High CO_2 increases the freezing sensitivity of plants: Implications for paleoclimatic reconstructions from fossil floras

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ABSTRACT

Reconstructions of coldest month mean temperature (CMMT) in the geologic past are important for understanding the dynamics of the climate system operating at that time. A common method for estimating CMMT involves applying the climatic tolerances of modern plant species to close analogues in the fossil record (the so-called nearest living relative [NLR] approach). Here we show that the leaves of seven modern species commonly used as NLRs to reconstruct CMMT from the fossil record freeze at warmer temperatures when grown at high CO_2 (800 vs. 400 ppmv). This CO_2 effect was found across all tested plant groups, including palms, taxodiaceous conifers, and two Southern Hemisphere evergreen species. This pervasive response indicates that many NLR-derived estimates of CMMT for times of high CO_2 should be adjusted by at least +1.5 to +3 °C. A revision of CMMT estimates toward warmer values further increases the disparity in continental interiors between CMMT indicators from the fossil record and the colder predictions from general circulation models.

Keywords: paleoclimate, paleotemperature, paleobotany, carbon dioxide.

INTRODUCTION

The coldest month mean temperature (CMMT) is an important climatic component for reconstructing seasonality in the geologic past. An understanding of seasonality, in turn, sheds light on regional topography, the potential for snowfall, and the seasonal importance of meridional heat transport. CMMT estimates derived from the rock record also provide important constraints on model predictions of CMMT. For example, general circulation models consistently underpredict CMMT in continental interiors by as much as 15 °C (Sloan and Barron, 1990, 1992; Barron et al., 1995) relative to estimates derived from the fossil record (Horrell, 1991; Wing, 1991; Wing and Greenwood, 1993; Markwick, 1994; Greenwood and Wing, 1995; Tarduno et al., 1998). Such model-data discrepancies are important to resolve in order to better understand the processes governing global climates, and to design more reliable models for predicting future climates (Parrish, 1998).

One approach for reconstructing CMMT for Cretaceous and Tertiary terrestrial climates involves identifying the nearest living relatives (NLRs) of as many taxa as possible in a given fossil flora and applying the range of CMMT of the NLRs to the fossil site (e.g., Schweitzer, 1980; Van der Burgh, 1984; Boyd, 1990; Horrell, 1991; Basinger et al., 1994; Greenwood and Wing, 1995; Wing and Greenwood, 1996; Utescher et al., 2000). Because many plants are highly sensitive to CMMT, the NLR approach can yield precise estimates. For example, the most frost-hardy palms today (e.g., *Trachycarpus*, *Chamaerops*) cannot survive temperatures below −10 °C (Larcher and Winter, 1981; Sakai and Larcher, 1987), and the poleward limit of palms today (the so-called palm line) corresponds to a CMMT of >5 °C (Van der Burgh, 1984; Greenwood and Wing, 1995). *Sequoia sempervirens* also cannot survive temperatures below −17 °C (Sakai, 1971, 1983), and its minimum CMMT today is ~6 °C (Van der Burgh, 1984).

Furthermore, there are few alternative methods for reconstructing CMMT. One method involves regressing multiple characters of leaf size and shape against a suite of climatic parameters, including CMMT (Wolfe, 1993; Wing and Greenwood, 1993). However, because mean annual temperature dominates the correlations with the leaf characters, estimates of CMMT and mean annual temperature are autocorrelated. Thus, independent estimates of CMMT are not possible with this method because CMMT simply tracks the present-day relationship with mean annual temperature (Jordan, 1996, 1997; Wing and Greenwood, 1996). Therefore, the floral analysis of nearest living relatives remains one of the more reliable methods for reconstructing CMMT.

Despite its importance as a key quantitative method for reconstructing CMMT, the NLR approach is undermined by the possibility that the climatic tolerances of fossil taxa are not fixed, and may diverge from their modern NLR counterparts as phylogenetic distance increases further back into geologic time (e.g., Chaloner and Creber, 1990) or if the geographic range of a given taxon becomes severely restricted (e.g., Hickey, 1977; Mai, 1994). Surprisingly, however, it is not known whether environmental factors such as the concentration of atmospheric CO_2 influence NLR-derived paleoclimate interpretations (Beerling, 1998). Recent evidence from a wide variety of CO_2-enrichment growth experiments indicates that the temperature at which leaves freeze (ice-nucleation temperature, INT) for several plant species is warmer at high CO_2 than their equivalents grown under low CO_2 (Table 1). Leaf INT and degree of frost damage show similar responses to CO_2 (Lutze et al., 1998), indicating that patterns in leaf INT reflect minimum temperature tolerances (T_{min}). CMMT, in turn, is currently correlated with T_{min} in the following fashion (adapted from Prentice et al., 1992):

$$d(CMMT)/d(T_{min}) = 0.77. \qquad (1)$$

Given that geochemical models and proxy measurements suggest significantly higher concentrations of atmospheric CO_2 than present-day levels during certain times in the Cretaceous and Paleogene (Berner and Kothavala, 2001; Chen et al., 2001; Ekart et al.,

Species	Month of sampling	CO$_2$ (ppm)	INT (°C)	CO$_2$ (ppm)	INT (°C)	Δ* (°C)	P	Source
Eucalyptus pauciflora	October	350	−5.7	700	−4.3	1.4	<0.02	Lutze et al., 1998
Ginkgo biloba	June	350	−10.1	700	−4.5	5.6	<0.001	Terry et al., 2000
	September	350	−7.8	700	−5.5	2.3	<0.001	
Empetrum hermaphroditum	June–Oct.[†]	350	−7.8	600	−6.7	1.1	<0.001	Beerling et al., 2001
Vaccinium myrtillus	June–Sept.[†]	350	−6.3	600	−6.2	0.1	>0.05	
V. uliginosum	June–Sept.[†]	350	−8.8	600	−7.3	1.5	<0.001	
V. vitis-idaea	June–Oct.[†]	350	−8.0	600	−7.2	0.8	<0.001	
Betula nana	N.D.[§]	276	−7.8	315	−7.6	0.2	0.34[#]	Beerling et al., 2002
Myrica gale	N.D.	290	−9.0	322	−7.4	1.6	0.003[#]	
Salix herbacea	N.D.	252	−6.8	287	−5.0	1.8	<0.001[#]	
S. repens	N.D.	263	−7.7	287	−6.1	1.6	0.03[#]	

* Δ = INT$_{[highCO_2]}$ − INT$_{[lowCO_2]}$.

[†] Leaves sampled three to four times throughout the growing season. Ice-nucleation temperatures represent seasonal means.

[§] N.D. = no data.

[#] Analysis of variance based on regression of a series of INT measurements spanning the listed CO$_2$ range.

Figure 1. Temperature trace for high- and low-CO$_2$–grown branchlet of *Taxodium distichum*. Exothermic spike corresponds to leaf's ice-nucleation temperature. Time axis represents duration of cooling event, beginning with corresponding leaf temperature of 0 °C.

1999; Ghosh et al., 2001), this CO$_2$ effect could introduce a significant, and previously unrealized, bias into CMMT estimates obtained from the analyses of fossil floras. However, all of the species investigated thus far, with the exception of *Ginkgo biloba* (Terry et al., 2000), do not have long fossil records and so are not directly relevant to the use of NLRs for reconstructing paleoclimates (see Table 1). It is therefore unknown whether this CO$_2$ effect is applicable to the fossil record.

Here we test the long-term (2 yr) influence of the concentration of atmospheric CO$_2$ on the INTs of leaves of five tree species with long fossil records, as well as two species of palms. All selected taxa have been previously used as NLRs in climatic reconstructions and include species with both deciduous and evergreen leaf habits. Although modern palms do not have long fossil records at the generic level, their leaf, stem, and root anatomy is indistinguishable from their fossil counterparts (e.g., presence of manoxylic wood), suggesting a similar cold tolerance (Wing and Greenwood, 1993, 1996; Greenwood and Wing, 1995).

EXPERIMENTAL DESIGN

All plants were grown in four fully replicated growth rooms located in Sheffield, UK. Each growth room is partitioned into a high- and low-CO$_2$ environment (800 and 400 ppmv) and is subject to a photoperiod equivalent to 68° latitude. Temperatures track ambient values plus 5 °C (daily means are ~10 °C in January and ~20 °C in July). These simulated conditions are representative of the high-latitude Cretaceous and Paleogene climates under which most of the plant lineages tested here grew, including the best-guess range of atmospheric CO$_2$ (see Beerling and Osborne [2002] for discussion and further details on experimental design).

Three 1-yr-old saplings of each species were placed in each of the eight growth rooms

in April 2000. These species included two deciduous conifers (*Metasequoia glyptostroboides*, *Taxodium distichum*), one evergreen conifer (*Sequoia sempervirens*), and two Southern Hemisphere evergreens (*Nothofagus cunninghamii*, *Araucaria araucana*). All of these taxa have long fossil records at the generic level (≥65 m.y.) (Chaney, 1951; Tralau, 1968; Hill, 1991; Kershaw and Wagstaff, 2001) and may therefore reflect the physiological processes expressed by their fossil representatives. We also grew two species of palms, *Trachycarpus fortunei* and *Chamaerops humilis*, that grow near the northern limit of palms today (Sakai and Larcher, 1987). They therefore represent good physiological surrogates for palms that grew near palms lines in the geologic past.

Ice-nucleation temperatures were measured by slowly cooling (0.1 °C·min^{-1}), detached, individual branchlets (for *Metasequoia*, *Taxodium*, and *Sequoia*) or leaves (for all other species) using an insulated peltier cooling plate coupled to a temperature controller (see Terry et al. [2000] for experimental design). Only leaves from the current year's growth were used. Multiple thermocouples (76 μm in diameter) were placed directly on the surface of the leaves to measure leaf temperature. When ice crystals form within leaf tissues, the exothermic reaction registers as a positive temperature excursion. The base of this excursion was taken as the ice-nucleation temperature (INT). An example of a temperature trace for a high- and low-CO$_2$–grown *Taxodium* is shown in Figure 1. Leaves from at least four plants (≥1 per growth room) were measured for each CO$_2$ treatment. Statistics were based on the mean INT per growth room for each species per CO$_2$ treatment ($n = 4$). Measurements were made at the end of the growing season in October–November 2001 and again at the beginning of the growing season in March–April 2002.
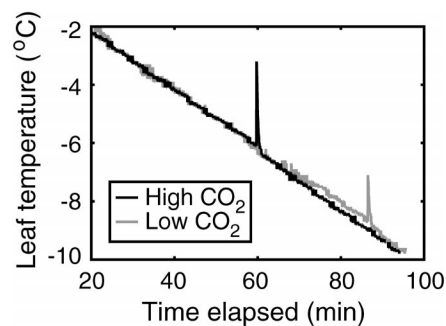
RESULTS

The leaves of all species grown at high CO$_2$ systematically froze at warmer temperatures relative to their low-CO$_2$ counterparts (Figs. 2 and 3). The mean temperature response to the high-CO$_2$ treatment during the fall was +1.5 °C (range: +0.6 to +2.3 °C); all species showed a significant response at the α = 0.05 level (see Fig. 2), with the exception of *Araucaria*. For the early spring, the mean temperature response increased to +2.6 °C (range: +1.7 to +3.7 °C); all species showed a significant response. Both the fall and spring mean responses were significantly different from the null response of 0 °C (P <0.0007; Student's t-test).

There were no strong differences in the CO$_2$ responses between the deciduous and evergreen species or among the major plant groups (e.g., palms, taxodiaceous conifers). In addition, plants with Northern and Southern Hemisphere native ranges responded similarly to CO$_2$. This result suggests that the leaf INT response to CO$_2$ is pervasive and fairly uniform, at least within the tested plant groups.

The CO$_2$ effect was more pronounced during the spring than during the fall (+2.6 vs. +1.5 °C; $P = 0.02$; Student's t-test; see Fig. 3). This same seasonal pattern has also been observed in *Ginkgo biloba* (Terry et al., 2000; see Table 1), but not in a range of subarctic ericaceous shrubs (Beerling et al., 2001). Our observations show that both the evergreen and deciduous plants form the bulk of their plant tissues during the spring. As a result, frost damage incurred during the spring is more detrimental to the overall fitness of these plants than during the fall, and thus the magnitude of the leaf INT response to CO$_2$ is most critical during this season. Using the transfer function of Prentice et al. (1992) (already described) and assuming a similar relationship between minimum temperatures and CMMT in the past, these springtime shifts of 2–3.5 °C
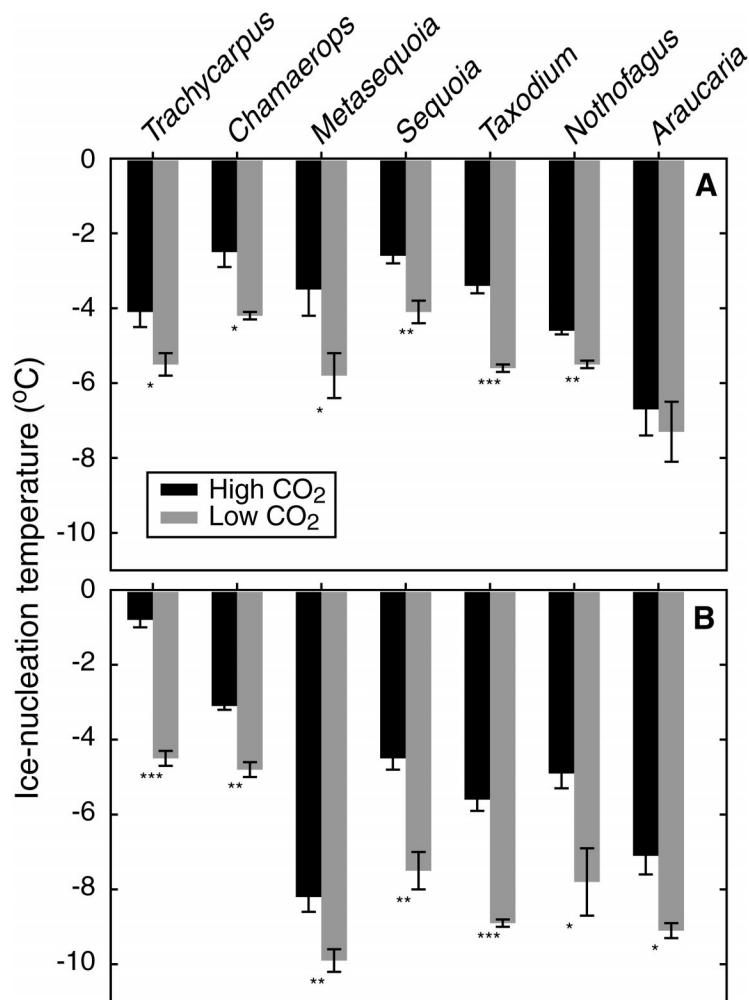
Figure 2. Mean leaf ice-nucleation temperatures for plants grown under two CO_2 treatments (800 and 400 ppmv) as measured during (A) fall and (B) spring. Error bars represent standard errors. Asterisks correspond to following levels of significance: *—$\alpha = 0.05$; **—$\alpha = 0.01$; ***—$\alpha = 0.001$.
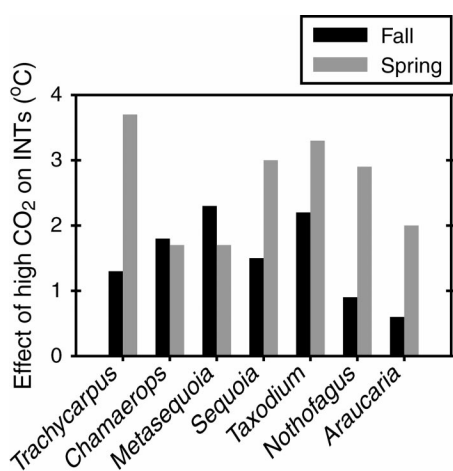

Figure 3. Difference in ice-nucleation temperatures (INTs) between plants grown under two CO_2 treatments ($\Delta = INT_{[high\ CO_2]} - INT_{[low\ CO_2]}$; raw data from Fig. 2). Positive values correspond to species whose leaves froze at warmer temperatures when grown under high CO_2.

were probably warmer during polar winters than previously realized. For example, the presence of palms during the Late Cretaceous to early Paleogene in the North American and Asian continental interiors (paleolatitude = 45–60 °N) (Horrell, 1991; Wing et al., 1993; Greenwood and Wing, 1995) and coastal Spitsbergen (paleolatitude = 70 °N) (Schweitzer, 1980) indicate a CMMT of >5 °C, which with the CO_2 effect observed here would increase to >8 °C. In contrast, general circulation models predict CMMTs of −3 °C or less for the North American and Asian continental interiors during these times (Sloan and Barron, 1990, 1992; Barron et al., 1995). These disparities further point toward the need for models to incorporate additional climate forcings such as high-latitude vegetative feedbacks (Otto-Bliesner and Upchurch, 1997), orbital variations (Sloan and Morrill, 1998), and other radiatively active greenhouse gases.

REFERENCES CITED

Barron, E.J., Fawcett, P.J., Peterson, W.H., Pollard, D., and Thompson, S.L., 1995, A "simulation" of mid-Cretaceous climate: Paleoceanography, v. 10, p. 953–962.

Basinger, J.F., Greenwood, D.R., and Sweda, T., 1994, Early Tertiary vegetation of Arctic Canada and its relevance to paleoclimatic interpretation, *in* Boulter, M.C., and Fisher, H.C., eds., Cenozoic plants and climates of the Arctic: Berlin, Springer-Verlag, p. 175–198.

Beerling, D.J., 1998, The future as the key to the past for palaeobotany?: Trends in Ecology and Evolution, v. 13, p. 311–316.

Beerling, D.J., and Osborne, C.P., 2002, Physiological ecology of Mesozoic polar forests in a high CO_2 environment: Annals of Botany, v. 89, p. 329–339.

Beerling, D.J., Terry, A.C., Mitchell, P.L., Callaghan, T.V., Gwynn-Jones, D., and Lee, J.A., 2001, Time to chill: Effects of simulated global change on leaf ice nucleation temperatures of subarctic vegetation: American Journal of Botany, v. 88, p. 628–633.

Beerling, D.J., Terry, A.C., Hopwood, C., and Osborne, C.P., 2002, Feeling the cold: Atmospheric CO_2 enrichment and the frost sensitivity of terrestrial plant foliage: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 182, p. 3–13.

Berner, R.A., and Kothavala, Z., 2001, GEOCARB III: A revised model of atmospheric CO_2 over Phanerozoic time: American Journal of Science, v. 301, p. 182–204.

Boyd, A., 1990, The Thyra Ø Flora: Toward an understanding of the climate and vegetation during the early Tertiary in the High Arctic: Review of Palaeobotany and Palynology, v. 62, p. 189–203.

in leaf INT correspond to shifts in CMMT of 1.5–3 °C.

IMPLICATIONS FOR PALEOCLIMATE RECONSTRUCTIONS

Our data therefore indicate that CMMTs estimated by using the NLR approach for times of high CO_2 (>800 ppmv) need to be adjusted by at least +1.5 to +3 °C. Because CMMT is correlated with other temperature parameters such as mean annual temperature, some NLR-derived estimates of mean annual temperature may also require an adjustment to warmer values for times when the atmospheric CO_2 concentration was higher than now. Overall, our study indicates that adopting a strictly uniformitarian approach when using fossil plants to reconstruct ancient climates can result in underestimating CMMT.

For times of high CO_2, our observations amplify the discrepancies between proxy estimates and climate-model predictions of CMMT and suggest that the high latitudes

Chaloner, W.G., and Creber, G.T., 1990, Do fossil plants give a climatic signal?: Geological Society of London Journal, v. 147, p. 343–350.

Chaney, R.W., 1951, A revision of fossil *Sequoia* and *Taxodium* in western North America based on the recent discovery of *Metasequoia*: American Philosophical Society Transactions, v. 40, p. 171–263.

Chen, L.-Q., Li, C.-S., Chaloner, W.G., Beerling, D.J., Sun, Q.-G., Collinson, M.E., and Mitchell, P.L., 2001, Assessing the potential for the stomatal characters of extant and fossil *Ginkgo* leaves to signal atmospheric CO_2 change: American Journal of Botany, v. 88, p. 1309–1315.

Ekart, D.D., Cerling, T.E., Montañez, I.P., and Tabor, N.J., 1999, A 400 million year carbon isotope record of pedogenic carbonate: Implications for paleoatmospheric carbon dioxide: American Journal of Science, v. 299, p. 805–827.

Ghosh, P., Ghosh, P., and Bhattacharya, S.K., 2001, CO_2 levels in the late Palaeozoic and Mesozoic atmosphere from soil carbonate and organic matter, Satpura basin, central India: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 170, p. 219–236.

Greenwood, D.R., and Wing, S.L., 1995, Eocene continental climates and latitudinal temperature gradients: Geology, v. 23, p. 1044–1048.

Hickey, L.J., 1977, Stratigraphy and paleobotany of the Golden Valley Formation (early Tertiary) of western North Dakota: Geological Society of America Memoir 150, 181 p.

Hill, R.S., 1991, Tertiary *Nothofagus* (Fagaceae) macrofossils from Tasmania and Antarctica and their bearing on the evolution of the genus: Linnean Society Botanical Journal, v. 105, p. 73–112.

Horrell, M.A., 1991, Phytogeography and paleoclimatic interpretation of the Maestrichtian: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 86, p. 87–138.

Jordan, G.J., 1996, Eocene continental climates and latitudinal temperature gradients: Comment: Geology, v. 24, p. 1054.

Jordan, G.J., 1997, Uncertainty in palaeoclimatic reconstructions based on leaf physiognomy: Australian Journal of Botany, v. 45, p. 527–547.

Kershaw, P., and Wagstaff, B., 2001, The southern conifer family Araucariaceae: History, status, and value for paleoenvironmental reconstruction: Annual Review of Ecology and Systematics, v. 32, p. 397–414.

Larcher, W., and Winter, A., 1981, Frost susceptibility of palms: Experimental data and their interpretation: Principes, v. 25, p. 143–152.

Lutze, J.L., Roden, J.S., Holly, C.J., Wolfe, J., Egerton, J.J.G., and Ball, M.C., 1998, Elevated atmospheric $[CO_2]$ promotes frost damage in evergreen tree seedlings: Plant, Cell and Environment, v. 21, p. 631–635.

Mai, D.H., 1994, Two conifers—*Tetraclinis* Mast. (Cupressaceae) and *Metasequoia* Miki (Taxodiaceae)—relicts or palaeoclimatic indicators of the past, *in* Boulter, M.C., and Fisher, H.C., eds., Cenozoic plants and climates of the Arctic: Berlin, Springer-Verlag, p. 199–213.

Markwick, P.J., 1994, ''Equability,'' continentality, and Tertiary ''climate'': The crocodilian perspective: Geology, v. 22, p. 613–616.

Otto-Bliesner, B.L., and Upchurch, G.R., 1997, Vegetation-induced warming of high-latitude regions during the Late Cretaceous Period: Nature, v. 385, p. 804–807.

Parrish, J.T., 1998, Interpreting pre-Quaternary climate from the geologic record: New York, Columbia University Press, 338 p.

Prentice, I.C., Cramer, W., Harrison, S.P., Leemans, R., Monserud, R.A., and Solomon, A.M., 1992, A global biome model based on plant physiology and dominance, soil properties and climate: Journal of Biogeography, v. 19, p. 117–134.

Sakai, A., 1971, Freezing resistance of relicts from the Arcto-Tertiary flora: New Phytologist, v. 70, p. 1199–1205.

Sakai, A., 1983, Comparative study on freezing resistance of conifers with special reference to cold adaptation and its evolutive aspects: Canadian Journal of Botany, v. 61, p. 2323–2332.

Sakai, A., and Larcher, W., 1987, Frost survival of plants: Berlin, Springer-Verlag, 321 p.

Schweitzer, H.-J., 1980, Environment and climate in the early Tertiary of Spitsbergen: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 30, p. 297–311.

Sloan, L.C., and Barron, E.J., 1990, ''Equable'' climates during Earth history?: Geology, v. 18, p. 489–492.

Sloan, L.C., and Barron, E.J., 1992, A comparison of Eocene climate model results to quantified paleoclimatic interpretations: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 93, p. 183–202.

Sloan, L.C., and Morrill, C., 1998, Orbital forcing and Eocene continental temperatures: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 144, p. 21–35.

Tarduno, J.A., Brinkman, D.B., Renne, P.R., Cottrell, R.D., Scher, H., and Castillo, P., 1998, Evidence for extreme climatic warmth from Late Cretaceous arctic vertebrates: Science, v. 282, p. 2241–2243.

Terry, A.C., Quick, W.P., and Beerling, D.J., 2000, Long-term growth of *Ginkgo* with CO_2 enrichment increases leaf ice nucleation temperatures and limits recovery of the photosynthetic system from freezing: Plant Physiology, v. 124, p. 183–190.

Tralau, H., 1968, Evolutionary trends in the genus *Ginkgo*: Lethaia, v. 1, p. 63–101.

Utescher, T., Mosbrugger, V., and Ashraf, A.R., 2000, Terrestrial climate evolution in northwest Germany over the last 25 million years: Palaios, v. 15, p. 430–449.

Van der Burgh, J., 1984, Some palms in the Miocene of the Lower Rhenish Plain: Review of Palaeobotany and Palynology, v. 40, p. 359–374.

Wing, S.L., 1991, "Equable" climates during Earth history?: Comment: Geology, v. 19, p. 539–540.

Wing, S.L., and Greenwood, D.R., 1993, Fossils and fossil climate: The case for equable continental interiors in the Eocene: Royal Society of London Philosophical Transactions, ser. B, v. 341, p. 243–252.

Wing, S.L., and Greenwood, D., 1996, Eocene continental climates and latitudinal temperature gradients: Reply: Geology, v. 24, p. 1054–1055.

Wing, S.L., Hickey, L.J., and Swisher, C.C., 1993, Implications of an exceptional fossil flora for Late Cretaceous vegetation: Nature, v. 363, p. 342–344.

Wolfe, J.A., 1993, A method of obtaining climatic parameters from leaf assemblages: U.S. Geological Survey Bulletin 2040, p. 1–71.