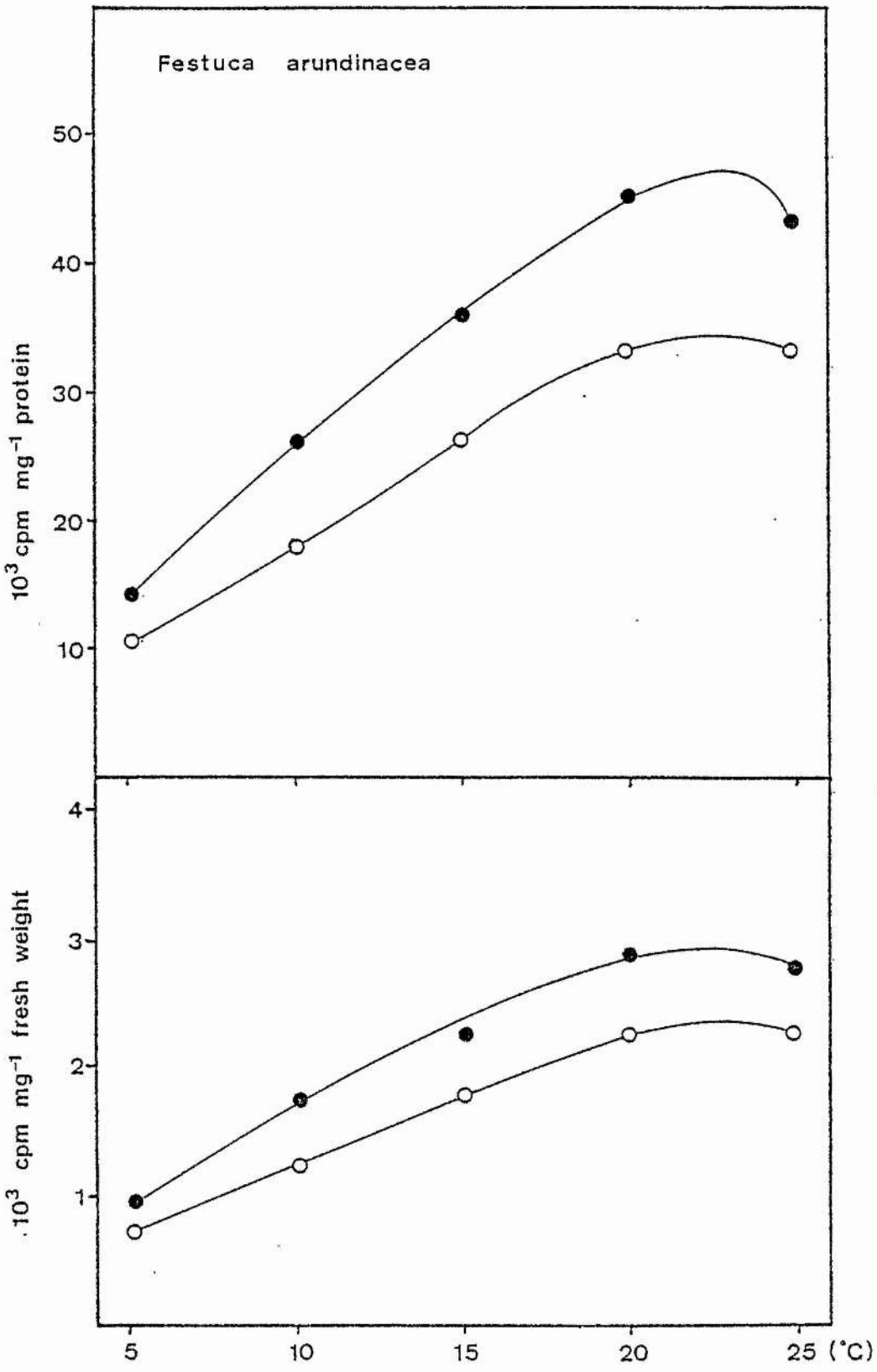


Fig. 6-4. RuBPCarboxylase activity in northern (●——●) and southern (○——○) provenances

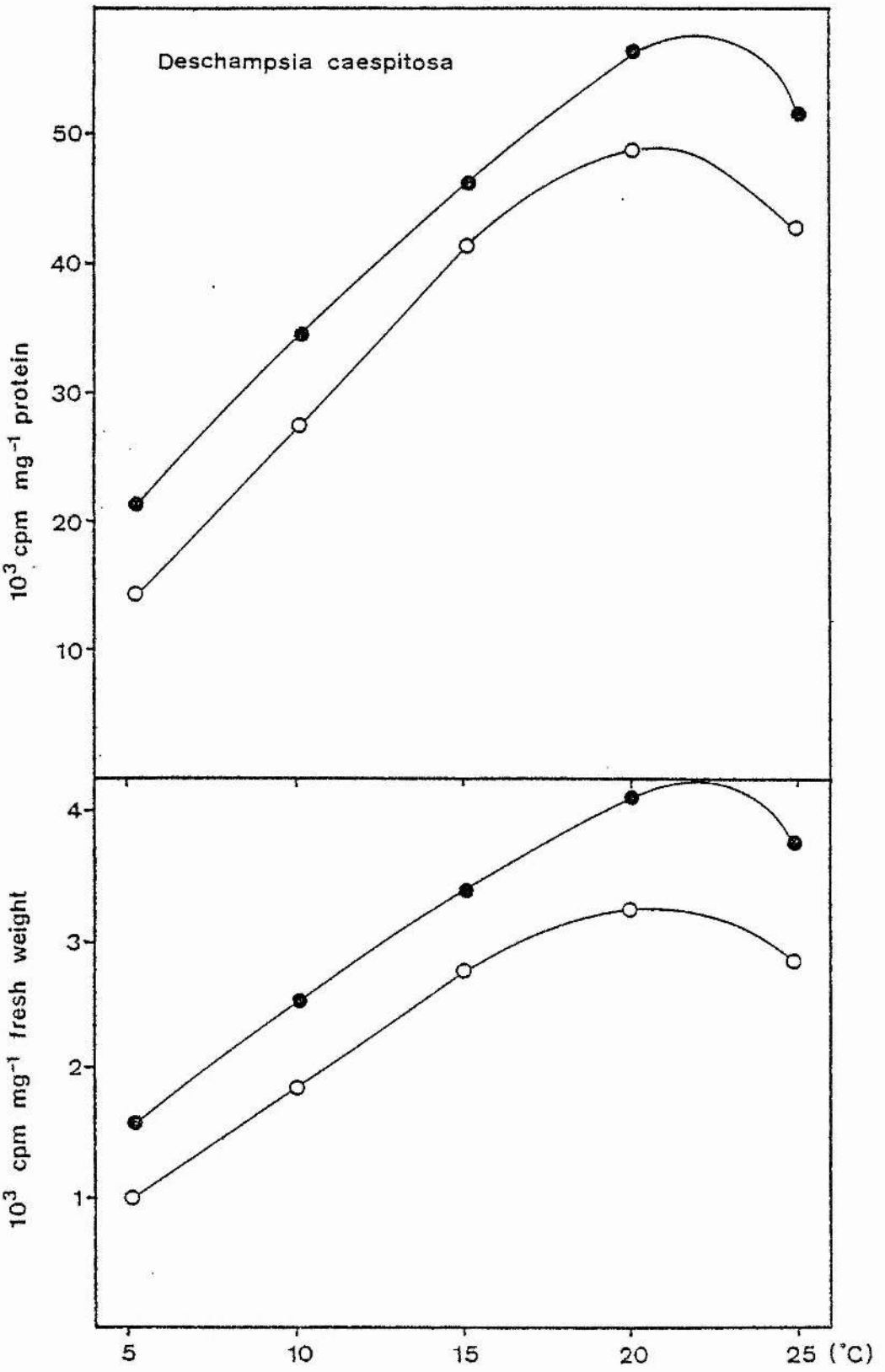


Fig. 6-5. RuBPCarboxylase activity in northern (●—●) and southern (○—○) provenances

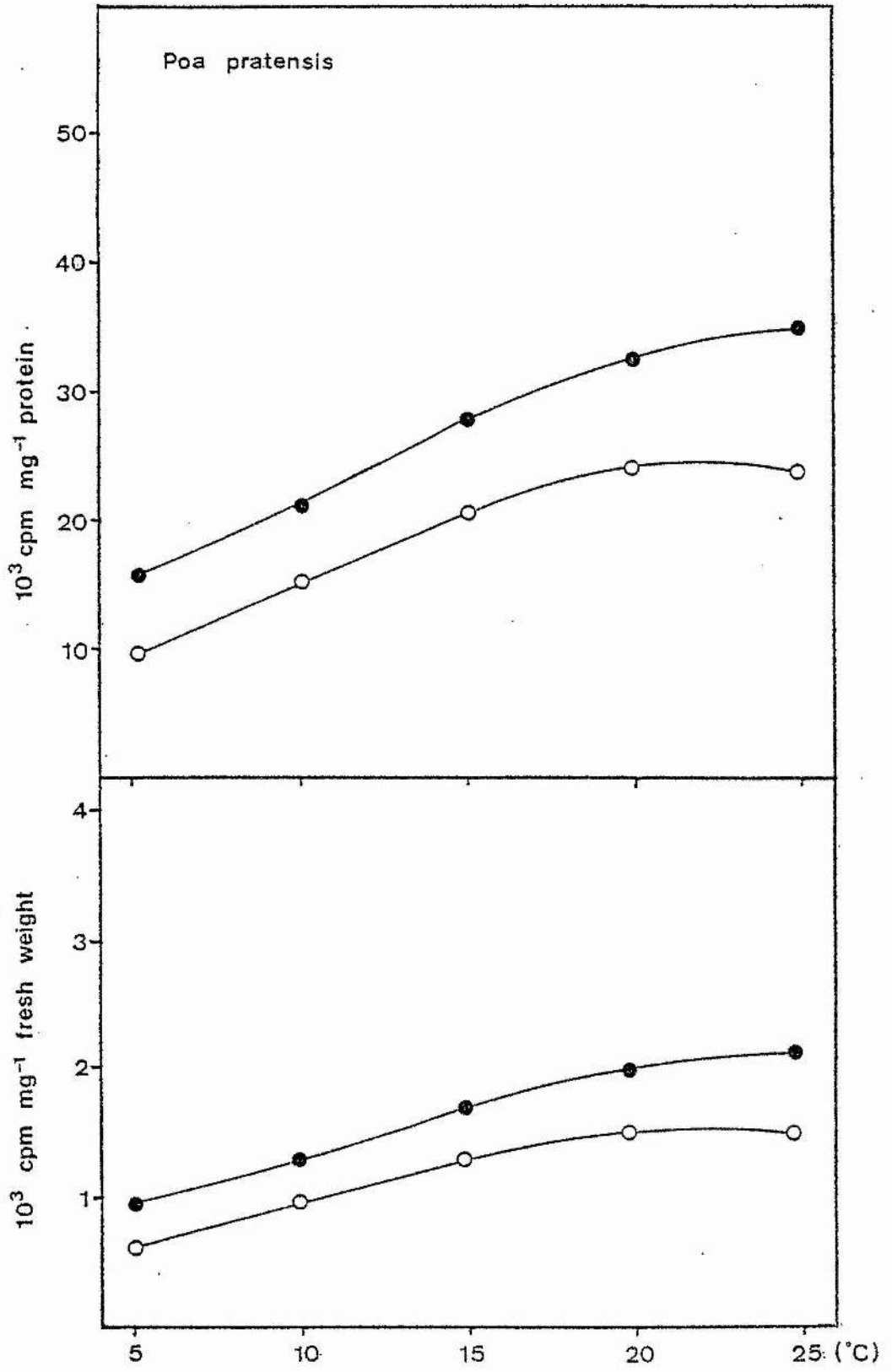


Fig. 6-6. RuBPCarboxylase activity in northern (●——●) and southern (o——o) provenances

mine temperature optima and thermal stabilities of the enzyme. Björkman and Badger (1976/77) cite reports that have demonstrated marked differences in the thermal stability of the photosynthetic apparatus among species native to habitats with contrasting thermal regimes. In the present experiments, RuBPCarboxylase from both northern and southern plants increases in its activity up to 20°C. Between 20°and 25°C a maximum is reached in many cases (see figs. 6-1 to 6-6). Does the inactivation of the enzyme at temperatures above 20°C show any correlation to the geographic origin? In table 6-2 the activities at 25°C have been expressed as percentage increase or decrease as compared to the activities at 20°C. There

Table 6-2. Change of RuBPCarboxylase activities, calculated as $\frac{\text{cpm } 25^\circ \cdot 100\ \%}{\text{cpm } 20^\circ} - 100\ \%$

Species	Origin	Change of activity
Dactylis glomerata	N	+ 0.6
	S	+ 4.2
Lolium perenne	N	- 9.2
	S	+ 2.3
Holcus lanatus	N	- 3.2
	S	-10.2
Festuca arundinacea	N	- 4.3
	S	+ 0.1
Deschampsia caespitosa	N	- 8.7
	S	-12.3
Poa pratensis	N	+ 7.5
	S	- 0.8

is apparently no pattern in these figures which would relate to the provenances of the plants. The temperature optimum of 20°-25°C in most examples contrasts with findings for other plants. The optimum was reported to be above 45°C for Oxyria digyna (Chabot et al.,1972), above 40°C for rice and barley (Ishii et al.,1977), 30°-32°C for Festuca arundinacea and 25°C for Lolium multiflorum (Nelson and Treharne,1974). The reason for these marked differences in activity is probably not that environmental stress selects for enzymes that are as much as 20°C apart in their optimum temperatures. It seems much more likely, that one cannot extrapolate from enzyme assays in vitro to the in vivo behaviour. Tieszen and Sigurdson (1973) have shown for the arctic grass genera Andropogon, Arctagrostis, Dupontia and Alopecurus, that the carboxylating enzymes were stable in vivo up to 40°C, whereas in vitro RuBPCarboxylase was considerably more temperature sensitive. Moreover, the optimum temperature for the uptake of CO_2 was lower than the optimum temperature for the carboxylation of ribulose-1,5-biphosphate. The authors conclude that the decrease in photosynthetic activity in arctic grasses at temperatures above 15°-25°C cannot be explained by inactivation of carboxylating enzymes but rather by complex interaction of metabolic reactions. These findings indicate that inactivation rates and optimum temperatures for RuBPCarboxylase from in vitro measurements have little meaning for the plant's response in nature.

It can be seen from figs. 6-1 to 6-6, that in the

range of 5°-15°C enzyme activities increased steadily without any flattening of the curves. Apparent energies of activation (E_a) were therefore calculated only for this temperature range. Only "apparent" activation energies could be calculated for three reasons: a) No initial velocities were measured and hence the enzyme activities do not equal the maximum catalytic activities; b) unpurified enzyme was used, and c) the pH of the buffer was not corrected at the several temperatures. The activation energies were calculated from Arrhenius plots where regression lines were fitted through the converted activity values obtained at 5°, 10° and 15°C (table 6-3).

Table 6-3. Apparent activation energies in kcal/mol for RuBPCarboxylase in the temperature range 5° to 15°C. r^2-values are the determination coefficients of the regression lines.

Species	Origin	E_a (kcal/mol)	r^2
Dactylis glomerata	N	14.3	0.9952
	S	14.7	0.9809
Lolium perenne	N	11.4	0.9742
	S	12.5	0.9960
Holcus lanatus	N	14.7	0.9809
	S	15.0	0.9591
Festuca arundinacea	N	13.9	0.9463
	S	15.0	0.9913
Deschampsia caespitosa	N	12.5	0.9831
	S	16.9	0.9792
Poa pratensis	N	9.5	0.9984
	S	12.5	0.9831

Activation energies for RuBPCarboxylase are all lower for plants from the north than for plants from the south. Activation energies of enzymic reactions may play an important role on an evolutionary scale in adapting an organism to its environment. A positive correlation has been suggested between the energy of activation of an enzymic reaction and the temperature to which the organism is adapted (Hochachka and Somero, 1973). In environments with limited thermal energy, natural selection may have favoured enzymes with lower activation energies. Thus, the lower activation energies noted for the grass species of northern origin may well reflect a certain degree of adaptation to the lower temperatures in the northern environment. On the other hand, Chabot et al.(1972) compared apparent energies of activation of RuBPCarboxylase in arctic and alpine populations of Oxyria digyna and failed to detect any significant differences. Alexandrov (1977) argues that higher activation energies for cold adapted populations can also be regarded as having its positive aspects in that only a slight increase in temperature would enhance the activity of a process considerably. I shall return to this ambiguity in the general discussion after presenting activation energies for other enzymic reactions in subsequent chapters.

Q_{10} values are frequently used as a measure of the temperature influence on the rate of a reaction. A simple relationship exists between the activation energy E_a and Q_{10}:

$$E_a = \frac{RT^2 \ln Q_{10}}{10}$$

where R is the gas constant and T the absolute temperature.

Lower values of E_a will thus be expected to be correlated with lower values for Q_{10}. This is the case for the examples in table 6-3 and is shown in table 6-4 column A which lists Q_{10} values for the temperature change of from 5° to 15°C. E_a values are often found to be constant with-

Table 6-4. Q_{10} values (column A) and activity differences (column B) of RuBPCarboxylase for the temperature difference 5°-15°C. The values in column B are calculated as (cpm/mg protein (15°C) - cpm/mg protein (5°C)) · 10^{-4}

Species	Origin	A	B
Dactylis glomerata	N	2.47	2.10
	S	2.48	1.46
Lolium perenne	N	2.04	1.75
	S	2.18	1.38
Holcus lanatus	N	2.52	1.36
	S	2.61	0.97
Festuca arundinacea	N	2.37	1.94
	S	2.55	1.60
Deschampsia caespitosa	N	2.18	2.53
	S	2.91	2.77
Poa pratensis	N	1.79	1.24
	S	2.18	1.11

in a range of temperatures of physiological importance or to show a rapid change at lower temperatures which is observed as a break in the Arrhenius plot of the reaction (Precht,1958). In a range of temperatures where E_a is constant, Q_{10} will decrease with increasing temperature. In cases where a break occurs in the Arrhenius plot, it is obvious that different Q_{10} values will exist for the

rate of the reaction at temperatures above and below this break. These and other facts show the only formal meaning of Q_{10}values and have led Precht (1956) to argue that the use of activation energies is more reasonable than the calculation of Q_{10}values.

It can be seen from the slopes in figs. 6-1 to 6-6 (pages 93-98), that RuBPCarboxylase activities in northern genotypes increase more with an increase in temperature than in southern genotypes. That is, temperature changes have greater influence in the northern than in the southern genotypes. Since the activities are different for northern and southern genotypes at all experimental temperatures, this relationship can only be seen if the activity differences between two temperatures are calculated rather than the Q_{10}values. This is shown in table 6-4 column B. With the only exception of RuBPCarboxylase from Deschampsia caespitosa, an increase in temperature from 5°to 15°C leads to a greater increase of activity in all preparations from northern plants.

It has been pointed out that results from in vitro measurements of optimum temperatures cannot be extrapolated to mean optimum temperatures under field conditions. It is of importance to an interpretation of the above results to know if an in vitro relationship of enzyme activities is meaningful for the plant as living organism. Studies of thermostabilities have demonstrated for leaf acid phosphatase (Feldman,1973) and for malate dehydrogenase (Simon,1979 a) that the enzymes are more stable in leaves than in extracts, but that the tendency of ge-

notypic differences *in vivo* is maintained *in vitro*. I assume that the data presented do not reflect the magnitude of differences *in vivo*, but that they are representative of their relation.

In conclusion, the results have demonstrated a higher activity of RuBPCarboxylase in grasses from higher latitudes. Thus, the activity of the key photosynthetic enzyme emerged as of considerable importance. Its higher activity and lower energies of activation in northern populations are interpreted as an adaptation on the enzyme level to the lower ambient temperatures in the north. The results can be seen in context with the studies of carbohydrate replenishment in chapter 5 and seem to explain, at least in part, the higher rates of carbohydrate accumulation in northern grass genotypes. It is also suggested that the rate of photosynthesis via the regulation of its rate by means of the carboxylating enzyme has more been subject of selection for fitness in the species investigated than their respiratory rates.

6.2. Ribulose-1,5-biphosphate carboxylase content in grasses from contrasting latitudes

6.2.1. Introduction

It was pointed out in section 6.1.3. that total protein content is a good basis to express RuBPCarboxylase activities. It is however not a perfect measure, for differences in the specific activity, in spite of the large quantities of RuBPCarboxylase present in the cells

of higher plants, could be due to different enzyme concentrations or other reasons. On the enzyme level, Hochachka and Somero (1973) have distinguished three mechanisms of metabolic compensation which function through alterations of a) enzyme concentrations, b) enzyme types and c) enzyme activities. The first mechanism is termed "quantitative strategy", and the authors emphasize that there are no studies which attempt to establish whether cold-adapted species contain more enzyme molecules than their warm-adapted counterparts. Only activity assays are available for the majority of enzymes, and activity is a ratio, the activity per amount of something. Either of the two parameters may change, with or without adaptive significance.

As far as RuBPCarboxylase is concerned, there are only indirect hints in the literature that the enzyme content might be important for the photosynthetic activity. Wareing and co-workers (1968) observed increased photosynthetic rates and increased protein levels after partial defoliation of bean, maize and willow plants. Gauhl (1976) subjected a sun-adapted variety of Solanum dulcamara which had been grown at low light intensity to bright light. The capacity for CO_2 fixation, the RuBP-Carboxylase activity and the protein synthesis increased within 6 days, and the author suggested that de novo synthesis of enzyme may account for the effects. Ku et al.(1979) found a linear relationship between RuBPCarboxylase activity and RuBPCarboxylase concentration in various C_3- and C_4-species, suggesting a causal rela-

tionship.

Due to its high concentration in photosynthetically active cells, RuBPCarboxylase is more suitable for researches into the quantitative strategy than any other plant enzyme. If we are to explain the 20 % or 30 % higher specific activities for the northern genotypes in terms of quantitative differences of enzyme, then one would expect appreciable differences in amount which may render possible quantitative determinations that overcome the limitations of inevitable measuring errors involved. The same genotypes as in the measurements of specific activity (table 6-1, page 89) have therefore been used to estimate the content of RuBPCarboxylase in relation to total protein.

6.2.2. Methods

Crude tissue extracts were subjected to vertical disc electrophoresis. After identification of the RuBP-Carboxylase band the amount of protein in all bands was measured by density scanning of the stained gels, and the content in RuBPCarboxylase was expressed as a percentage of total protein.

Preparation of crude extracts

0.5 g leaf material was homogenized with 5 ml of extraction buffer in an ice-chilled mortar. The medium of section 6.1.3. was used as extraction buffer, but with addition of 10 % sucrose to increase its specific weight. The slurry was pressed through 6 layers of mus-

lin and the suspension was centrifuged for 2 minutes in a Beckman Microfuge B. 30 µl of the supernatant was used per gel for electrophoretic separation, corresponding to roughly 100 µg protein. For each provenance two independent extractions were carried out, and gels were run in duplicate from each extract. Hence, 8 gels were run for each species, resulting from 2 provenances, 2 extractions for each provenance and 2 replicate gels for each extraction.

Disc electrophoresis

7 % acrylamide running gel was used. The gels were 0.5 x 6 cm in size and prepared freshly before each experiment. Unless stated differently, the procedure of disc electrophoresis was that described by Davis (1964). Eight gel tubes were arranged concentrically around the two electrodes in a perspex apparatus (Shandon Southern Instruments Ltd.). On each gel were placed 5 µl of bromphenol blue solution (0.04 mg/ml; 10 % sucrose) and 30 µl of crude extract. Electrophoresis was carried out at 5°C buffer temperature in a refrigerator. Stacking lasted for 20 minutes at 2 mA per gel. Running time was approximately 2 hours at 200 V.

The gels were then removed from the tubes and transfered to test tubes with staining solution (1 l of 20 % trichloro acetic acid in water plus 20 ml of dye solution composed of 0.25 g Coomassie Brillant Blue R 250 in 91 ml 50 % ethanol and 9 ml glacial acetic acid) where they were left for 6 hours. The staining solution was then

replaced by washing solution (250 ml methanol plus 75 ml glacial acetic acid plus 675 ml water). A little Whatman DE 52 ion exchanger was added to each test tube to bind the dye and keep the solution clear. After clearing overnight, the gels were stored in 7 % acetic acid until they were required for scanning.

Localization of the RuBPCarboxylase band on the gel

Extracts of Dactylis glomerata from Orkney were run as described above. After termination of electrophoresis, one of the gels was cut longitudinally into two halfs. The one half was stained, the other half was cut into transverse sections of 1 mm thickness with a gel slicer, starting at the origin of the running gel. Two and two of the 48 sections obtained were then transfered into 24 small ice-chilled test tubes containing 0.2 ml Tris extraction medium as in 6.1.2. The test tubes were placed in a water bath at 15°C and preincubated with 0.1 ml ribulose-1,5-biphosphate solution (ca. 0.1 µMol) for 5 minutes. 0.05 ml $NaH^{14}CO_3$ (5 µCi) were then added to each test tube. After 1 hour of incubation, the reaction was stopped by adding 0.1 ml 6 M acetic acid to each test tube. 0.1 ml aliquots from each test tube were spread out on planchets, dried for 30 minutes at 70°C and counted for 10 minutes per planchet as described in 6.1.2.

The result is plotted in fig. 6-7 where the stained half of the gel is arranged as abscissa in a graph to show the activity for each 2 mm section of the gel. It

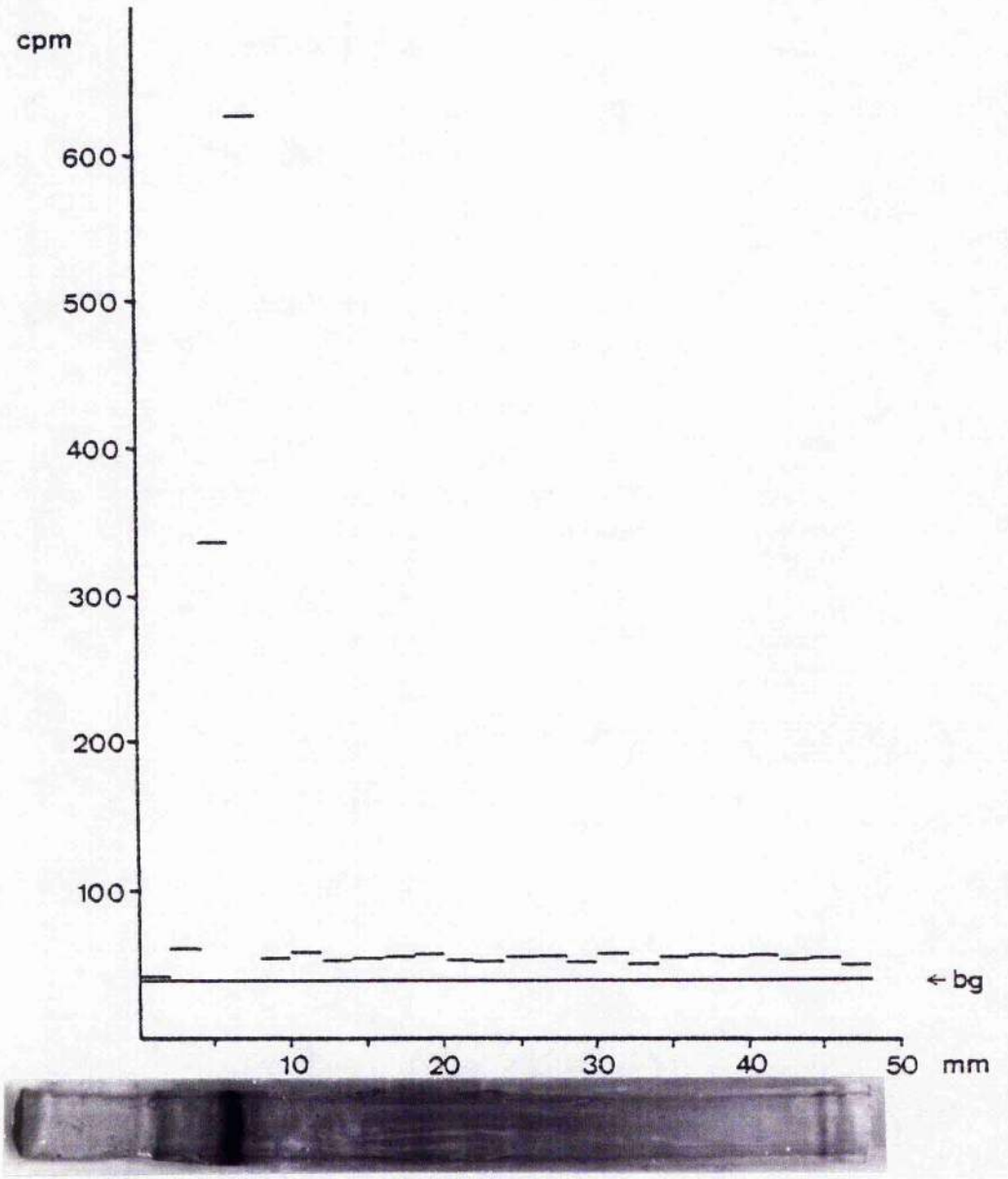


Fig 6-7. Localization of RuBPCarboxylase on the gel. Activity is found only in the intensely stained band close to the origin of the gel on the left. Background activity = bg

is clear that only in the strong band close to the origin of the gel there is RuBPCarboxylase activity. All other slices showed only slightly enhanced background activity. The strong band has not migrated far as would be expected from the high molecular weight of RuBPCarboxylase of about 560 000. This band appears not only in the Dactylis glomerata preparations but in all other species tested (see figs. 6-8 and 6-9, pages 113 and 114). This is evidence that the strongest band contains the RuBPCarboxylase.

Scanning of the gels on the densitometer

Weber et al.(1972) have shown that the amount of protein in each band can be determined by scanning the gel. This procedure relies on three assumptions (Weber et al.,1972): a) that different polypeptide chains have very similar colour yields; b) that the intensity of stain for a given component is a linear function of the amount of protein present; and c) that the staining procedure is highly reproducible. Points a) and b) have been confirmed in comparisons of the Coomassie blue dye binding method with the method of protein estimation after Lowry (Chiappelli et al.,1979). Point c) was secured in the present work by using 4 replicates of one species. Their staining pattern and intensity were identical (figs. 6-8 and 6-9, pages 113,114).

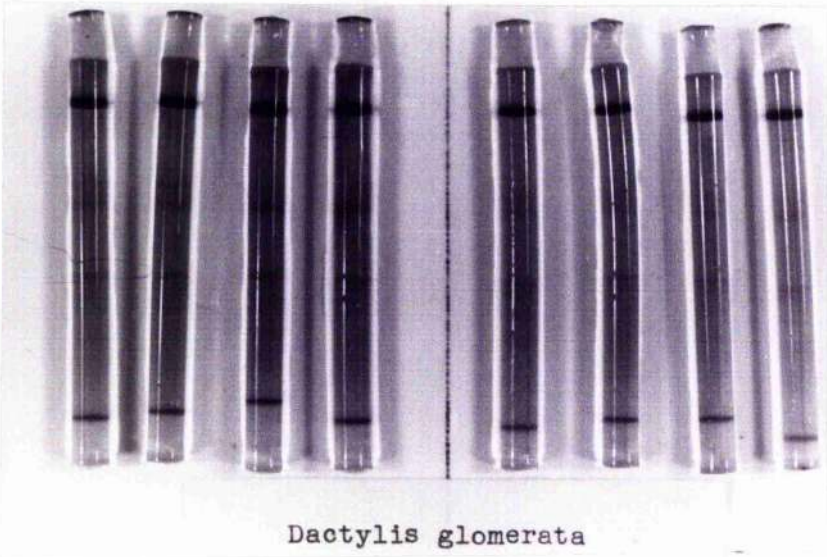
To measure the amount of RuBPCarboxylase and of total protein, each gel was scanned on a Vitatron TLD 100 Densitometer with recorder Vitatron UR 400. The absorp-

tion maximum of Coomassie blue is 555 nm, at 575 nm the absorption is still 83 % of the maximum absorption (Fazekas de St.Groth et al.,1963). Density scanning was done at 570 nm. Total protein and RuBPCarboxylase were calculated from the areas under the curves recorded. These were measured with a computer programme. In preliminary measurements the absorption in a gel which had been run without protein was compared with that of a clear gel which had not undergone electrophoresis and staining. These were found to be equal. So, since there was no background staining, no corrections needed to be made and the density of protein bands could be used directly as a measure of amounts.

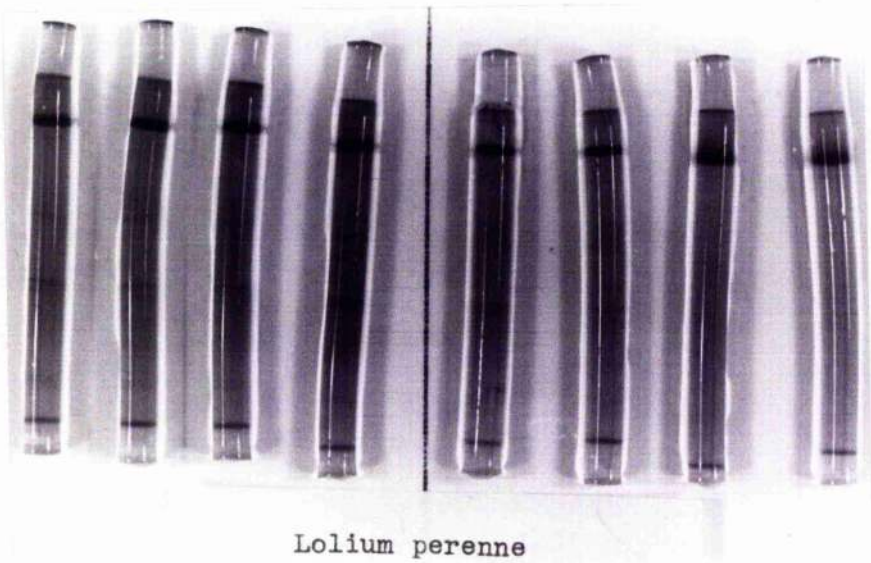
6.2.3. Results and Discussion

All gels were photographed (figs. 6-8 and 6-9) and their relative content of RuBPCarboxylase was estimated visually before scanning. (One replicate of the northern Festuca arundinacea was damaged; one replicate of the northern Poa pratensis could not be used for scanning because the front had run off the gel.) In no case were any differences visible between northern and southern samples of one species. The RuBPCarboxylase bands appeared to be somewhat denser in northern samples of Dactylis glomerata, Lolium perenne and Deschampsia caespitosa, but there was generally more staining on these gels, indicating a higher protein content in the crude extracts.

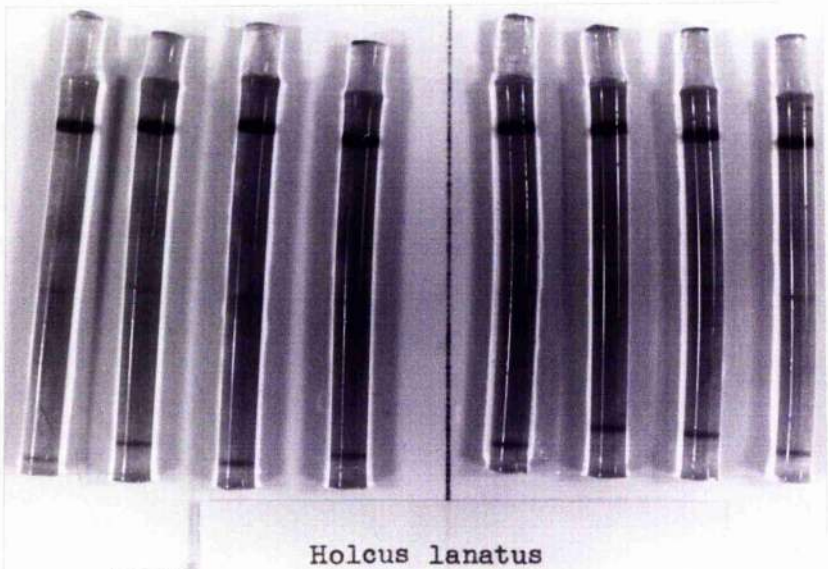
The results of the densitometric scanning of the gels are given in table 6-5 (page 115). The percentages of



Dactylis glomerata



Lolium perenne



Holcus lanatus

N S

Fig. 6-8. Electrophoresis gels of northern (left) and southern (right) provenances

Fig. 6-9. Electrophoresis gels of northern and southern provenances

RuBPCarboxylase are within the range of 23% - 59% reported for other C_3 species (Singer et al., 1952; Ku et al., 1979). Unfortunately, it was noticed that the resolution of the method employed does not permit statements about intraspecific differences in enzyme content. The t-test (table 6-5) shows that none of the differences noted for northern and southern plants are statistically significant. The main reason for the large standard

Table 6-5. Percentages RuBPCarboxylase of total protein in northern (N) and southern (S) genotypes. T-values and degrees of freedom in t-test: The critical values for t are 2.571 at 5 degrees of freedom and 2.447 at 6 degrees of freedom. I.e., none of the percentages for northern and southern genotypes are significantly different.

Species	% RuBPC. ± S.E. (N)	% RuBPC. ± S.E. (S)	t	deg. of freed.
Dactylis glomerata	38.4±3.8	36.6±2.3	0.40	6
Lolium perenne	37.8±6.1	44.1±6.8	-0.69	6
Holcus lanatus	50.6±4.1	43.8±1.9	1.52	6
Festuca arundinacea	27.0±5.6	25.7±2.0	0.25	5
Deschampsia caespitosa	34.5±1.9	30.9±3.5	0.90	6
Poa pratensis	39.1±8.6	33.1±1.1	0.82	5

errors in table 6-5 seems to be the relative insensitivity of the densitometer as compared to the human eye and its sensitivity for a slight unevenness of the gel. The eye is able to detect bands where the scanner does not record peaks. Minute scratches in the gel surface on the other hand, easily caused by rimming the gel when remo-

ving it from the glass tube, cause notable absorbance. Another complication may be a limitation of the validity of Lambert-Beer's law for the linearity of the relationship between content and absorbance. For paper electrophoresis and Coomassie blue, Fazekas de St.Groth et al. (1963) state a range of linearity for protein concentrations between 0.5 and 20 µg per cm. For gel electrophoresis, 1 - 15 µg protein have been indicated as a suitable range for gels of 6 mm diameter and 10 cm length (Weber et al.,1972). It is conceivable that the dye concentration in the RuBPCarboxylase bands was too high to give accurate readings.

For these reasons, I tend to trust the visual evaluation more than the quantitative results from scanning. The eye does not work quantitatively, but if no differences were detectable at all, this suggests that in fact the enzyme content in northern and southern plants was equal or very similar. But even if some meaning is admitted to the quantitative results, the differences in content could not account for the differences in activity. This is seen in table 6-6 where the differences are listed as a percentage when activity and content are taken as 100 % in northern plants. Under conditions of sufficiently high substrate concentrations, i.e. under unnatural conditions, the speed of an enzymic reaction may be doubled by using the double amount of enzyme. This means that if enzyme levels were higher in northern plants by up to 15 % as compared to southern ones, this would not cause an increase in enzyme activity of as

Table 6-6. Differences in content and activity of RuBP-Carboxylase between northern and southern provenances. Content and activity in the northern plants have been taken as 100 %. Activity differences are mean values of the measurements taken at 5°,10° and 15°C.

Species	content (%)	activity (%)
Dactylis glomerata	4.7	30.3
Lolium perenne	-16.7	29.3
Holcus lanatus	15.3	31.5
Festuca arundinacea	4.8	26.7
Deschampsia caespitosa	10.4	20.7
Poa pratensis	15.3	31.5

much as 20 % or 30 %. Under natural conditions where the substrate CO_2 is rate limiting the amount of RuBPCarboxylase would have to increase by considerably more than 30 % to cause an increase in activity of 30 %. It is therefore suggested that enhanced enzyme levels are at least not the only mechanism by which the species under investigation bring about higher enzyme activities in the northern populations. It is known that RuBPCarboxylase varies in its detailed structure and composition according to plant species (Strøbaek and Gibbons,1976). It is conceivable therefore that grasses use modified enzyme molecules in different climates, i.e. use the qualitative strategy of adaptation on the enzyme level.

In conclusion, no evidence has been found that the higher activity of RuBPCarboxylase from grasses of northern latitudes is due to a higher content of that en-

zyme. Higher contents may be a contributing factor. Numerous other causes can be thought up to explain higher specific activities. The determination of RuBPCarboxylase quantities from electrophoresis gels is a method not sufficiently accurate to allow conclusions as to the significance of concentration differences. There are further sources of error in this method which have not yet been mentioned: Other protein bands could be superimposed on the RuBPCarboxylase band, and changes in the amount of RuBPCarboxylase relative to total protein could be due to both changes in the amount of RuBPCarboxylase and changes in the amount of all other proteins. The more sophisticated techniques of density gradient centrifugation, gel chromatography or immunochemical methods will have to be employed to separate and purify RuBPCarboxylase from other proteins, in order to decide whether higher assimilation rates and higher activities of the carboxylating enzyme in grasses from the north can be attributed to higher enzyme concentrations.

7. TEMPERATURE COEFFICIENTS AND ACTIVATION ENERGIES OF SUCCINATE DEHYDROGENASE IN INTACT MITOCHONDRIA FROM LEAVES OF PERENNIAL GRASSES FROM LATITUDINALLY DIVERSE POPULATIONS

7.1. Introduction

The previous chapter described attempts to trace back to the enzyme level the differences in temperature response of carbohydrate accumulation in northern and southern populations. The higher potential for carbohydrate replenishment and the greater temperature influence on the speed of carbohydrate formation in northern plants could be correlated with corresponding properties for CO_2 fixation of the carbon fixing enzyme in these plants. Since all experiments were performed with plants grown under the same conditions, the results reflect genetically rather than physiologically based differences. These findings provide further support for theories which suggest that, on an evolutionary scale, enzymes are subject to selection to fit a particular thermal environment. Most studies into thermal adaptations on the enzyme level (reviewed by Hochachka and Somero, 1973) have used ectothermic animals such as fishes and amphibians, whereas few and inconsistent analyses are available for higher plants. Experiments reported in this and the following chapter were carried out to establish whether genetically based differences were detectable also with enzymes other than RuBPCarboxylase which could be interpreted as contributing to fitness through

their regulation of essential metabolic processes.

Somero (1969) has suggested that selection for interenzymic differences is more likely to occur with enzymes that are rate-limiting catalysts in a particular metabolic pathway than with enzymes which are not rate-limiting and therefore without regulatory function. May (1976) cites evidence that succinate dehydrogenase (EC 1.3.99.1) functions as a regulator of the Krebs cycle and of oxydative phosphorylation. It can thus be regarded as a key enzyme. It is a lipoprotein with a molecular weight of approximately 200 000 and forms part of the succinate-cytochrome oxidase system (Dixon and Webb, 1958). In his initial examination of the temperature response of succinate dehydrogenase in plants, May (1976) found minimum Q_{10} values in the temperature range approximating that of the natural habitat in Taraxacum officinale. Although his estimations were carried out with saturating substrate concentrations and have therefore probably led to misleadingly high Q_{10} values, they do reflect climate related differences which may be of adaptive significance.

The present investigation of the temperature response of succinate dehydrogenase in grasses used, in contrast to May's examination, a range of different species. Furthermore, they were from latitudinally rather than altitudinally diverse origins (except for a preliminary experiment which was to establish whether thermal enzyme properties could be expected to differ with the site of origin). May did not determine whether the diffe-

rences in the temperature response were genetically based or result of physiologically based short-term acclimatization. Plants in this study have been cultivated identically so that short-term acclimatization effects should be excluded. Finally, May found that enzyme-substrate affinities did not differ significantly between temperatures or populations; activation energies were not determined. The measurements with grasses were done so that activation energies could be calculated, but enzyme-substrate affinities were not estimated.

Since succinate dehydrogenase is a membrane-bound enzyme, intact mitochondria were used in order to allow experimentation in an undisturbed state. Earlier manometric studies of isolated mitochondria have suggested that ectotherms native to cold habitats should be selected for enzymes with low activation energies (Vroman and Brown, 1963), and that the oxidative rates of mitochondria are genetically controlled and higher in populations of colder growing sites (Klikoff, 1966).

7.2. Materials and Methods

A first experiment used leaves of Deschampsia caespitosa plants which were collected at two different altitudes on the west coast of Scotland. For all subsequent experiments, leaves were harvested from the stock collection of latitudinal races growing in the glasshouse. The species used are shown in table 7-1.

The method of mitochondria isolation and enzyme assay was modified after May (1976). Ten g of mature leaves were

Table 7-1. Species and provenances used for the measurement of the temperature response of succinate dehydrogenase in intact mitochondria.

Species	Provenances	Latitude
Deschampsia caespitosa	Ben Vair,Scotland,sea level	57°N
	Ben Vair,Scotland,565 m	57°N
Dactylis glomerata	Orkney Is.,Scotland	59°N
	Lazio, Italy	42°N
Poa pratensis	Fife, Scotland	56°N
	Lazio, Italy	42°N
Festuca arundinacea	England	52°N
	Morocco	32°N
Lolium perenne	Orkney Is.,Scotland	59°N
	Lazio, Italy	42°N
Holcus lanatus	Orkney Is.,Scotland	59°N
	Corfu, Greece	39°N

washed and cut into small sections. These were homogenized for 30 seconds with 50 ml of ice-chilled homogenizing medium (0.25 M sucrose; 1.5 mM EDTA; 0.5 % bovine serum albumin; 6.0 % polyvinyl pyrrolidone m.w. 40 000; in 0.05 M HEPES-NaOH buffer pH 7.4) with an Ultra Turrax TP 18/10 Homogenizer. The slurry was squeezed through 6 layers of muslin into an ice-chilled beaker. The filtrate was centrifuged twice at 5000 g and 2°C for 5 minutes and the pellets were discarded. The supernatant was then centrifuged for 30 minutes at 20 000 g and 2°C. The supernatant was discarded and the pellet resuspended in 10 ml ice-cold suspension medium (0.25 M sucrose; 0.02 M glucose; in HEPES-NaOH buffer 0.05 M pH 7.4), using a glass homogenizer. The protein content in the mitochondrial

suspension was estimated with the Folin method (Plummer, 1971).

To estimate the succinate dehydrogenase activity, dichlorphenolindophenol (DCIP) was used as an electron acceptor in a system of coupled reactions:

Succinate → FAD → Leuco-DCIP (colourless)

Fumarate ← $FADH_2$ ← DCIP (blue)

Assays were performed by incubating cuvettes containing 0.5 ml HEPES-NaOH buffer 0.05 M pH 7.4; 0.1 ml DCIP (1.7×10^{-3}M in buffer; fresh solution every week); 0.1 ml KCN 0.1 M; 0.1 ml $MgSO_4$ 0.25 M; 1.6 ml H_2O and 0.4 ml mitochondrial suspension. The cuvettes were placed in the jacket of a Unicam SP 1800 Ultraviolet Spectrophotometer which was connected to a water bath maintained at a selected temperature. The absorbance of the reaction mixture was recorded at 600 nm. Incubation was continued until absorption or absorption change was constant. Following incubation, 0.2 ml of sodium succinate (0.17 M) were added and the reduction of DCIP was recorded at 600 nm.

Since there is no satisfactory method for the isolation of mitochondria from green leaves (Leech, 1977), the mitochondrial suspensions contained chloroplast fragments. The decrease in absorption of DCIP could nevertheless be followed as chlorophyll absorbs very little at the absorption peak of DCIP at 600 nm. The concentration of succinate (11.3 mM in the reaction mixture) was found by May (1976) to saturate the enzyme in all populations and at all temperatures. In random samples with grasses, increasing the concentration of succinate did not result

in increased reaction velocities. So, the above concentration of succinate was used throughout. Assay controls without mitochondrial suspension did not show any reduction of DCIP. It was however noticed that the mitochondrial suspension, although kept on ice, was not stable and lost most of its activity in the course of 24 hours. Assays were therefore performed immediately after preparation.

Enzyme activities were calculated from the difference in the rate of absorption change before and after addition of substrate. Statements of the extinction coefficient of DCIP at 600 nm vary in the literature from 15.6 $cm^2/\mu Mole$ to 19 $cm^2/\mu Mole$ (Bergmeyer,1965). The value of 16.0 $cm^2/\mu Mole$ was adopted here. All assays were carried out in triplicates which agreed within 20 %.

7.3. Results

A pilot experiment was performed with population samples of Deschampsia caespitosa from two altitudes. Deschampsia caespitosa is a rather variable grass, especially in size and length of the leaves (Hubbard,1976). It was therefore considered likely that morphological differences are accompanied by physiological differences. The plants were taken from Ben Vair near Fort William on the west coast of Scotland. One lot was collected at sea level and one at the timber line at an altitude of 565 m. The plants from the two sites differed markedly in size and leaf length. They were taken back to the laboratory in plastic bags, stored in the open air at about $3^{\circ}C$

night and 9°C day temperature and assayed on the two days following sampling.

Mean temperatures decrease with increasing altitude. The Meteorological Office has adopted a standard temperature lapse rate of 6.0°C per 1000 m rise in elevation (Taylor, 1976). Accordingly the two populations have been growing at annual mean temperatures which were 3.4°C apart. It was calculated from the climatic data given by the Meteorological Office (1958) that these mean temperatures were approximately 9.2°C and 5.8°C respectively.

Since all measurements in this study were made at saturating substrate concentrations, the results indicate the maximum velocity of succinate oxidation catalyzed by the succinate dehydrogenase preparations. Fig. 7-1 illustrates the findings for the two populations of Deschampsia caespitosa. It must be noted that the degree of activity per se has no biological significance in these experiments as the results could not be expressed in terms of specific activity. The mitochondrial suspensions contained still considerable amounts of protection protein (bovine serum albumin), estimated as between 30 and 70 % of total protein. The protein assays carried out in most of the experiments were only to make sure that the amounts of total protein were not drastically different in different preparations. Bovine serum albumin from extraction could be expected to constitute a fairly constant proportion of protein in the mitochondrial suspensions.

The Arrhenius plot (bottom graph in fig. 7-1) shows no straight lines for succinate dehydrogenase activity in

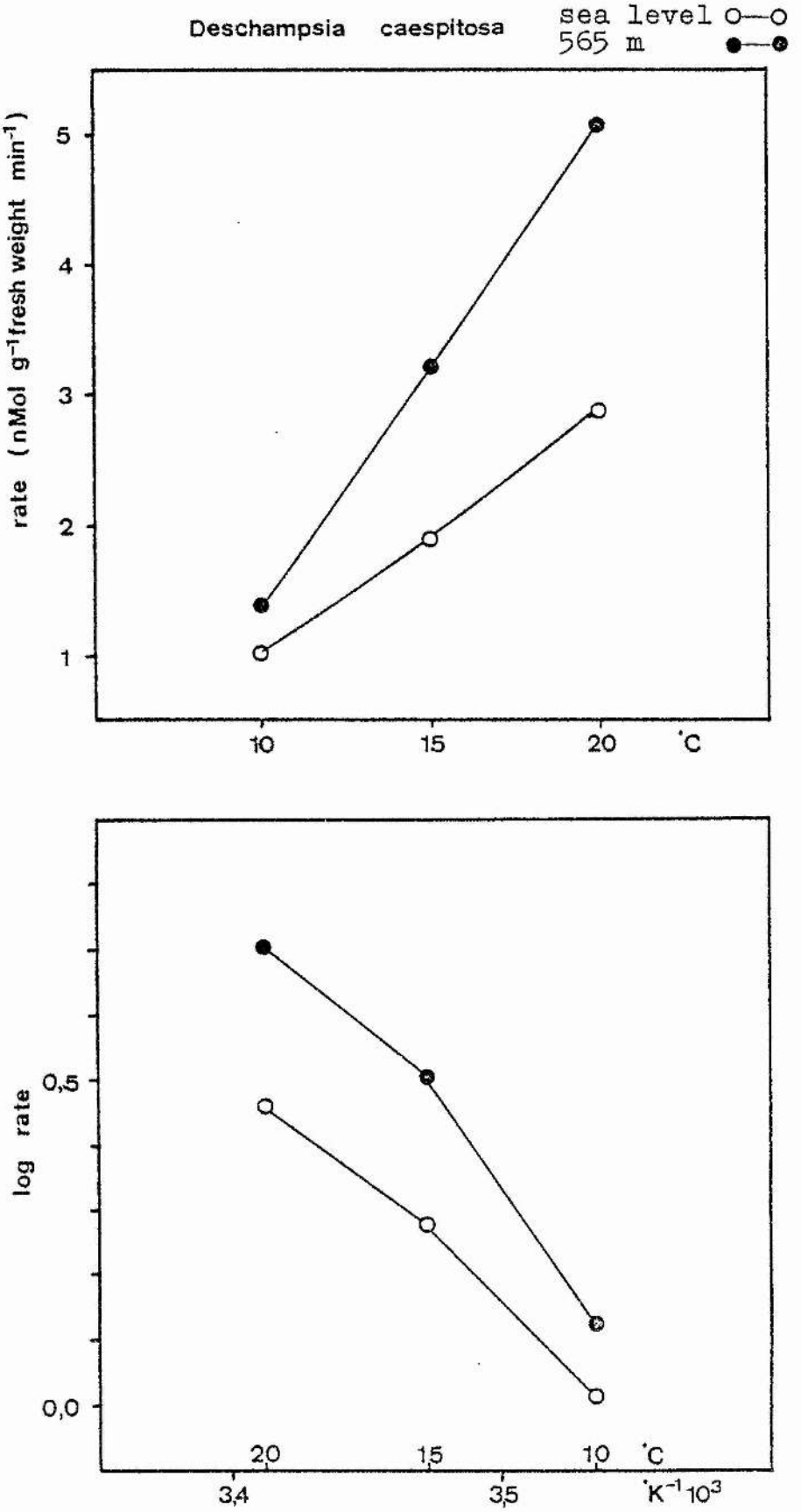


Fig.7-1. Activity (top) and Arrhenius plots (bottom) of succinate dehydrogenase preparations from Deschampsia caespitosa from two different altitudes

the range 10°-20°C. Temperature coefficients rather than activation energies were therefore calculated. These were

	Q_{10} (10°-20°C)
Deschampsia caespitosa, 0 m	2.80
Deschampsia caespitosa, 565 m	3.79

Thus, as in May's investigation with Taraxacum officinale, population samples from different altitudes differed in their temperature coefficients of succinate dehydrogenase. This difference could be due to physiological or genetical differentiation or both.

In subsequent experiments five pairs of population samples from identically grown grass species were used to look for genetical differentiation. The temperature response of the mitochondria preparations was measured at 5°, 10°, 15°, 20° and 25°C. The results are shown in the top graphs in figs. 7-2 to 7-6. As pointed out above, higher activity of succinate dehydrogenase does not indicate higher in vivo activities. This is particularly true as higher activities occurred in conjunction with higher protein contents in the mitochondrial suspensions (see figs. 7-2 to 7-6 and table 7-2, page 133).

When the temperature coefficients are calculated for the two temperature ranges 5°-15°C and 15°-25°C (table 7-2), it is seen that only the latter show a pattern related to the geographic origin of the plants. Higher Q_{10} values for 15° to 25°C were consistently found for the plants of northern origin.

Since higher Q_{10} values are synonymous with higher

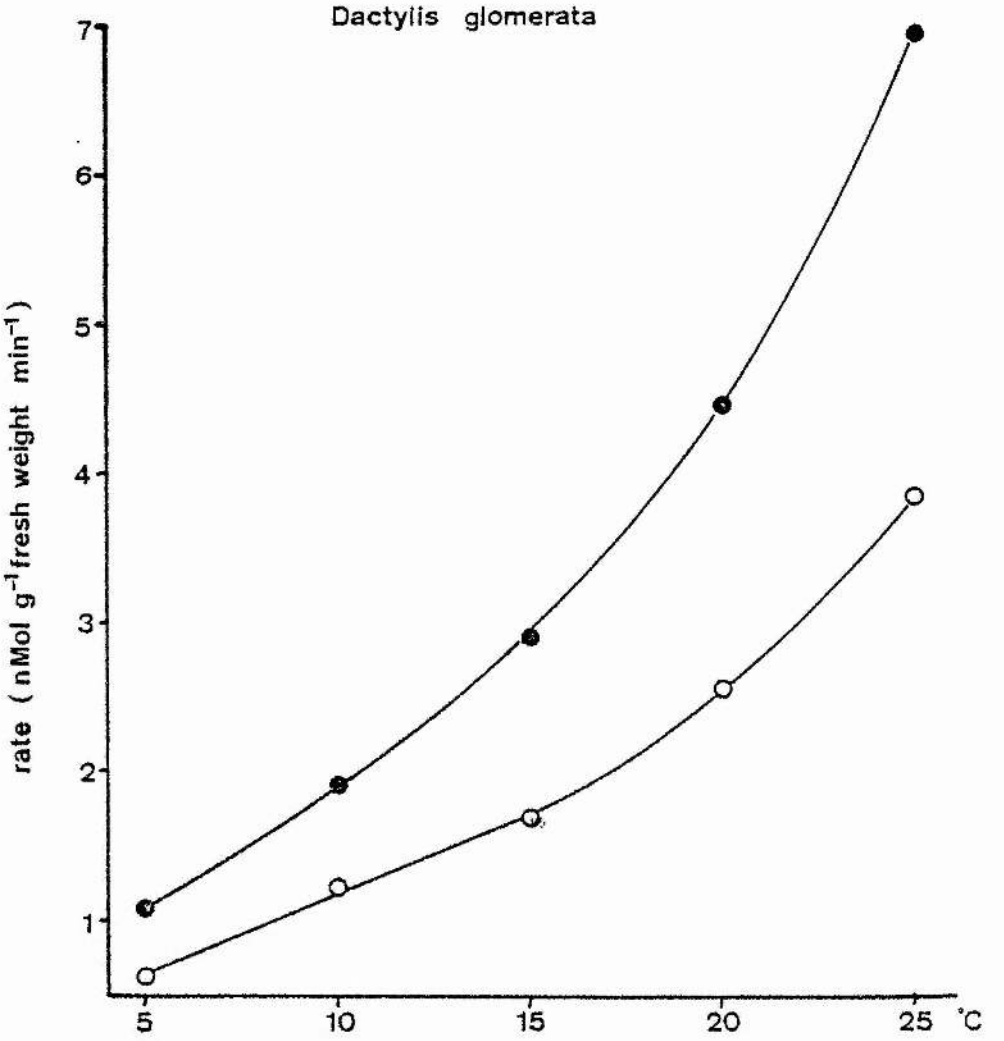
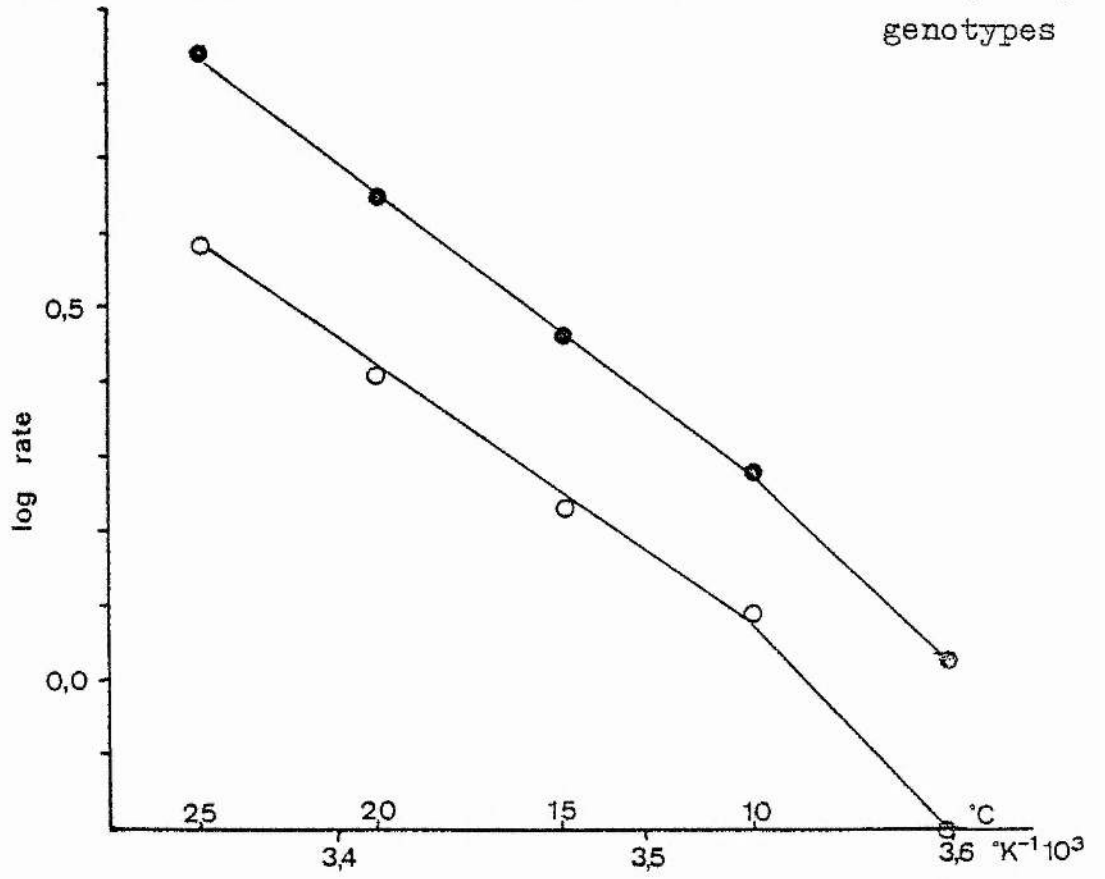


Fig. 7-2. Temperature response of succinate dehydrogenase in mitochondria of northern (●——●) and southern (○——○) genotypes



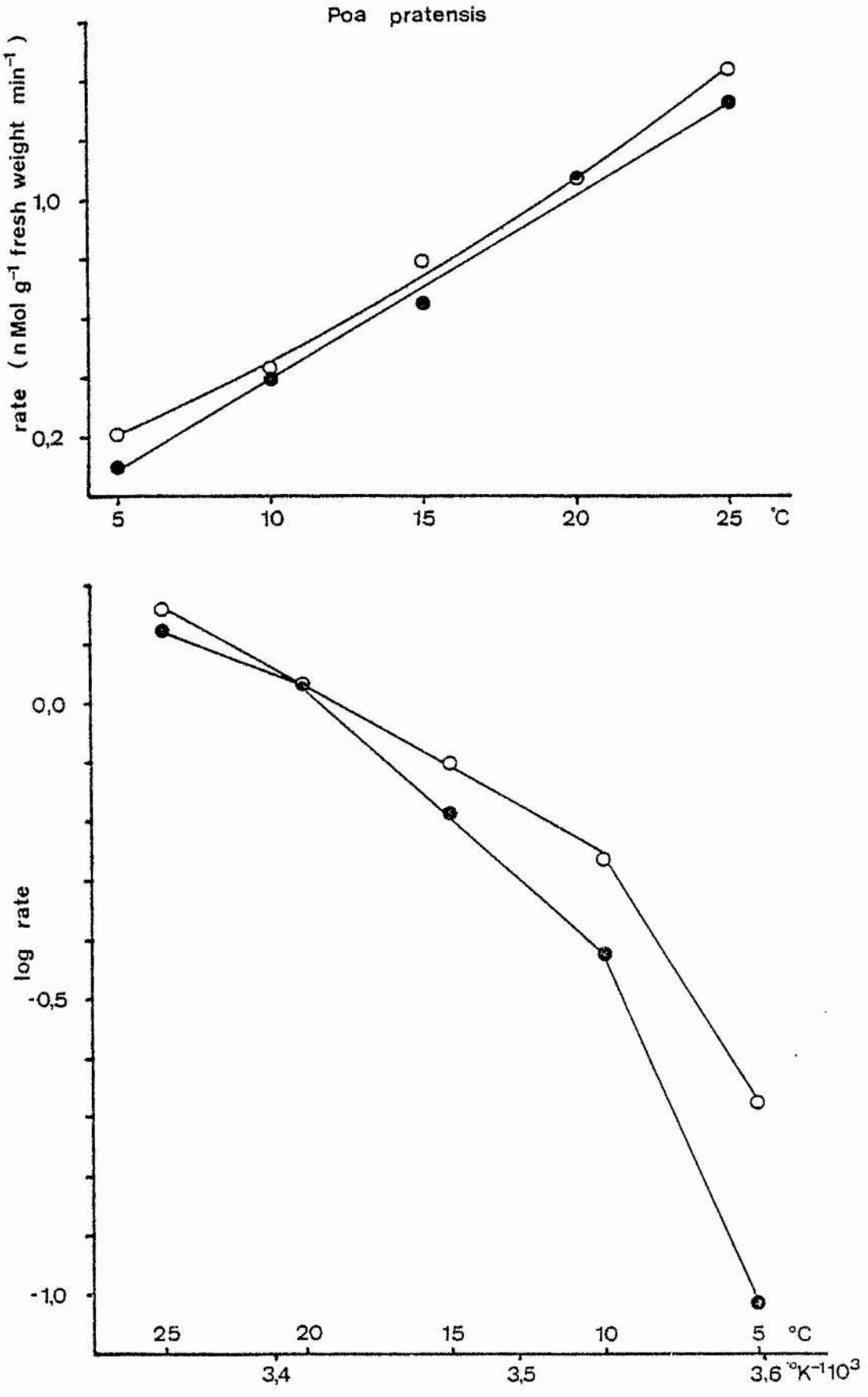


Fig. 7-3. Temperature response of succinate dehydrogenase in mitochondria of northern (●——●) and southern (○——○) genotypes

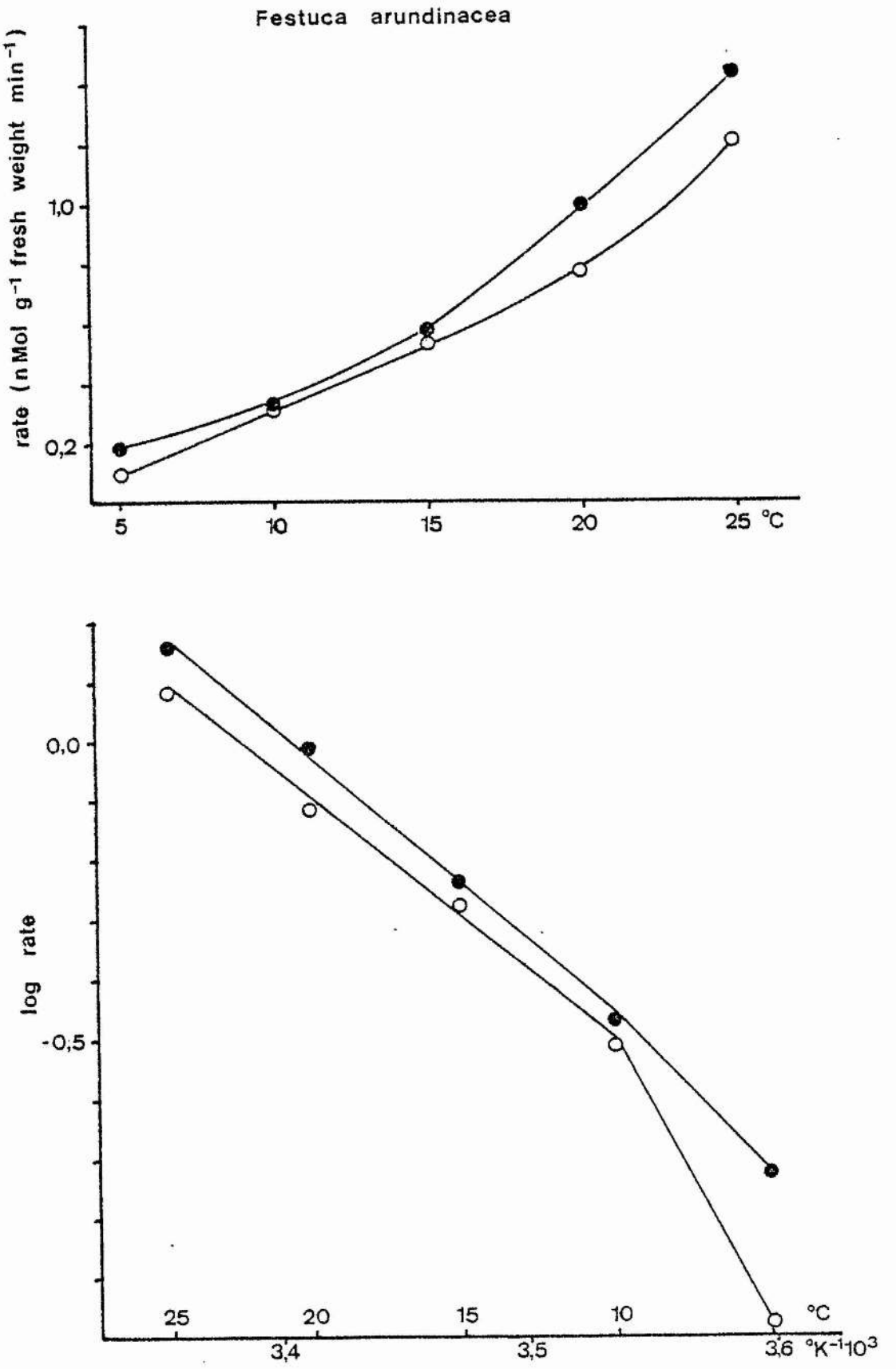


Fig. 7-4. Temperature response of succinate dehydrogenase in mitochondria of northern (●——●) and southern (○——○) genotypes

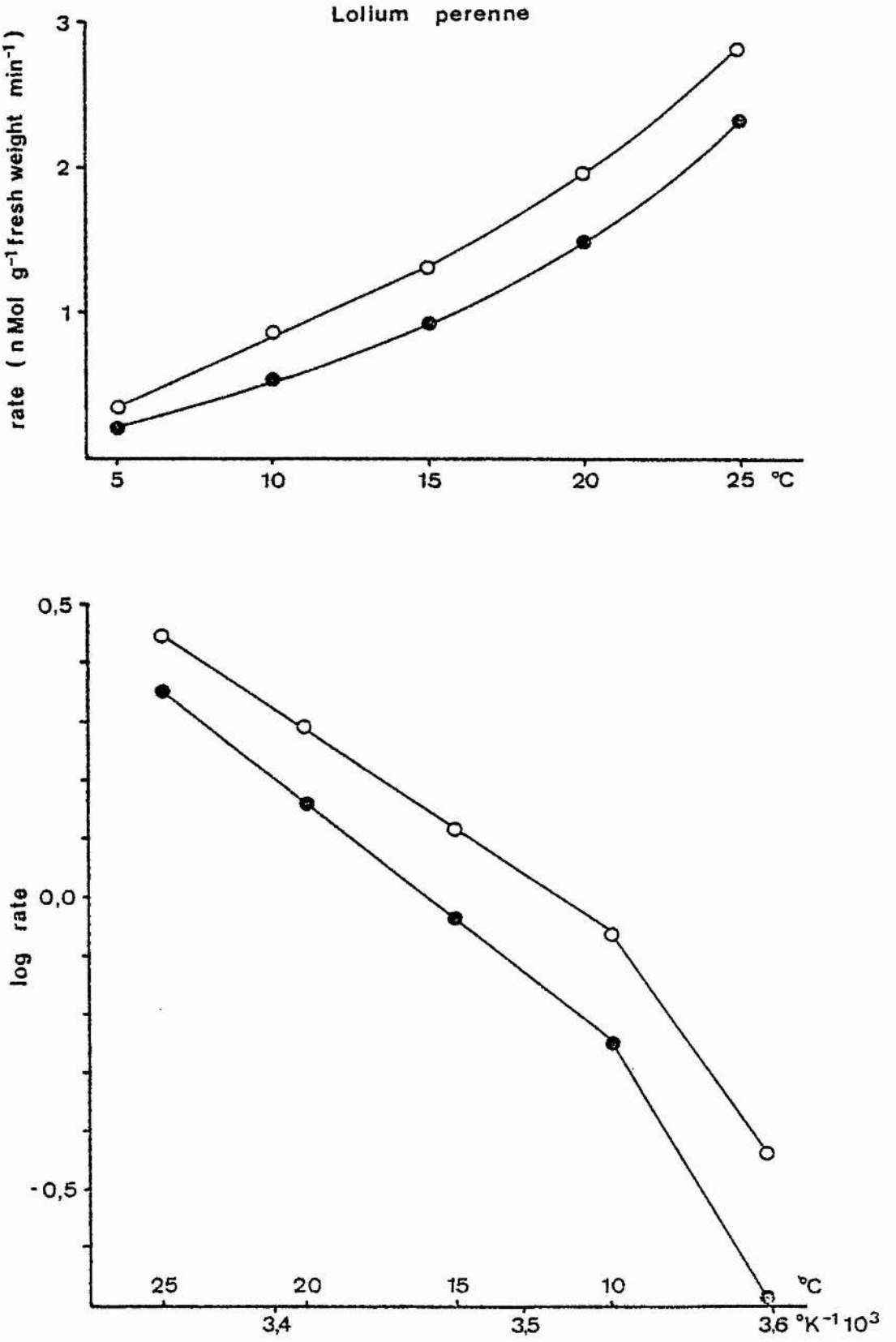


Fig. 7-5. Temperature response of Succinate dehydrogenase in mitochondria of northern (●——●) and southern (○——○) genotypes

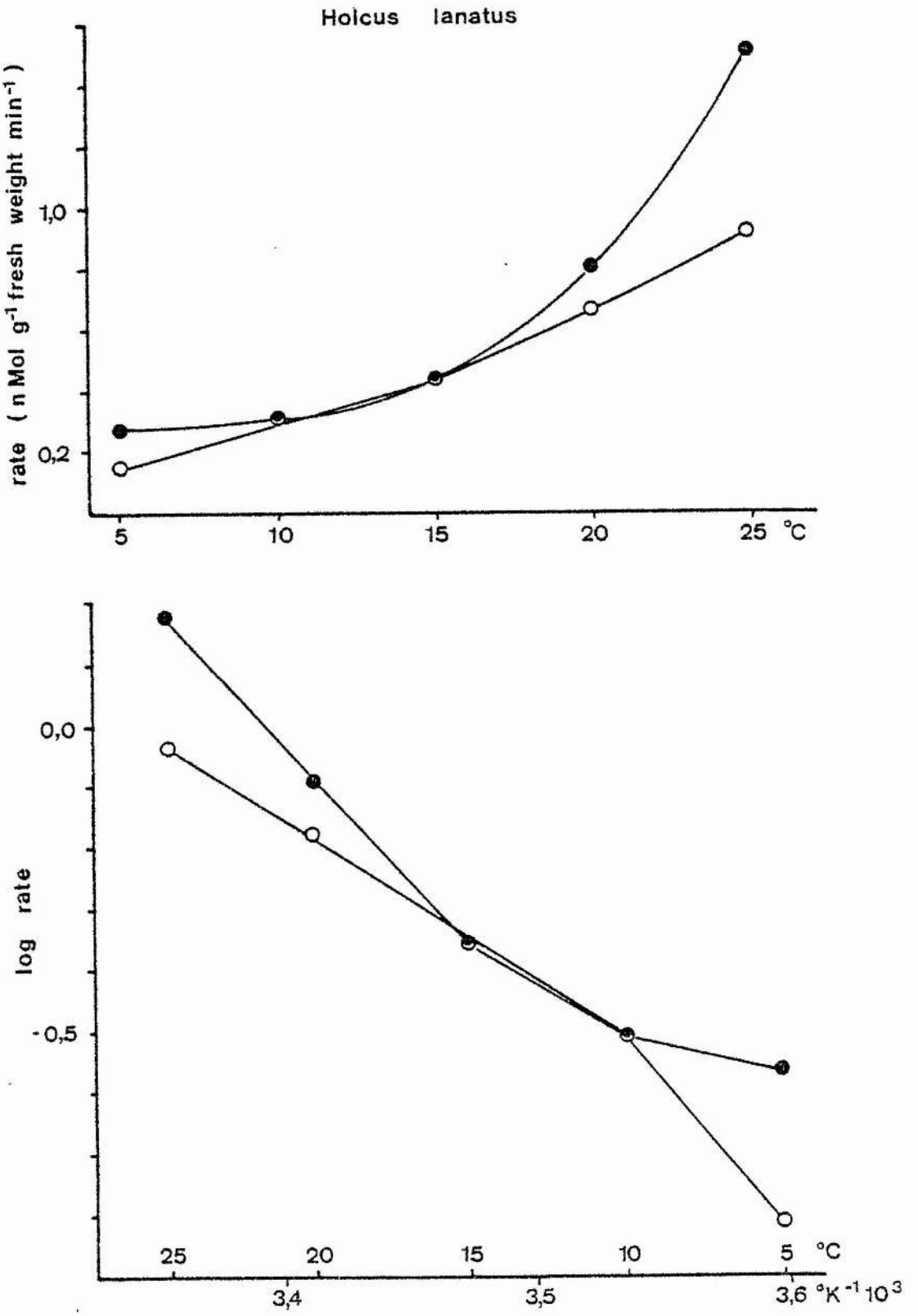


Fig. 7-6. Temperature response of succinate dehydrogenase in mitochondria of northern (●——●) and southern (○——○) genotypes

Table 7-2. Protein contents of the mitochondrial suspensions, temperature coefficients (Q_{10}) and energies of activation for five pairs of samples from latitudinally diverse populations. Coefficients of determination r^2 of the regression lines in the Arrhenius plots.

		Tot.protein in suspension (mg/ml)	Q_{10} (5°-15°)	Q_{10} (15°-25°)	E_a (kcal/mol)	r^2
Dactylis glomerata	N	7.9	2.69	2.40	14.5	0.9991
	S	6.7	2.72	2.28	12.8	0.9936
Poa pratensis	N	4.2	6.65	2.04	14.3	0.9723
	S	4.7	3.71	1.82	13.1	0.9720
Festuca arundinacea	N	4.2	3.06	2.49	16.2	0.9968
	S	4.1	5.06	2.26	15.0	0.9957
Lolium perenne	N	5.5	4.45	2.44	15.4	0.9999
	S	6.1	3.54	2.14	13.1	0.9997
Holcus lanatus	N	5.8	1.60	3.42	17.7	0.9783
	S	-	2.11	2.83	12.4	0.9979

activation energies, the northern plants will also possess higher activation energies for succinate dehydrogenase. The bottom graphs in figs. 7-2 to 7-6 show straight lines in the Arrhenius plots in the temperature range 10° to 25°C for most examples. Activation energies could therefore be calculated with higher accuracy using the four points at 10°, 15°, 20° and 25°C rather than just the two points from the Q_{10} estimate. E_a's are listed in table 7-2. All higher values belong to northern provenances. There was consistently a change of activation energies between 5° and 10°C towards higher values at the lower temperature.

7.4. Discussion

The experiments have shown higher Q_{10} values for the cold adapted plants from northern latitudes and for the high altitude Deschampsia caespitosa. The differences between the populations from contrasting latitudes must be genetically-based. The higher activation energies in northern populations are in contrast with suggestions derived from studies of ectothermic animals according to which cold adapted species should be selected for lower activation energies to improve catalytic efficiencies. For example, Vroman and Brown (1963) found the activation energy of succinate dehydrogenase in the rat was three times as high as in the frog; and Somero and co-workers (1968) determined the extremely low value of 3.78 kcal/mole for succinate dehydrogenase from the Antarctic fish Trematomus bernacchi in the temperature range 5°-30°C. However, these authors have not made comparisons between ectotherms acclimatized at different temperatures. Furthermore, their determinations were done manometrically rather than spectroscopically, and Somero et al.(1968) have emphasized that for comparison of results similar methods should be employed.

This is the first examination of activation energies for succinate dehydrogenase from higher plants. It would be premature to draw conclusions from higher temperature coefficients and higher activation energies in cold adapted populations to the adaptive significance of these properties. Caution is also needed in relating these findings to the results of dark respiration measurements reported

in chapter 4. Since the mitochondria were isolated from their natural environment, membrane effects are not controlled as in the compartmentation of the cell. Higher activation energies do not necessarily mean that chemical reactions in plants proceed more slowly at low temperatures (Bagnall and Wolfe,1978). More data are required to decide whether the higher activation energies for succinate dehydrogenase in cold-adapted grasses are a contribution to their fitness.

In studies with chill-sensitive and chill-resistant plants, Lyons and Raison (1970) found discontinuities in the Arrhenius plot for the oxydative rates of isolated mitochondria only for chill-sensitive plants. Discontinuous Arrhenius plots have often been observed in reactions where membrane bound enzymes are involved (Alexandrov,1977). It has been suggested (Sutcliffe,1977) that the "break" in the Arrhenius plot occurs at the temperature where chilling injury becomes irreversible. The above results cast doubt on this interpretation, for temperate perennial grasses are chilling-resistant. Detailed studies have furthermore shown that what is observed as a break in the Arrhenius plot when measurements are made at only a few temperatures is in fact a continuous transition within a narrow temperature range (Bagnall and Wolfe,1978). It is conceivable that a relation between the temperature in the population habitat and the activation energy exists also for the low temperature range below 5°-10°C. To establish such a pattern measurements would have to be made in small intervals of low positive temperatures.

8. ACTIVITY, ACTIVATION ENERGY AND SUBSTRATE BINDING ABILITY OF MALATE DEHYDROGENASE IN GRASSES FROM LATITUDINALLY DIVERSE POPULATIONS

8.1. Introduction

Chapter 6.2. dealt with the question whether grasses originating from colder climates contain higher enzyme concentrations than grasses from warmer habitats. It was suggested that for RuBPCarboxylase this "quantitative strategy"- thermal compensation by increasing enzyme quantities - is at most a mechanism contributing to fitness at the enzyme level. The quantitative strategy is however only one possible mechanism of rate compensation to temperature. Higher enzyme concentrations cannot account for the diverse activation energies observed for succinate dehydrogenase (chapter 7). These must be due to different types of enzymes or to modification of enzymes, phenomena which were termed qualitative and modulation strategy and for which various mechanisms have been proposed (Hochachka and Somero,1973). Modification of kinetic properties of enzymes may be an important mechanism of response to both short term temperature fluctuations and climatic conditions.

For this study with grasses, another important enzyme was selected to investigate its thermal properties in different populations. This was malate dehydrogenase (MDH; EC 1.1.1.37) as previous research has revealed intraspecific differentiation of this enzyme which could be correlated to habitat temperatures. MDH from clones of

Typha latifolia from a climatic gradient could be ordered by activation energy, thermostability and activity in a pattern related to the climatic origin: Higher E_a's and thermostabilities were found in plants from warmer sites, whereas activity was most closely correlated to the frost free period (McNaughton,1974). For two populations of Potentilla glandulosa from continental and coastal sites, thermostability of the enzyme was not found to differ, but the enzyme-substrate affinity showed a temperature dependence which was related to the temperature fluctuations at the site of origin (Teeri and Peet,1978).

MDH is a major enzyme in carbon metabolism. It is compartmented in the cell into mitochondrial, cytosolic and microbody MDH, according to its functions in redox regulation, nonautotrophic CO_2 fixation, ionic and osmotic adjustments and photorespiration (Ting et al.,1975). The multiple regulatory functions suggest that environmental pressures may have acted to select for enzyme forms with thermal properties that are adapted to the temperature conditions in the plant's environment.

The experiments reported here were designed to determine three kinetic enzyme properties, specific activity, activation energy and substrate binding ability in the physiological temperature range of from 5° to 25°C for the cytoplasmatic enzyme from leaves. Thermostability of MDH was not estimated because it was considered unlikely that heat inactivation of the enzyme plays an important role in the plant in situ. MDH is an enzyme remarkably

insensitive to thermal denaturation. The enzyme from Typha latifolia extracts lost only 35 - 70 % of its activity after exposure to 50°C for 15 minutes (McNaughton, 1974). In leaves the enzyme is even more stable than in homogenates (Simon,1979 a). Temperatures in this range are unlikely to occur during the growing season of temperate grasses. Modification of MDH thermostabilities will therefore be of little importance for the maintenance of metabolic rates under various regimes of environmental temperatures.

8.2. Materials and Methods

Six pairs of climatic provenances were investigated (table 8-1). Fully developed leaves were used for the en-

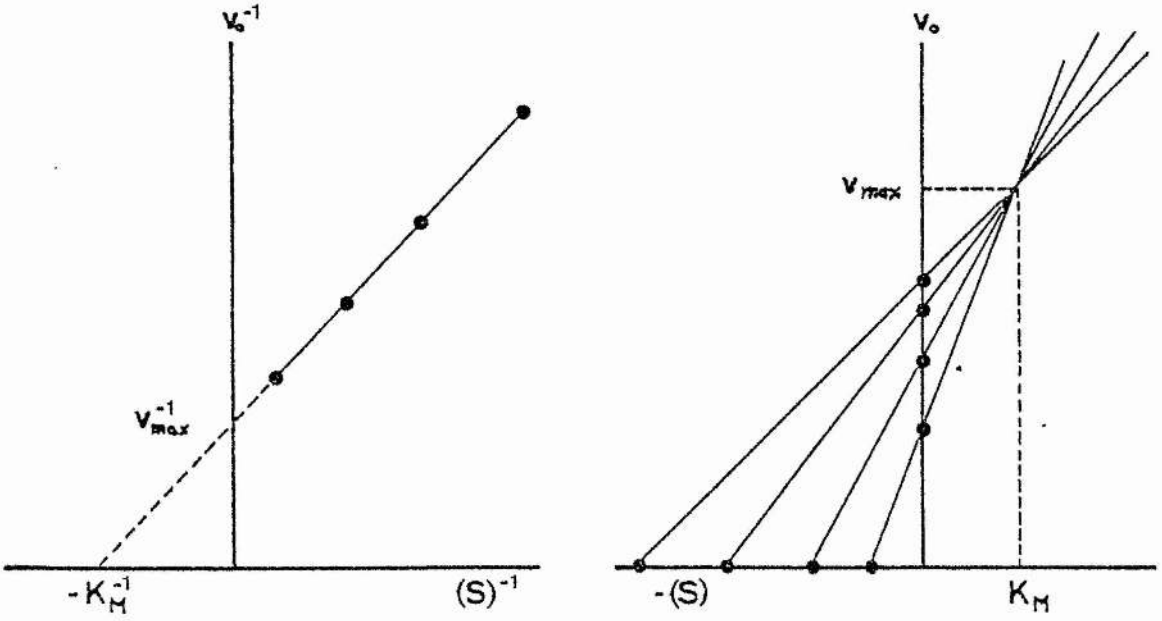
Table 8-1. Species and provenances used for the measurement of kinetic properties of malate dehydrogenase

Species	Provenances	Latitude
Poa pratensis	Fife, Scotland	56°N
	Lazio, Italy	42°N
Festuca arundinacea	England	52°N
	Morocco	32°N
Lolium perenne	Trondheim, Norway	64°N
	Lazio, Italy	42°N
Deschampsia caespitosa	Iceland	65°N
	Fife, Scotland	56°N
Dactylis glomerata	Orkney Is., Scotland	59°N
	Lazio, Italy	42°N
Brachypodium sylvaticum	Fife, Scotland	56°N
	Barcelona, Spain	41°N

zyme extracts. The procedure of enzyme preparation was that used by Teeri and Peet (1978), except that 1 g of fresh weight was used and homogenized with 10 ml of extraction medium in an Ultra Turrax TP 18/10 Homogenizer. The extracts were diluted 1:10 or 1:5 with distilled water and so used for the assays.

For the estimation of K_M values and temperature coefficients, reaction mixtures were prepared with substrate concentrations ranging from 0.0333 mM to 0.2666 mM. The reaction mixtures were prepared in glass cuvettes and contained 1.9 ml phosphate buffer (KH_2PO_4-NaOH buffer 0.1 M, pH 7.4); 0.1 ml enzyme extract in appropriate dilution; 0.1 ml $NADH_2$ (6.7 mM); 0.1-0.8 ml oxaloacetic acid 1 mM and water to make up to 3.0 ml. The activity of MDH was assayed by following the oxidation of $NADH_2$ at 340 nm with a Unicam SP 1800 Ultraviolet Spectrophotometer. The reactions were started by addition of the $NADH_2$. Samples without substrate showed no activity, i.e. there was no $NADH_2$ oxidation activity in the enzyme extracts. Measurements were made at 5°,10°,15°,20° and 25°C in duplicates which agreed within 20 %. Apparent K_M and v_{max} values were calculated from Lineweaver Burk plots and from Direct Linear Plots (Eisenthal and Cornish-Bowden,1974), using computer programmes for both procedures. The principles of both methods are illustrated in fig. 8-1 (page 140). Protein in the diluted enzyme extracts was analyzed with the Folin method (Plummer,1971).

Fig. 8-1. Principles of the calculation of K_m and v_{max} from Lineweaver Burk plots and Direct Linear Plots



Lineweaver-Burk plot

Direct Linear Plot

8.3. Results

Levels of specific activity at the five assay temperatures are shown in figs. 8-2 and 8-3. The amount of activity varies with plant species, but consistently higher activities were found for genotypes from northern latitudes. This is still true when activity values are refered to fresh weight rather than to total protein. This is seen in table 8-2 (page 143) where v_{max} values are listed for the reaction mixtures in the cuvettes. Since the same dilutions of the enzyme extracts were employed for northern and southern population samples of one species, the MDH in both assays stemmed from the same amount of fresh weight. Very similar values of v_{max} were obtained from calculation from Lineweaver Burk and Direct Linear Plots.

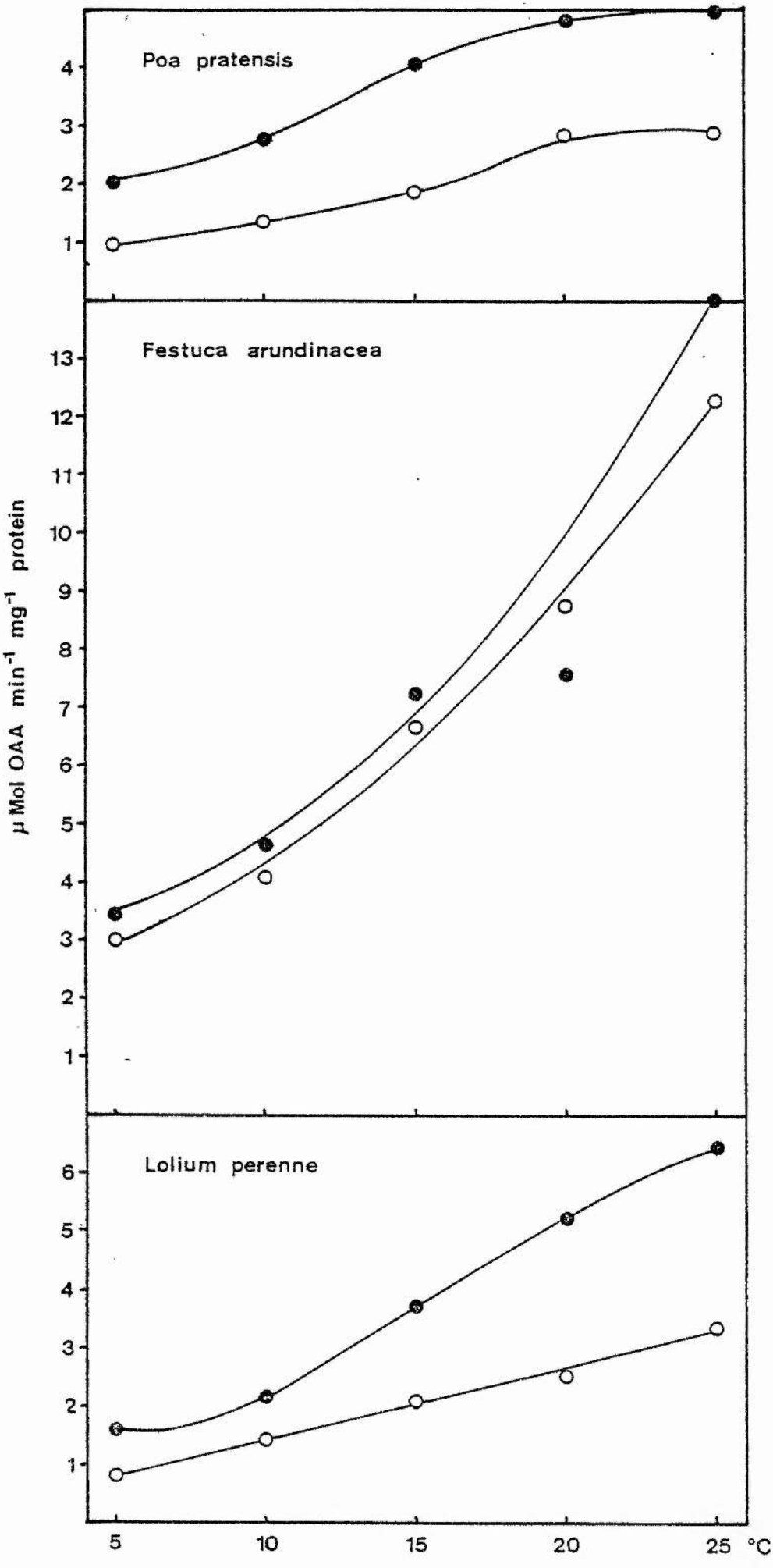


Fig. 8-2. Specific activity of malate dehydrogenase in northern (●—●) and southern (○—○) provenances

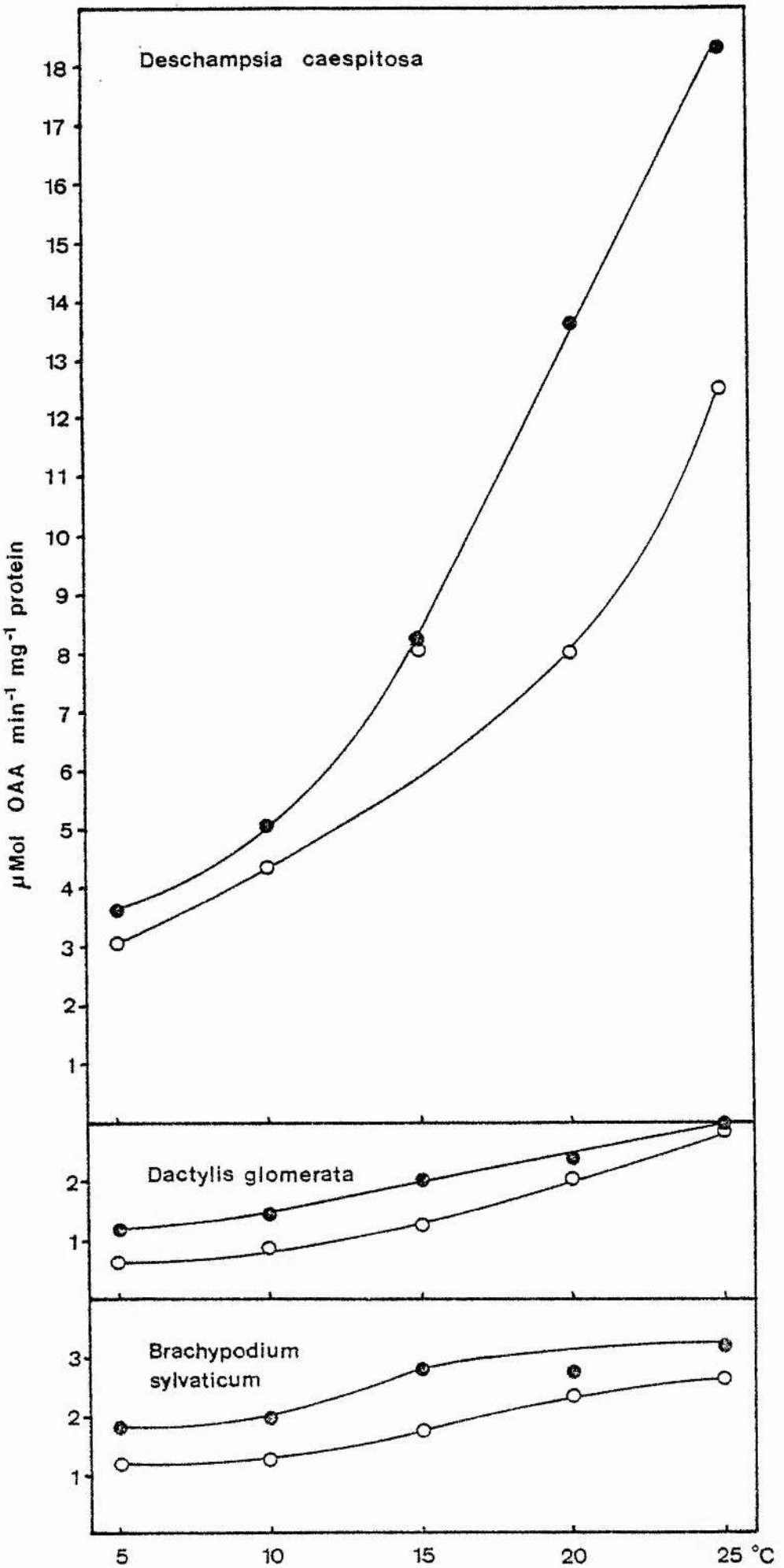


Fig. 8-3. Specific activity of malate dehydrogenase in northern (●—●) and southern (○—○) provenances

energies of activation. Units: K_M in 10^{-5}M ; E_a in kcal/Mole.

		v_{max}(cuvette) A	v_{max}(cuvette) B	K_M A	K_M B	$r^2(K_M)$	$E_a(5^o\text{-}25^o)$ A	$E_a(5^o\text{-}25^o)$ B	$r^2(E_a)$ A	$r^2(E_a)$ B
Poa pratensis										
	5°	0.0428	0.0431	7.8	8.3	0.9955				
	10°	0.0584	0.0547	8.8	7.8	0.9965				
N	15°	0.0850	0.0822	12.7	12.1	0.9990	7.8	8.2	0.9359	0.9732
	20°	0.1008	0.0955	15.0	12.3	0.9466				
	25°	0.1054	0.1129	12.0	13.1	0.9918				
	5°	0.0353	0.0366	5.5	5.7	0.9949				
	10°	0.0502	0.0474	7.8	7.3	0.9919				
S	15°	0.0685	0.0705	9.2	9.9	0.9981	9.6	10.6	0.9563	0.9369
	20°	0.1047	0.1203	14.5	17.9	0.9946				
	25°	0.1053	0.1146	12.2	15.2	0.9860				
Festuca arundinacea										
	5°	0.1141	0.1076	11.0	9.9	0.9973				
	10°	0.1517	0.1314	13.9	11.2	0.9962				
N	15°	0.2383	0.2115	18.8	15.9	0.9981	10.9	9.5	0.9495	0.9767
	20°	0.2498	0.2642	17.2	18.8	0.9958				
	25°	0.4619	0.3189	40.6	24.9	0.9879				
	5°	0.0836	0.0859	6.1	6.2	0.9906				
	10°	0.1143	0.1133	6.8	6.9	0.9939				
S	15°	0.1867	0.1705	12.9	11.3	0.9697	11.8	10.7	0.9938	0.9922
	20°	0.2439	0.2127	17.7	13.6	0.9953				
	25°	0.3431	0.3181	25.9	22.2	0.9956				
Lolium perenne										
	5°	0.0581	0.0634	5.3	6.7	0.9855				
	10°	0.0772	0.0833	7.1	7.9	0.9907				
N	15°	0.1347	0.1129	12.5	10.7	0.9958	12.1	10.5	0.9817	0.990
	20°	0.1887	0.1787	16.7	15.6	0.9989				
	25°	0.2328	0.2136	17.3	15.8	0.9961				
	5°	0.0343	0.0332	6.0	5.5	0.9971				
	10°	0.0596	0.0565	10.9	9.3	0.9567				
S	15°	0.0873	0.0724	14.5	11.1	0.9841	11.2	11.1	0.9684	0.984
	20°	0.1042	0.1016	14.8	14.0	0.9973				
	25°	0.1416	0.1329	21.8	20.5	0.9975				
Deschampsia caespitosa										
	5°	0.1128	0.1151	7.1	7.3	0.9949				
	10°	0.1571	0.1814	8.2	10.5	0.9736				
N	15°	0.2535	0.2352	13.2	11.6	0.9959	13.9	12.9	0.9926	0.991
	20°	0.4226	0.3946	23.1	21.5	0.9965				
	25°	0.5682	0.5553	29.7	28.8	0.9923				
	5°	0.1006	0.1087	5.8	6.6	0.9765				
	10°	0.1442	0.1727	9.5	12.3	0.9763				
S	15°	0.2670	0.2475	16.9	15.5	0.9983	11.3	10.4	0.9446	0.970
	20°	0.2638	0.2750	11.8	12.8	0.9971				
	25°	0.4126	0.4149	20.3	20.5	0.9914				
Dactylis glomerata										
	5°	0.1097	0.0940	12.8	10.3	0.9739				
	10°	0.1325	0.1350	10.5	10.6	0.9925				
N	15°	0.1882	0.1936	12.0	12.3	0.9947	6.9	7.8	0.8853	0.909
	20°	0.1710	0.1789	9.8	10.5	0.9967				
	25°	0.2750	0.2639	17.1	15.9	0.9671				
	5°	0.0578	0.0607	6.5	6.8	0.9346				
	10°	0.0755	0.0908	5.8	7.0	0.8431				
S	15°	0.1122	0.1204	8.5	8.9	0.9335	12.5	11.9	0.9912	0.996
	20°	0.1775	0.1829	14.1	15.1	0.9936				
	25°	0.2500	0.2620	19.0	20.2	0.9933				
Brachypodium sylvaticum										
	5°	0.1753	0.1810	9.5	10.0	0.9971				
	10°	0.1870	0.1997	6.0	6.5	0.9855				
N	15°	0.2714	0.2870	8.6	9.6	0.9879	4.8	7.0	0.8783	0.913
	20°	0.2621	0.3909	5.3	13.7	0.8655				
	25°	0.3040	0.3747	5.9	10.4	0.9517				
	5°	0.0900	0.0856	10.2	9.7	0.9954				
	10°	0.0939	0.0992	4.9	5.2	0.9749				
S	15°	0.1314	0.1592	7.3	11.6	0.9776	7.2	8.0	0.9541	0.889
	20°	0.1749	0.2128	10.8	15.1	0.9761				
	25°	0.1973	0.1958	9.0	9.0	0.9933				

Table 8-2. Survey of kinetic data of malate dehydrogenase

To assess the effect of temperature on MDH activity in northern and southern populations, the differences in specific activities were calculated from the individual measuring points in figs. 8-2 and 8-3 for the three temperature intervals 5°-15°, 10°-20° and 15°-25°C (table 8-3). In the column for 5-15°C, the higher values are

Table 8-3. Differences of specific activity of MDH between two temperatures in 10°C-intervals. (Data as uMole oxaloacetate min^{-1} mg^{-1}protein)

Species		5°-15°	10°-20°	15°-25°
Poa pratensis	N	2.01	2.02	0.97
	S	0.90	1.47	1.00
Festuca arundinacea	N	3.76	2.97	6.78
	S	3.68	4.63	5.58
Lolium perenne	N	2.13	3.10	2.73
	S	1.26	1.06	1.29
Deschampsia caespitosa	N	4.54	8.56	10.15
	S	5.04	3.63	4.41
Dactylis glomerata	N	0.86	0.42	0.95
	S	0.63	1.17	1.58
Brachypodium sylvaticum	N	1.01	0.79	0.34
	S	0.56	1.09	0.89

assoziated with northern provenances, indicating that a rise in temperature from 5° to 15°C increases the MDH activity more in the northern than in the southern population samples. The only exception in this feature in Deschampsia caespitosa where the erratic value for the

southern sample at 15°C (see fig. 8-3) has produced a higher figure. In the other two temperature intervals, there is no such pattern related to climatic origins.

Despite some irregularities, especially for Brachypodium sylvaticum, K_M values show generally the tendency to increase with temperature in both northern and southern population samples (table 8-2). There is no pattern of distinction between provenances. Unlike with maximum velocities, the use of the alternative methods of Lineweaver burk and Direct Linear plots has resulted in sometimes markedly differing K_M values. This is most drastically seen for the northern Festuca arundinacea at 25°C and for the southern Brachypodium sylvaticum at 20°C.

Activation energies were calculated from Arrhenius plots. Since the graphs of log rate versus the reciprocals of absolute temperature appeared to be straight lines, the values were calculated for the full range of experimental temperatures of 5° to 25°C. When the v_{max} values from the Direct Linear Plot are used, E_a's are lower for the northern population samples in 5 out of 6 species. When v_{max} values are taken from Lineweaver Burk plots, this pattern is found for 4 of the 6 cases, for the E_a relationship is reversed for Lolium perenne (table 8-2). Lower activation energies were also found for RuBPCarboxylase in northern genotypes (chapter 6.1.). These results contrast with findings for succinate dehydrogenase (chapter 7) which displayed higher activation energies in southern genotypes.

The temperature dependence of an enzymic reaction is

largely determined by the temperature dependence of the enzyme-substrate affinity. The larger the temperature dependent change in K_M, the lower will be the Q_{10} value of the reaction at any given level of substrate below saturating level (Hochachka and Somero,1973). This important relationship for immediate temperature compensation is shown in table 8-4. With very few exceptions (Brachypodium sylvaticum in the ranges 5°-15°C and 15°-25°C and Dactylis glomerata 10°-20°C), Q_{10} values at saturating substrate concentrations are markedly higher than at lower concentrations. The tendency for Q_{10} values to increase with increasing substrate concentrations is most pronounced in the temperature ranges 5°-15°C and 10°-20°C. It is furthermore seen from the rows in table 8-4 – although variability is higher in this case – that Q_{10} values tend to decrease with increasing temperatures at all levels of substrate concentration. These patterns occur in both northern and southern genotypes without fundamental differences in the extent of modulation.

8.4. Discussion

The higher activity values for MDH recorded in northern genotypes supplement findings for RuBPCarboxylase where a similar pattern was observed (chapter 6.1.). For both enzymes, higher activity was assoziated with lower activation energies in northern genotypes. The level of specific activity was still low when compared with the specific activity of purified MDH from pig heart. The latter is approximately 1200 units per g

Table 8-4. Dependence of the Q_{10} on substrate concentration [S] and temperature. The Q_{10} values at substrate saturation are calculated after the v_{max} values from the Direct Linear Plot.

[S] (mM)	Q_{10}(5°-15°)		Q_{10}(10°-20°)		Q_{10}(15°-25°)	
	N	S	N	S	N	S
Poa pratensis						
0.0333	1.36	1.37	1.10	1.24	0.97	0.89
0.0666	1.53	1.43	1.37	1.35	1.13	1.34
0.1000	1.64	1.61	1.43	1.55	0.99	1.29
0.1333	1.52	1.67	1.47	1.57	1.22	1.35
0.2666	1.71	1.70	1.60	1.79	1.27	1.42
Satur.	1.90	1.93	1.75	2.54	1.38	1.62
Festuca arundinacea						
0.0333	1.34	-	1.10	1.01	0.97	-
0.0666	1.43	1.53	1.37	1.28	1.13	1.15
0.1000	1.54	1.52	1.43	1.32	0.99	1.25
0.1333	1.60	1.74	1.47	1.32	1.22	1.16
0.2666	1.67	1.75	1.60	1.51	1.27	1.29
Satur.	1.97	1.98	2.01	1.88	1.51	1.87
Lolium perenne						
0.0333	1.22	1.30	1.25	1.40	1.33	1.17
0.0666	1.60	1.64	1.46	1.42	1.38	1.11
0.1000	1.29	1.64	1.72	1.20	1.72	1.25
0.1333	1.64	1.74	1.59	1.50	1.51	1.35
0.2666	1.71	1.88	1.75	1.93	1.52	1.40
Satur.	1.94	2.18	2.15	1.79	1.74	1.84
Deschampsia caespitosa						
0.0333	1.41	1.17	1.13	1.52	1.13	1.33
0.0666	1.61	1.48	1.57	1.72	1.19	1.36
0.1000	1.67	1.70	1.44	1.58	1.27	1.46
0.1333	1.68	1.63	1.54	1.60	1.56	1.25
0.2666	1.86	1.77	1.67	1.65	1.47	1.51
Satur.	2.04	2.28	2.18	1.59	2.36	1.68
Dactylis glomerata						
0.0333	1.84	1.65	1.38	1.17	1.13	1.13
0.0666	1.81	1.42	1.28	1.65	0.97	1.64
0.1000	1.45	2.18	1.34	1.48	1.27	1.22
0.1333	1.80	1.59	1.22	1.66	1.42	1.45
0.2666	2.12	1.87	1.51	1.64	1.25	1.66
Satur.	2.07	1.97	1.33	2.02	1.36	2.18
Brachypodium sylvaticum						
0.0333	1.70	1.91	1.58	1.10	1.48	1.26
0.0666	1.61	1.69	1.32	1.17	1.27	1.53
0.1000	1.48	1.56	1.34	1.28	1.35	1.37
0.1333	1.68	1.68	1.61	1.59	1.19	1.35
0.2666	1.73	1.81	1.60	1.55	1.35	1.30
Satur.	1.58	1.85	1.96	2.15	1.30	1.98

protein at 25°C, whereas the highest value noted in the experiments is that of Deschampsia caespitosa at 25°C, about 18 units per g protein. The total protein content in the diluted extract from Deschampsia caespitosa was 0.31 mg/ml. This means that MDH constitutes only about 50 % of the protein in the extract. This, of course, is only a rough estimate since it is not known whether the enzymes from grasses and pigs agree in activity. For most other species, the values are considerably lower than 50 %. Consequently, it cannot be excluded on grounds of the above measurements that higher MDH concentrations contribute to the higher activities of high latitude genotypes. However, the assaziation of higher activities with lower activation energies in most cases for both RuBPCarboxylase and MDH suggests that lower activation energies faciliate the reactions in the temperature range examined. Through the reduced energy barriers in northern populations, these plants would achieve a particular reaction rate at lower temperatures than plants from southern populations. This feature would be advantageous in low temperature environments. As in the case of RuBPCarboxylase, an increase in temperature from 5° to 15°C enhances the activity of MDH more in northern than in southern populations. Thus, metabolic homeostasis for the reactions catalyzed by these enzymes appears to be more pronounced in southern populations.

The higher specific activities and lower activation energies for MDH in grasses from colder climates contrast with findings by McNaughton (1974) for MDH from

climatic races of Typha latifolia, where a close positive relationship was noted between the frost free period at the original site of the plants and the MDH activity. The author gives no interpretation of this phenomenon. A recent extensive study of thermal properties of MDH in Lathyrus japonicus populations from climatically contrasting sites (Simon,1979 a and c) has similarly revealed higher activity levels in warm-adapted genotypes. Conversely, activities of the respiratory enzymes MDH, isocitrate dehydrogenase and glycollate oxidase from Festuca arundinacea populations were highest in plants grown at low temperature and lowest in plants grown at high temperature (Nelson and Treharne,1974). This is indicative of temperature compensation during acclimatization. Higher rates in cold-adapted populations were also found for RuBPCarboxylase (Chabot et al.,1972) and for the oxidative rates of mitochondria (Klikoff,1966). On the whole, more studies support the principle of temperature compensation through elevated enzyme activities as a response to cold acclimatization. So far there are no obvious reasons for the inconsistencies found for MDH.

K_M values were in the range of 0.05 to 0.4 mM oxaloacetic acid in the temperature range from 5° to 25°C. This is the order of magnitude found for MDH from Potentilla glandulosa (Teeri and Peet,1978), Lathyrus japonicus (Simon,1979 a) and other plants (Ting et al.,1975). The general pattern of K_M is that of positive thermal modulation (Hochachka and Somero,1973). The increase of K_M-values as a positive function of temperature is an impor-

tant mechanism of temperature compensation. Decrease of temperatures and thus decrease of kinetic energy results in enhanced enzyme-substrate affinities to maintain levels of metabolic activity. This mechanism of enzyme modulation is of particular importance for the immediate compensation of short-term temperature fluctuations which occur too rapidly for modifications of enzyme concentrations or de novo synthesis and degradation of isoenzymes to be involved. However, for successful adaptation to a plant's environment, all three mechanisms may be realized to act synergistically. A recent study of the speed of acclimatization for activation energies of MDH (Simon, 1979 b) has indeed suggested that the physiological processes of gene activation or gene induction are involved to regulate the synthesis of MDH isoenzymes.

The lack of population differentiation with respect to enzyme-substrate affinities is in accordance with Simon's (1979 d) work with MDH in Lathyrus japonicus. These investigations also failed to detect a clear pattern of distinction in the K_M values for plants from climatically different origins. This may be explained by the fact that enzyme-substrate affinity changes, unlike the other two mechanisms, are of little importance for long-term temperature compensation processes such as evolutionary adaptation or winter acclimatization. It was pointed out by Hochachka and Somero (1973), that cold-adapted organisms do not usually possess lower K_M values of enzymic reactions in comparison to warm-adapted organisms, for small increases in substrate concentrations would cause

saturation of enzymes with too high substrate affinities and thus impede metabolic regulation.

The property of positive thermal modulation is consistent with findings for MDH from Lathyrus japonicus (Simon,1979 d) where enzyme-substrate affinities also decreased with increasing temperatures when the plants were cultivated in temperature regimes of up to 25°C. This pattern of response was also found for ß-galactosidase in Escherichia coli (Langridge and McWilliam,1967) where substrate affinities decreased steadily between 10° and 60°C. Lowry and Passonneau (1972) have pointed out that K_M values would be expected to increase with temperature. An indication of this kind of modulation is the drop of Q_{10} values at lower substrate concentrations noted in table 8-4 (page 147). There are however discrepancies as to the type of modulation of different enzymes and even of one enzyme in different species. If no changes occured in the Q_{10} of an enzymic reaction at different substrate concentrations, then the K_M of the enzyme would be independent of temperature. An example for this non-modulation is the acid phosphatase in Feldman's (1973) study with spring and summer species of Leucojum. In both species, K_M of the enzyme proved to be the same in the temperature range from 10° to 30°C. Again another type of modulation is that enzyme-substrate affinities first increase and then decrease with rise of temperature. That is, distinct ranges exist in which the enzyme is regulated negatively and positively; there is an intermediate temperature at which substrate affinity is highest. This

is the pattern described for MDH from Potentilla glandulosa populations (Teeri and Peet, 1978). The minimal K_M values were measured near the average habitat temperature in each population, and the breadth of the thermal optimum was related to the temperature range likely to occur in their respective habitats. Negative thermal modulation in the low temperature range for MDH in Potentilla glandulosa means that reductions of kinetic energy and enzyme-substrate affinity act synergistically to cause rapid reductions of enzyme activity. The temperature dependence of the reaction will be very high under these conditions. It is not known at present why MDH should show positive thermal modulation in some species and partly negative thermal modulation in others. It has been demonstrated for the cytosolic as well as for the mitochondrial and microbody forms in Lathyrus japonicus (Simon, 1979 a) that the enzyme consists of a number of isoenzymes. Differences in the kinetic properties may therefore be due to different isoenzyme compositions.

It might also be speculated that the range of negative thermal modulation is related to chill- and frost hardiness of plants. Small temperature drops suffice to bring about large reductions of the reaction rate. If several key enzymes are governed by negative thermal modulation, then only a slight decrease of temperatures below the value where enzyme-substrate affinities are greatest would reduce metabolic rates below maintenance level. This may result in stages of dormancy or in cold injuries. Temperate grasses are known to be chilling re-

sistant at all temperatures above freezing point. It is therefore possible that ranges of negative thermal modulation do exist for MDH from the species examined, but at temperatures below those applied in the experiments.

In conclusion, the measurements have documented the occurrence of the modulation strategy of thermal compensation for MDH from several grass species. It was suggested that positive thermal modulation as a rate compensatory mechanism is of importance only for immediate compensation. This would explain the lack of K_M differences in different populations. However, a strict proof of the relevance of positive thermal modulation of MDH for immediate temperature compensation would not only have to demonstrate that MDH is, in fact, a rate-limiting enzyme, but also to determine the actual substrate concentrations available to the enzyme in the cell.

Some comments should be added regarding the two methods used for the calculation of K_M values and maximal reaction velocities. Lineweaver Burk plots and Direct Linear Plots have produced very similar values for v_{max}. K_M values however differ markedly in some instances. Activation energies are lower for northern population samples in 5 cases when they are based on v_{max} values calculated after the Direct Linear Plot, in 4 cases for calculation after Lineweaver Burk plots. Calculation from Direct Linear Plots has yielded K_M values that do not show some of the fluctuations which result when Lineweaver Burk plots are used (see Festuca arundinacea N, Dactylis glomerata N and S and Brachypodium sylvaticum N)

and therefore seem more realistic. The coefficients of determination (r^2) for activation energies are improved in all but two cases (Poa pratensis S and Brachypodium sylvaticum S; see table 8-2, page143). Direct Linear Plots avoid the overaccentuation of measurements at low substrate concentrations where errors are greatest. Finally, the use of medians of a series of measurements in the Direct Linear Plot was shown to be a far better approximation to the true K_M than the use of mean values (Cornish-Bowden and Eisenthal,1974). The median is to a large extent unaffected by erratic values. The computer programme used here has further restricted the effect of erratic values by the exclusion of intersections with negative K_M's. With these and the reasons stated by Markus et al.(1976), the Direct Linear Plot appears as the preferable method for the calculation of v_{max} and K_M. Its advantages more than outweigh the shortcoming that estimates of accuracy can only be obtained from diagrams or from the calculation of the area enclosed by all intersections.

9. GENERAL DISCUSSION AND CONCLUSIONS

For successful growth and reproduction it is essential that plants maintain their metabolic rates in a range of temperatures. Depending on the duration of a particular temperature regime to which plants are subjected, three time-courses of metabolic compensation were distinguished, termed evolutionary, seasonal and immediate compensation (Hochachka and Somero, 1973). Given that plants in all natural environments have to cope with temperature fluctuations and would consequently be expected to possess mechanisms for immediate compensation, the major objective of this thesis was to describe physiological properties which could be interpreted as relevant in the context of evolutionary compensation. It was conceivable that temperature might have exercised a selective pressure, so that populations have evolved with particular fitness for the temperatures prevailing in their respective environments. In transplant studies these populations would display different responses to certain temperature regimes.

To investigate temperature responses in grasses, a comparative approach was employed throughout this thesis, using plants from contrasting climates. Temperature is an important factor discriminating the collection sites of the plants under study. The population samples were all cultivated under the controlled conditions of temperature and photoperiod in the glasshouse to obviate the possibility of interaction of different mechanisms of compensation. Thus, population differences in temperature response should not be superimposed with short term

effects, and should reflect temperature adaptations that have occurred on an evolutionary scale.

In the general introduction the question was posed whether there is a general pattern of differences between northern and southern populations of a series of grass species in their response to temperature. Such systematic differences were found in great number. Starting from the fact that northern and southern genotypes differ in their growth response at low temperatures, the experiments were designed to elucidate the physiological basis of temperature adaptations. Since the basic problem of metabolic temperature compensation is the regulation of enzymic activity, this work has concentrated on adaptations at the enzyme level. With the exception of the measurement of dark respiration which showed no fundamental differences in the temperature response of leaves and roots, all experiments were done with leaves only. Leaves as the photosynthesizing organs were considered of major importance in the adjustment of metabolic processes to ambient temperatures. It is worth mentioning here again the work of Hansen (1977) with Lolium multiflorum which has demonstrated the dependence of the respiration rates of roots on the extent of photosynthesis and thus of light intensity. The importance of the photosynthetic apparatus for survival at low temperatures has also emerged from studies of tropical grasses (West,1973) where the amount of starch accumulation was greatly influenced by the temperature given during the photosynthetically active periods.

The experimental results from the examination of various physiological processes in this thesis can be ordered under three categories: 1. Temperature effects upon rates. 2. Dependence of rate changes on temperature changes, i.e. temperature influence on rates. 3. Kinetic data of enzymic reactions.

1. Temperature effects upon rates. It was shown for Lolium perenne, Dactylis glomerata and Holcus lanatus, that the growth rate of seedlings in water culture at 12°C was greater for samples from southern populations. These findings were in accordance with similar reports from the literature and were said to be related to the cool growing season activity in grasses from the Mediterranean.

The measurement of dark respiration rates of leaves and roots of grasses cultivated in the glasshouse at 18°-22°C did not yield population differences. However, when the plants had been pre-cultivated at 10°C, then respiratory rates of leaves were enhanced in all northern samples when compared with those pre-cultivated at a higher temperature. This response pattern was found for only three samples of southern genotypes, namely Anthoxanthum odoratum, Festuca arundinacea and Poa pratensis, whereas southern provenances of Holcus lanatus, Dactylis glomerata and Lolium perenne have depressed their respiration rate as a reaction to cultivation at 10°C.

The rate of carbohydrate accumulation after dark starvation was higher in northern population samples

(1 exception) at both 12° and 20°C. This property could be related to the levels of RuBPCarboxylase activity which were also found to be higher in northern plants in the range of from 5°to 25°C, regardless whether activities were expressed on a fresh weight or total protein basis. The examination of RuBPCarboxylase contents, expressed as a percentage of total protein, has suggested - with some reservations, that there must be other reasons for the higher activities than solely elevated enzyme concentrations.

Finally, the rates of the reaction catalyzed by MDH, on protein and fresh weight basis, were higher in northern provenances when measured in the temperature range 5° -25°C.

2. Effect of temperature changes upon rate changes. In spite of the higher growth rates of southern genotypes of Lolium perenne and Dactylis glomerata, it was the northern samples of these grasses which increased their productivity more when the temperature was raised from 12° to 20°C. A similar effect was noted for carbohydrate accumulation. Temperature increase from 12° to 20°C increased the carbohydrate content in detatched leaves from high latitude samples more than in Mediterranean counterparts (1 exception). As in the case of rate effects, this property was also found for RuBPCarboxylase where a rise of temperature from 5° to 15°C increased the enzyme activity more in northern than in southern population samples (1 exception).

The assays of the MDH-reaction have shown that it

is again the northern plants which amplify their advantage in activity more than the southern ones when temperature increases from 5° to 15°C.

3. Kinetic data of enzymic reactions. Activation energies of all three enzymes examined showed a pattern related to the geographic origin of the plants. For succinate dehydrogenase in isolated mitochondria E_a's were higher in plants of northern origin, but lower E_a's were found for northern plants with respect to RuBPCarboxylase and MDH (1 exception). The immediate temperature compensation which was noted as increase of temperature coefficients with increasing substrate concentrations and decreasing enzyme-substrate affinities with increasing temperatures occurred in both northern and southern provenances without fundamental distinction.

Positive and negative results with respect to differences between populations are summarized in table 9-1. The lower growth rates at low temperature, the higher rates of carbohydrate accumulation and the higher activity of RuBPCarboxylase in northern plants are properties which may act to maintain a positive carbon balance. The higher respiration rates after cold acclimatization in northern plants and their higher MDH activities suggest that there is generally a higher energy turnover in these provenances.

It is conspicuous that species from northern latitudes have higher rates in most processes examined, and that they increase their rate relatively more when the temperature is raised within a physiological range than

Table 9-1. Survey of positive and negative findings with respect to latitudinal population differentiation

Positive	Aspect investigated	Negative
At 12°C higher productivity of seedlings of southern grasses than at 20°C Rising the temperature from 12° to 20°C causes a greater increase of growth rates in seedlings from the north.	Growth	No population differences in mean shoot-root ratios of seedlings.
Acclimatization at 10°C increased the respiration rate in all northern but only in some southern plants. In others cultivation at 10°C has reduced the respiration rate when compared with that for higher acclimatization temperatures.	Respiration	No differences in respiration rates of leaves and roots of plants grown at 18°-22°C.
Higher accumulation rates in northern plants at 12° and 20°C (1 exception). Temperature increase from 12° to 20°C increases the carbohydrate content more in northern plants (1 exception).	Carbohydrates	
Higher activity in plants from the north. Activity increase with temperature rise is more pronounced in northern samples (1 exception). Lower activation energies in northern plants.	RuBP-carboxylase	No differences in thermostability of the enzyme in vitro. Differences in enzyme content cannot explain the differences in activity.
Higher activation energies in northern population samples.	Succinate-dehydrogenase	
Higher activities in northern plants. Temperature increase from 5° to 15°C amplifies this difference more in northern than in southern samples (1 exception). Lower activation energies in northern plants (1 exception).	Malate-dehydrogenase	Similar K_M- temperature dependence in all population samples.

southern counterparts. In principle the same response pattern was found for photosynthetic rates of Dactylis glomerata from Norway and the Mediterranean (Treharne and Eagles, 1970). The fact that this pattern was found in this study for several physiological processes and several species suggests that higher rates are one factor by which members of the grass family adjust to long-term temperature conditions in their habitat. The reduced temperature sensitivity in southern population samples complements well with the findings cited in chapter 3 that temperate grass species from the Mediterranean keep growing over the winter when transplanted to Great Britain, whereas species indigenous to this country cease to grow in October. The overall pattern of physiological activity contrasts with the result that with respect to plant productivity the southern populations are more successful at low temperatures, while northern plants, despite their higher temperature dependence, still display higher activities in the temperature ranges examined. This apparent discrepancy cannot be readily explained, but it should be noted again that there exist many difficulties when comparing in vivo and in vitro responses.

Vroman and Brown (1963) have suggested that ectotherms native to cold habitats should be selected for enzymes with low activation energies to increase catalytic efficiency. This relation was found for RuBPCarboxylase and malate dehydrogenase, but succinate dehydrogenase had consistently higher activation energies

in grasses from cold climates. In chapter 6 it was already alluded to the fact that the ecological significance of E_a values is still controversial. Alexandrov (1977) cites a large number of references concerning Q_{10} and E_a of enzymic reactions in bacteria, animals and plants living at different temperatures. Numerous contradictory data support his conclusion that to date there is no evidence for the significance of temperature coefficients and activation energies for the temperature ecology of a species. Higher activation energy should only mean that a reaction proceeds more slowly at low temperature (Bagnall and Wolfe, 1978). A critical question in analyzing the temperature response of a single process is whether this process can be regarded as a master reaction which determines the rate of a complex reaction sequence. The concept of activation energies was derived for individual chemical reactions. Thus it is not meaningful to state activation energies for processes which consist of a sequence of coupled reactions. In a reaction system such as respiration, a higher activation energy for an individual reaction does not mean that the whole process continues more slowly at low temperatures, unless there is evidence that this reaction is the rate-limiting reaction in the system. Succinate dehydrogenase catalyses only one reaction step in the respiratory chain. Lower activation energies of succinate dehydrogenase obviously do not result in higher rates of the whole reaction system of respiration at low temperatures. It is perhaps no coinci-

dence that, contrary to the findings with RuBPCarboxylase and malate dehydrogenase, higher activation energies in plants from colder climates occurred for an enzyme which is membrane-bound. The properties of the lipid layer may play an important role in the determination of catalytic activities.

The coherent pattern of activation energies for RuBPCarboxylase and malate dehydrogenase in relation to climatic origins can hardly be mere chance. As for RuBP-Carboxylase, there is no doubt that this enzyme catalyzes the reaction which is rate-limiting in the Calvin cycle. For MDH the situation is less clear, but its multiple functions suggest that the regulation of this enzyme is of great importance for the balance of metabolism. Furthermore, the finding of lower energies of activation of MDH in plants from cold environments parallels the results by McNaughton (1974) and Simon (1979 a and c) with Typha latifolia and Lathyrus japonicus from contrasting latitudes. These patterns suggest modifications of both RuBP-Carboxylase and MDH enzymes in the manner of the qualitative strategy of temperature compensation proposed by Hochachka and Somero (1973). The results reported in this thesis are not a direct indication of catalytic efficiency since they do not measure the free energy of activation $\Delta G^{\ddagger}$. It is however likely, at least for MDH, that differences in E_a between genotypes are positively correlated with differences in $\Delta G^{\ddagger}$. This was shown for MDH from Lathyrus japonicus populations by Simon (1979 c).

The work of this author (Simon, 1979 a) has also pro-

vided further evidence that the results of *in vitro* measurements do not reflect the exact magnitude of differences *in vivo*, but are representative of them. This was established for the thermostability of MDH in intact leaves and enzyme extracts at various degrees of purification and parallels the results by Feldman (1973) for acid phosphatase. In addition, the effect of enzyme purification on E_a and activity values was also determined. Higher purification has resulted in somewhat higher values of activation energy and specific activity, but the correlation between genotypic differences was maintained at all stages of purification. These findings suggest two things. First, the results from *in vitro* measurements of enzyme characteristics are biologically meaningful insofar they reflect the basic pattern of response *in vivo*. Second, to compare measurements from different sources quantitatively, the systems studied must agree in their degree of purity. Some of the diversity in the relationship of activation energy and environmental temperature may result from the fact that systems with various degrees of purity have been investigated.

A number of differences in the temperature response of physiological processes was shown to exist in populations of a variety of grass species. The fact that these differences are genetically-based suggests that they are a result of selection for fitness in certain climatic conditions, amongst which temperature is an important factor. In view of population differences which might be regarded

as temperature adaptations a central question arises: To what extent do grasses in northern populations actually grow at temperatures lower than those experienced by their southern counterparts during their growing season? Do northern populations simply "wait" till the season is advanced and the temperature regime is similar to that during the main growing season in the south? Unfortunately, no studies have directly attacked this phenological problem. When the length of the growing season is calculated as the number of days in which day temperatures are above the 6°C-threshold, this would not take into account that evolutionary differentiation may have resulted in populations that differ in their temperature requirements. Furthermore, 6°C as a threshold for active growth is only a rough estimate and may vary from species to species. This is suggested by the fact that different species in temperate Europe reach certain developemental stages at different minimum day temperatures. Several examples of this phenomenon are listed in Walter (1960).

Citing Ihne and Kirchhoff, Walter also presents phenological data for Syringa vulgaris. The initiation of flowering in this species takes place before the middle of April in middle and southern Italy, but not until the middle of June in middle Scandinavia. Analyzing the Phenological Reports of the Royal Meteorological Society, Jeffree (1960) found that the mean flowering date, averaged over 57 years and 11 species, was 21 days later in north than in south Britain. A wealth of phenological data indicates that, apart from interferences through

mountains and the Golf Stream, the phenological isopleths are almost parallel to the degrees of latitude (Walter, 1960).

Climatic contrasts as characterized by the statement of mean temperatures for the hottest month and frost free periods (chapter 2) are not a direct indication of contrasting thermal regimes during the growth period of plants in different populations. A better measure would be accumulated temperature over the period of active growth. However, phenological data are available for a multitude of developemental events in the course of the annual vegetation period, but no studies have been made to determine the dates when growth starts and ceases in different climates.

It is obvious that the necessity for thermal compensation in plants can be circumvented, in part, by adjusting the seasonality of growth to periods when temperatures are favourable. The foregoing paragraphs have recalled that plants make use of this phenological strategy. On the other hand, the degree of diversity found between species from contrasting climates indicates that phenological adjustment is only a contributory factor of thermal adaptation. For a fuller understanding of these adaptations it is desirable to know in more detail the relationship between growth periods and climate.

10. SUMMARY

1. Genetically-based population differences of the temperature response of several physiological processes were investigated in a group of related species. These were perennial grasses because they provide a single life-form and wide geographical distribution. A comparative approach was employed throughout by using pairs of species that have been collected from lowland sites up to 2000 km apart on a north-south direction.

2. The collection sites differ markedly in their annual temperature regimes. By comparing population samples from thermally contrasting climates it was thought to gain insight into the physiological mechanisms underlying temperature adaptations in grasses. The plants studied were cultivated in the glasshouse at 18°-22°C and 16-hour days. Mature leaves were used in all experiments since it was known from reports in the literature that the photosynthetic apparatus shows many modifications which can be related to climatic conditions.

3. The fresh weight increase of seedlings in water culture was measured at 12° and 20°C with Lolium perenne and Dactylis glomerata. With Holcus lanatus the experiment was carried out at 12°C only. In all three species the fresh weight increased more in Mediterranean than in northern provenances when grown at 12°C. The effect of a temperature rise on growth rate increase was more pronounced in northern samples.

4. Measurements of dark respiration were performed manometrically. With the exception of Festuca rubra, no differences of the respiration rate in northern and southern samples were detected when the plants were taken from the glasshouse. Acclimatization at 10°C has increased the respiration rate in all northern samples. For southern samples the response pattern was not uniform. Some species increased their respiration rate after acclimatization at 10°C, others reduced their respiration rate.

5. The potential to replenish carbohydrates after 48 hours dark starvation was measured by exposing detatched leaves to light in a controlled temperature chamber and analysing their content of nonstructural carbohydrates after different times of illumination. Northern plants had higher rates of carbohydrate accumulation at 12° and 20°C. Temperature change from 12° to 20°C caused a greater rate change in northern than in southern plants.

6. The specific activity of ribulose-1,5-biphosphate carboxylase was higher in northern samples at 5 different temperatures in the range 5° to 25°C. Increasing the temperature from 5° to 15°C led to a higher increase of activity in the northern samples. The enzyme had lower energies of activation in the northern samples.

The relative content of ribulose-1,5-biphosphate carboxylase in relation to total protein was measured by densisy scanning of acrylamide gels. No evidence was found that higher activities are due to higher enzyme concentrations in northern plants.

7. Succinate dehydrogenase activities were determined in isolated mitochondria. The Q_{10} (10°-20°) for the reaction catalyzed was lower for a low altitude sample of Deschampsia caespitosa than for a high altitude sample. The apparent energies of activation for 5 latitudinal pairs of species were higher for the northern population samples in the range of 10°-25°C.

8. Higher specific activities were found for malate dehydrogenase from northern plants at 5°, 10°, 15°, 20° and 25°C. A temperature rise from 5° to 15°C increased the specific activity more in preparations from northern plants. With one exception, the enzyme had lower apparent energies of activation in northern plants. The enzyme displayed positive thermal modulation in the temperature range examined, but this property occurred similarly in northern and southern plants.

9. The population differences in the response to a range of physiological temperatures were ordered under three headings: a) Rates; b) effect of temperature changes on rate changes; c) kinetic properties of enzymes. The overall pattern of temperature responses suggested that the observed differences are important in the adaptation to the temperature conditions of the native habitat.

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