HUGHES *ET AL.* REPLY—Varley *et al.*'s[1] comments are based largely on misunderstandings of dendroclimatological techniques[2-7]. We agree that it is not appropriate to use mean ring-width chronologies. We did not do so but rather used standardised indices of ring-width[2] for well-documented reasons[5,7,8]. We did not use regression analysis of ring-widths on monthly meteorological figures as suggested[1]. We stated clearly[2] that ring-width indices were regressed on the eigenvectors of climatic data. Consequently our results should not be compared with those referred to by Varley *et al.*[1,9] where no account has been taken of the non-orthogonality of the data. We have maximised tree-ring variance attributable to the eigenvectors of climate, not the number of significant response function elements. The two are not simply related[5]. We discussed differences between the reported response functions. Such 'inconsistency' was ecologically expected and dendroclimatologically welcome[5,10]. The choice of meteorological variables related to climatic reconstruction, not tree physiology[5].

In their reference to defoliators Varley *et al.* miss the fundamental point of dendroclimatology. The material selected for site chronologies conforms strongly to a common pattern of ring-width variation[2]. This could only be produced by factors acting similarly and synchronously on all the trees selected. No sound evidence for caterpillars doing this on British oaks is produced[1]. The European examples[11,12] concern aggregate effects on stand samples, not dendrochronologically selected material. The English example[9,13] rests on a regression analysis of % mean latewood increment and % mean caterpillar numbers (5 trees, 20 yr) where no test of difference of the gradient from zero is given. As Varley[13] uses the intercept to estimate effect of caterpillars it is surprising that no confidence limits are given. Incomplete statistics are given for an ecologically incomplete analysis[14].

Our results are inevitably reproducible within sites as only strongly cross-dated trees are used. Chronologies from subsets of 13 and 10 trees (S. J. Milsom, unpublished data) at Maentwrog are extremely similar ($t = 15.4$, 261 yr overlap)[15] as is the Rostrevor chronology[16] and one from 11 nearby trees ($t = 17.4$, 223 yr overlap). Consequently they produce very similar response functions except for two differences in precipitation elements between the Rostrevor sites.

We will not use response functions to reconstruct climate. For this, we will use transfer functions between time-series of spatial anomalies of tree-rings and climatic variables[3-5]. Recent work (B.G., unpublished data) has shown there to be spatially coherent, temporally consistent patterns of ring-width variability. Very similar patterns for the first five eigenvectors accounted for 65% (1825–94) and 70% (1895–1964) of variance in a 15-chronology tree-ring grid between 50–60° N and 10° W–10° E. Patterns of UK mean monthly temperature (B. Gray, unpublished data) and annual rainfall (R. Tabony, unpublished data) share similar properties. It is such coherent, consistent large-scale patterns of tree-ring variation that will be calibrated for use in reconstructing past climate. These new results further support our expectation that tree-ring chronologies from the British Isles will provide valuable proxy climatic records[2] and bring the generation of testable reconstructions closer.

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Cell density is determined by a diffusion-limited process

FROM the culture of 3T3 cells in media with various viscosities, Whittenberger and Glaser[1] have concluded that the saturation density of these cells is not dependent on a diffusion-limited factor. A flaw exists in the design of their experiments. In their culture conditions the cell density of 3T3 cells is limited primarily by the total supply of serum growth factors in the medium, not simply by the concentration of serum. As high viscosity does not alter the total supply of medium constituents, it is not to be expected that the cell density attained should be affected by viscosity in their conditions.

Table 1 Effect of viscosity on cell density

Treatment	Cell density ($\times 10^{-4}$ cm^2)
1% Serum	9.5 ± 1.7
1% Serum plus 1% Methocel	3.8 ± 0.6
1% Serum plus 1.5% Methocel	2.5 ± 0.7
1% Serum plus 2% Methocel	1.3 ± 1.1

Swiss 3T3 (clone 4A) cells were plated on 14-mm glass coverslips in Dulbecco modified Eagle's medium containing 10% serum. After 1 d single coverslips, with cells at an initial density of 3×10^3 cells cm^{-2}, were transferred, one coverslip each, to 15-cm plastic culture dishes. To each dish 50 ml of medium was added with or without methylcellulose (Methocel A4M, Dow) as indicated above. Treatments were in triplicate. After a further 5 d incubation in a CO_2 incubator, the coverslips were removed, washed gently with Tris–saline buffer, and the cells were fixed in formol–saline. The cells were stained and 10 fields per coverslip were counted under a microscope with an eyepiece grid. (Means and s.d. are given for the 30 fields counted per treatment.) Observations under the microscope indicated that there was no loss of cells during the washing and fixing procedures. The cell densities at 5 d approach equilibrium densities[2]. Approximately the same variation of cell density with viscosity was observed when the coverslips were placed individually in 100-ml glass beakers containing 50 ml of medium with the same Methocel concentrations listed above, indicating that the geometry of the culture vessel is not critical. The low cell density observed in medium with 2% Methocel was also found when the cells, on a coverslip, were beneath a 3-mm layer of viscous medium (2% Methocel) in the bottom of a beaker, and 50 ml of medium without Methocel was placed above the viscous layer. Therefore, it is not the absence of convection currents in the bulk of the medium that leads to the lower cell density. The Methocel used was shown to be non-toxic by culturing the cells in a small volume of medium (0.2 ml cm^{-2} of cells) with 1% serum, with and without Methocel, in normal culture conditions. As was observed by Whittenberger and Glaser[1], Methocel had little effect on the cell density attained. As Dextran and Ficoll give lower than normal cell densities at 5 d in normal culture conditions[1] (attributed by the authors to 'toxicity' of these agents), these materials were not investigated.

In the culture conditions that were used, with the usual small volume of medium (0.2 ml cm^{-2} of cells), quiescent 3T3 cells destroy serum growth factors quickly after each medium change and before the cells have been exposed long enough for many cells to initiate DNA synthesis. Any increase in cell number after a medium change is balanced by cell loss when the concentrations of serum growth factors reach their minimum levels before the next medium change. High viscosity, if it has any effect, will prolong the period of destruction of serum factors in these conditions, and this prolongation will lengthen the exposure of the cells to a serum concentration high enough to initiate DNA synthesis. This might even increase the cell density. Therefore, the experiments of Whittenberger and Glaser