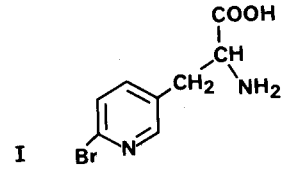
128 μmoles dm^{-3} of compound **I**. As figure 2, a, indicates, the average number of shoots from the leaf rosette increased with increasing concentration of compound, from 1, in the case of untreated seedlings, to around 8 in the case of seedlings grown on nutrient containing 128 μmoles dm^{-3} of the pyridylalanine. In contrast there was no significant difference in overall growth, as measured by total rosette shoot length, between single-stemmed untreated plants and multi-stemmed treated plants (figure 2, b).



I

When apical dominance in a normal single-stemmed *Arabidopsis* plant is suppressed by decapitating the stem, the 1st shoot to emerge from the rosette leaf axils quickly reestablishes dominance, so that only 1, or at the most 2, shoots develop; in the latter case one usually becomes dominant. The observation that several shoots arise more or less simultaneously from plants grown in the presence of the pyridylalanine (**I**) suggests that this compound overcomes the normal apical dominance control mechanism and allows the various axillary buds to initiate shoots independently of each other. The lack of a significant difference in total shoot length between single-stemmed untreated and multi-stemmed treated seedlings suggests that nutrient uptake could well be a growth limiting factor.

Although a large number of compounds have been assayed for potential PGR activity using *A. thaliana* as the test species, the bromopyridylalanine (**1**) was the first to produce multi-stemmed seedlings. Subsequently 2 closely related derivatives, β-(6-chloropyridin-3-yl)alanine and β-(5,6-dibromopyridin-3-yl)alanine were found to behave similarly. Since both growth-enhancing auxins and cell-division promoting kinins have been implicated in apical dominance control[3] it could be suggested that these pyridylalanines act by interference with either the biosynthesis, transport or activity of one or other of these hormones within the plant.

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Temperature acclimation of $Mg^{2+}Ca^{2+}$-myofibrillar ATPase from a cold-selective teleost, *Salvelinus fontinalis*: a compromise solution

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Summary. Brook trout (*Salvelinus fontinalis*, Mitchill) were acclimated over 15 weeks to either +4 °C or +24 °C. The effects of temperature on myofibrillar $Mg^{2+}Ca^{2+}$-ATPase activities were investigated. In contrast to goldfish, temperature acclimation does not alter the kinetic properties of the brook trout myofibrillar ATPase. Activation energy ($\Delta G^{\ddagger}$) is lower and substrate turnover number is higher than values previously reported for cold-adapted stenotherms. Properties of brook trout ATPase appear to be a compromise enabling function across a broad temperature range. The different strategies of adapting to seasonal temperature variations are briefly discussed.

Many species of fish show a partial or complete compensation in their locomotory capacities following acclimation from summer to winter temperatures[2]. Studies on the eurythermal goldfish *(Carassius auratus)* have shown differences in the properties of skeletal muscle $Mg^{2+}Ca^{2+}$ myofibrillar ATPase following temperature acclimation[3-6]. Cold acclimation is associated with a significant increase in ATPase activity and modifications in thermodynamic activation parameters[3,4].

In contrast to goldfish, a number of eurythermal fish will select (wherever possible) a relatively narrow temperature range in their natural habitat and yet retain a wide temperature tolerance. In the present study the effect of temperature acclimation on the properties of $Mg^{2+}Ca^{2+}$ myofibrillar ATPase has been determined on one such species (brook trout) and compared with previous findings on goldfish.

Materials and methods. Brook trout (*Salvelinus fontinalis*, Mitchill) approximately 220–270 mm standard length were obtained during April from the West of Scotland Trout Farm (Renfrewshire, Scotland). Groups of about 20 fish were acclimated to +4 °C or +24 °C over a period of a least 15 weeks. Experiments were carried out approximately 6 weeks after the final temperatures were attained.

Fish were stunned by a blow to the head and killed by spinal cord transection. Myofibrils were prepared from the fast (white) trunk muscle as previously described[7]. Contamination with nonfibrillar ATPases was reduced to a low level (< 0.1%) by treatment with triton-X 100[7]. $Mg^{2+}Ca^{2+}$-activated ATPase activities were assayed over a temperature range of 0–31 °C in a volume of 1 ml of 40 mM Tris-HCl pH 7.4 (at 10 °C); 5 mM $MgCl_2$; 100 μM $CaCl_2$; 6 mM disodium-ATP, and at a myofibril concentration of 0.4–0.6 mg · ml^{-1} and ionic strength of 0.12 (adjusted with KCl). Preparations were 90–95% Ca^{2+} sensitive (assayed in presence of 5 mM EGTA). Reactions were started by the addition of ATP to preincubated myofibrils, and terminated at intervals by the addition of 1 ml of 15% (w/v) trichloroacetic acid. Precipitated protein was removed by centrifugation at 3000 × g for 5 min and inorganic phosphate (P_i) determined in an aliquot of the supernatant[8] Protein concentrations of the myofibril suspensions were determined by a biuret method[9].

ATPase activities at different assay temperatures were represented as Arrhenius plots and thermodynamic parameters calculated as described previously[10].

Results. Temperature dependence of the brook trout ATPase does not alter significantly between groups of fish acclimated to +4 °C or +24 °C ($p > 0.10$) (table; fig. 1). Substrate turnover number is higher, and activation energy ($\Delta G^{\ddagger}$) is significantly lower than values previously reported for stenothermal species adapted to either of these

temperature regimes ($p < 0.001$) (fig. 2; table). Calculated values for the thermodynamic activation parametes were as follows:

Free energy of activation	$(\Delta G^{+}) = 15{,}545 \pm 44$ cal · mole^{-1}
Activation enthalpy	$(\Delta H^{+}) = 16{,}853 \pm 83$ cal · mole^{-1}
Activation entropy	$(\Delta S^{+}) = 4.19 \pm 0.12$ cal · mole^{-1} K^{-1}

All values means of 8 fish, ± 1 SEM.

Discussion. Natural selection in stenothermal fish has favoured the evolution of enzymes 'tailored' for optimal function at particular environmental temperatures[11,12]. For instance, when assayed at 0 °C, myofibrillar ATPases from cold-adapted species have activities which are 10- to 30-fold higher than the homologues from warm-water species (table).

Previous studies on fish myofibrillar ATPase have shown a close correlation between activation free energy (ΔG^{+}) and adaptation temperature. Cold-adaptation is also associated with a significant reduction in activation enthalpy (ΔH^{+}) and an increase in activation entropy (ΔS^{+})[10,12]. Compensatory modifications in thermodynamic activation parameters are associated with changes in the susceptibility of myosin ATPases to thermal denaturation. For example, the half-life of thermal denaturation of fish muscle myofibrillar ATPases differs by several orders of magnitude between polar and tropical stenotherms[13,14]. Many eurythermal species show a partial or complete compensation of metabolic rate following acclimation from summer to winter temperatures[2]. At the cellular level this is largely achieved through increases in the concentrations of aerobic enzymes[2,11,12,15]. For example, Wilson found that acclimation of goldfish from 25 °C to 5 °C resulted in, respectively, a 66% and 45% increase in concentration and activity of muscle cytochrome oxicase[16].

However, in the case of contractile proteins, an increase in concentration (more myofibrils/muscle cross-section) would effect the maximum tension generated but not the maximum speed of contraction of the muscle. Thus increases in protein concentration are not available as an option for increasing contractile performance at low temperatures. Fish of the carp family (Cypriniidae) have been shown to synthesize kinetically-dinstinct variants of their myofibrillar ATPase following a period of acclimation mimicing the seasonal temperature regime[4-6]. As in the ATPases of low-temperature stenotherms, cold-acclimation is associated with reduction in activation enthalpy (ΔH^{+}), increase in substrate turnover number and an enhanced susceptibility to thermal denaturation. It would appear that these acclimation-induced variants involve primarily adaptation in the calcium-regulatory sub-units of the tropomyosin/troponins complex[5]. This constitutes a relatively efficient mechanism of achieving compensation in contractile function, since the Ca^{2+}-regulatory proteins constitute only a small proportion of the total myofibrillar mass.

In contrast to goldfish temperature acclimation in brook trout is not associated with the synthesis of kinetically-distinct myofibrillar ATPases (fig. 1). Interestingly the values of activation free energy (ΔG^{+}) and enthalpy (ΔH^{+}) do not adhere to the relationship shown for cyprinids and for a wide range of stenothermal species (fig. 2a and b). Activation energy (ΔG^{+}) is significantly lower than might be anticipated from figure 2a, and activation enthal-

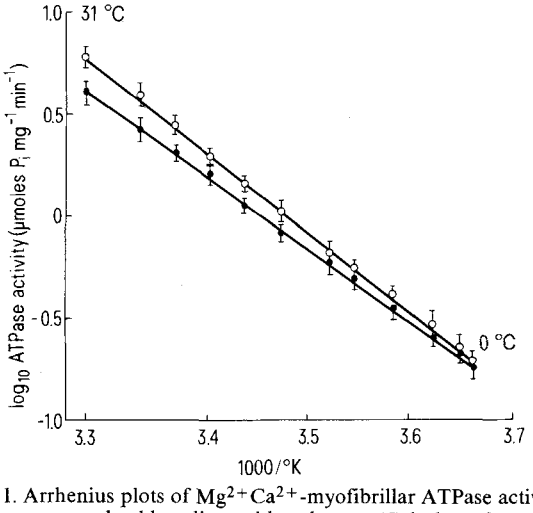

Fig. 1. Arrhenius plots of $Mg^{2+}Ca^{2+}$-myofibrillar ATPase activities from warm- and cold-acclimated brook trout *(Salvelinus fontinalis)*. +4 °C acclimated fish; +24 °C acclimated fish. Means ± 1 SEM of 8 fish. Assay conditions given in text. Slopes of regression lines are not statistically significant ($p > 0.05$).

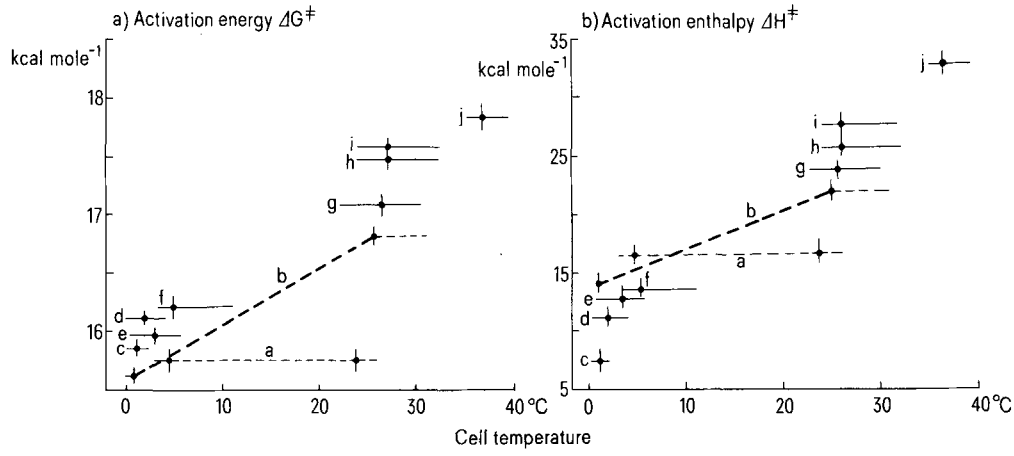

Fig. 2. Relationships between Gibbs free energy of activation (ΔG^{+}) (a) and activation enthalpy (ΔH^{+}) (b), and cell temperature for a range of eurythermal and stenothermal teleosts. Vertical bars represent mean ± 1 SEM of 8 or more preparations; horizontal and oblique bars correspond to temperature range experienced by each species in the natural environment. Values for stenothermal species are taken or derived from previous publication from this laboratory[10,13,14,18,19]. a, *Salvelinus fontinalis* (this study); b, *Carassius auratus* (European lakes)[3]; c, *Champsocephalus gunnari* (Antarctic marine)[14]; d, *Notothenia neglecta* (Antarctic marine)[19]; e, *Notothenia rossii* (Antarctic marine)[10]; f, *Cottus bubalis* (North Sea); g, *Abudefuf oxydon* (Indo-Pacific marine)[14]; h, *Pomatocentrus unicellatus* (Indo-Pacific marine)[14]; i, *Dascyllus carneus* (Indo-Pacific marine)[10]; j, *Tilapia grahami* (equatorial hotsprings; Lake Magardi, Kenya)[13].

Substrate conversion rates of $Mg^{2+}Ca^{2+}$-myofibrillar ATPases from eurythermal and stenothermal fish inhabiting different environmental temperatures

Species [Common name: habitat]	Environmental temperature (°C) [Range of habitat temperatures]	Substrate turnover number (moles ATP split · mole myosin^{-1} sec^{-1}) Assay at 0 °C	Assay at physiological temperature (°C)	Reference
Salvelinus fontinalis [brook trout: European rivers]	acclimated to +4	2.69±0.26	4.00±0.32 (4)	This study
	acclimated to +24	2.89±0.16	40.69±2.36 (24)	
Carassius auratus [common goldfish: European lakes]	acclimated to +1	1.40	1.45 (1)	3
	acclimated to +26	0.39	5.11 (26)	
Notothenia rossii [South Georgia cod: antarctic marine]	+2 [−1 to +4]	3.56	6.22 (4)	10
Gadus morhua [cod: North Sea]	+12 [+3 to +15]	1.02	8.96 (12)	13
Salmo trutta [brown trout: European lakes]	+15 [+5 to +24]	1.36	9.20 (15)	10
Abudefuf oxydon [neon reef perch: Indo-Pacific coral reefs]	+25 [+24 to +30]	0.15	4.76 (25)	10
Amphiprion sebae [Anenome Fish: Indo-Pacific coral reefs]	+25 [+24 to +30]	0.19	8.44 (25)	18
Tilapia nigra [Cichlid spp.: East African lakes]	+28 [+24 to +33]	0.25	6.67 (28)	13

Values are means of 6 or more preparations; SEM in each case < 15% of mean (see original references for further details). Substrate turnover numbers are calculated assuming a myosin content of 54% and a mol.wt of 240,000 daltons per enzyme active site (2 sites per myosin molecule)[16].

py ΔH^+) is comparable to that of a stenothermal species adapted to 8–10 °C (figure 2b).

It would appear that the properties of brook trout myofibrillar ATPase constitutes a compromise between the optimum kinetic forms for either the higher or the lower acclimation temperature.

The upper lethal limit for many salmoniids (22–24 °C) is much less than body temperatures routinely encountered by cyprinids in the natural habitats[17]. It is suggested that a combination of behavioural temperature regulation and a 'compromise' ATPase provides one alternative strategy for adaptation of locomotory function in eurythermal fish.

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Distribution of chromium in red kidney beans (*Phaseolus vulgaris* L.)

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Summary. Distribution of both Cr^{3+} and CrO_4^{2-} in bean shoots followed a markedly acropetal gradient. Chemical fractionation of radiochromium accumulated in the edible bean pods indicated the greatest association (70–75%) with ionic forms (extractable by weak mineral acids).

Chromium, which is present as a soil and water pollutant due to chromium wastes released from various industrial sources, and chromium-51, a gamma-emitting activation product released in controlled or accidental discharges from nuclear installations, could enter the human food-chain through rivers, groundwater and irrigated soil. Con-