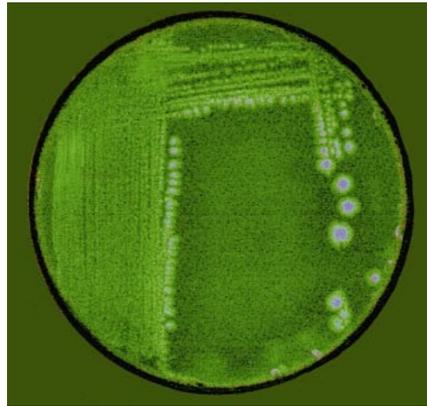


# *Clostridium* used in mediaeval dyeing

Before chemical methods of reduction were introduced in the past century, dyeing using the pigment indigo required a fermenting woad vat to reduce the insoluble indigo to a soluble form<sup>1,2</sup>. We have reproduced mediaeval techniques of woad preparation<sup>3</sup> and fermentation, and find that a thermophilic *Clostridium* bacterium is responsible for the reduction of indigo.

Leaves from the woad plant (*Isatis tinctoria*) were prepared for the woad vat according to the mediaeval practice<sup>1</sup> described<sup>3</sup>. Couched woad<sup>1,2</sup> provided the substrate for setting up the woad vat, which has been successfully used to dye wool and cotton. Spontaneous fermentation in the woad vat resulted in the evolution of gases (identified as CO<sub>2</sub> and H<sub>2</sub>), acidification of the medium (neutralized by addition of alkali to pH 9), and solubilization of the indigo, causing the vat to turn greenish brown with a surface layer of oxidized blue indigo. The redox potential of the fermenting woad was -560 to -595 mV relative to the standard calomel electrode. The woad vat evolved a noxious odour that was notorious even in mediaeval times<sup>1</sup>. Analysis by gas chromatography mass spectrometry of the organic headspace volatiles showed they were predominantly dimethyl sulphide (45%), dimethyldisulphide (24%) and methanethiol (18%) (C. Duckham and J. Ames, unpublished data), which are characteristic volatile products of the anaerobic breakdown of cruciferous plant material<sup>4</sup> as well as of microbial metabolism<sup>5</sup>.

We enriched five isolates of indigo-reducing Gram-positive anaerobic bacteria from the fermenting woad vat by using indigo-supplemented, reinforced clostridial agar (pH 9) incubated at 47 °C. Reduction of indigo was indicated by the medium turning colourless under anaerobic conditions, and becoming blue on exposure to air (Fig. 1). All of the isolates were rod-shaped,



**Figure 1** Indigo reduction by *Clostridium* isolated from a woad vat. The plates were initially brown because, even though they contained indigo, the dye was not divided finely enough to colour the agar blue. *Clostridium* reduced the indigo anaerobically to its colourless form, then, when exposed to air, the indigo was reoxidized and the agar turned blue.

produced oval terminal endospores, and were negative for the enzyme catalase. Phylogenetic analysis and 16S ribosomal RNA gene sequence analysis<sup>6</sup> of a representative strain (Wv6) showed that the unknown bacterium was a member of rRNA cluster I *Clostridium*, related to *C. carnis*. *Clostridium* strain Wv6 is a phylogenetically distinct species for which the name *C. isatidis* will be formally proposed elsewhere<sup>7</sup>.

We conclude that it was the reduction of indigo by *Clostridium* that enabled indigo to dissolve and so allowed its use as a dye for textiles in mediaeval times. This finding constitutes another important biotechnological application of anaerobic microbes<sup>8</sup>.

The mediaeval vat operators were aware of several requirements for the system to work: they had to keep the vat alkaline, adding wood ash<sup>1,2</sup> to counter acid produced by fermentation; they provided the *Clostridium* with polysaccharide substrate,

adding bran to maintain the fermentation for several months<sup>1,2</sup>; and they had to maintain the temperature at around 50 °C (refs 1,2) as the *Clostridium* is thermophilic. The *Clostridium* also reduced indigo when inoculated as a pure culture in reinforced clostridial medium (pH 9), its growth being accompanied by the production of acetic, formic and lactic acids.

In current industrial practice, the dyeing of cotton yarn for denim production consumes about 2 kg of sodium dithionite for every 3 kg of indigo reduced<sup>9</sup>. Disposal of the by-products of dithionite oxidation is an environmental burden, which could be eliminated by replacing modern chemical methods of indigo reduction with one based on the fermentative biochemistry of indigo-reducing *Clostridium*.

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## Scale of mast-seeding and tree-ring growth

The synchronous production of large seed crops by a population of plants<sup>1,2</sup>, known as mast-seeding, and synchronous tree-ring growth<sup>3,4</sup> within sites are well known phenomena among trees in the temperate zone. But information about the geographic or taxonomic extent of such synchronous growth or reproduction, or about the geographic extent of switching between them, is sparse. We have detected synchrony in growth and reproduction, both within and

among genera of Northern Hemisphere boreal trees, across geographical areas almost the size of a continent. Furthermore, we found a significant negative correlation between seed production and tree-ring growth at sites up to 1,000 kilometres apart, implying that there are trade-offs between them. This discovery suggests that mast-seeding is an evolved strategy that occurs on a geographic scale far larger than previously suspected<sup>5</sup>.

Annual seed or cone production values were obtained from the literature and standardized so that data acquired using different scales and methodologies could be combined. We used 298 data sets from

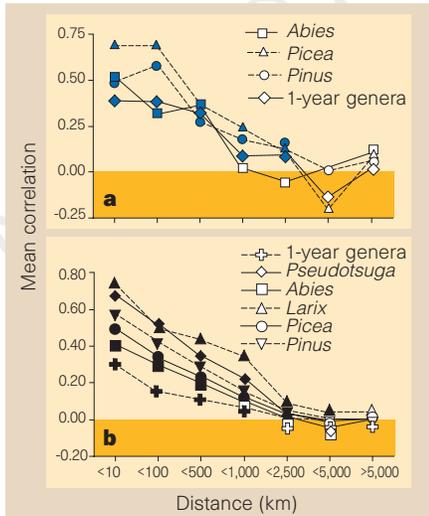
seven genera of boreal coniferous trees (*Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Thuja* and *Tsuga*) containing a minimum of four years of data (mean, 12.4 years). As a proxy of vegetative growth, we used dendrochronologies obtained from the International Tree-Ring Data Bank (version 2.2), a repository of more than 1,200 site-standardized chronologies from around the world.

Both data sets consisted of annual site means. Analyses included within-genus comparisons of sites measuring congeners and among-genera comparisons performed by pairing different genera. This latter analysis included firs (*Abies*), larches (*Larix*),

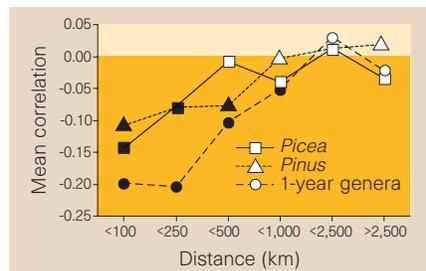
spruces (*Picea*), Douglas-firs (*Pseudotsuga*), cedars (*Thuja*) and hemlocks (*Tsuga*), but excluded pines (*Pinus*), which require more than a year to mature cones. Spatial autocorrelations and cross-correlations were calculated using a modified correlogram procedure<sup>6</sup>.

Our results reveal statistically significant spatial autocorrelation over large areas. Synchrony in the production of seeds and cones was detectable between sites from 500 km apart (*Abies*) up to 2,500 km apart (*Pinus*) (Fig. 1a). Synchrony in tree-ring growth was found between sites from 500 km apart (*Abies*) up to 5,000 km apart (*Larix*) (Fig. 1b). Assuming a circular area with a diameter corresponding to these distances, this indicates that detectable synchrony in cone production occurs over areas of  $0.2\text{--}4.9 \times 10^6 \text{ km}^2$ , and tree-ring growth patterns over areas of  $0.2\text{--}19.6 \times 10^6 \text{ km}^2$ . For comparison, the approximate land area of the North American continent is  $24.3 \times 10^6 \text{ km}^2$ , so reproduction and radial growth of conifers are statistically synchronous over subcontinent- to continent-wide areas.

Cone production and tree-ring growth patterns are both generally correlated with climatic factors, such as temperature or rainfall<sup>3,4,7</sup>. If the large-scale patterns of variable growth and reproduction are positively correlated with climate, they may involve only environmental tracking and require no further evolutionary explanation. Alternatively, mast-seeding may be an



**Figure 1** Correlations between synchronous activity of Northern Hemisphere coniferous trees depending on the geographical distance between sites. **a**, Mean annual seed or cone crop; **b**, mean annual tree-ring growth. Within-genus comparisons (*Abies*, *Larix*, *Picea*, *Pseudotsuga* and *Pinus*) include all sites measuring any species within the genus. The one-year genera category involves among-genera correlations using *Abies*, *Larix*, *Picea*, *Pseudotsuga*, *Thuja* and *Tsuga*, and excluded all within-genus comparisons. Filled symbols are significant at  $P < 0.05$  by randomization tests.



**Figure 2** Cross-correlation between mean annual seed or cone crops and mean annual tree-ring growth of Northern Hemisphere coniferous trees depending on the geographical distance between sites. Within- and between-genus comparisons are as for Fig. 1; only *Picea* and *Pinus* had sufficient data to be tested individually. Filled symbols are significant at  $P < 0.05$  by randomization tests.

evolved strategy, conferring fitness advantages by means of economies of scale involving seed predation, seed dispersal, or wind pollination<sup>8,9</sup>. The critical prediction, given these alternatives, is that there must be a trade-off between vegetative and reproductive growth on a population scale<sup>1</sup>.

We tested for such trade-offs by using cross-correlation analyses between the tree-ring and seed-production data sets. These yielded a negative relation between growth and reproduction for sites up to 250 km apart for spruces, 500 km apart for pines, and 1,000 km apart for one-year genera combined (Fig. 2). These inverse correlations between growth and reproduction, which are indicative of resource switching, are detectable within genera across areas of up to 196,000 km<sup>2</sup> and between genera across areas of up to 785,000 km<sup>2</sup>.

Our results show that mast-seeding involving switching of resources between growth and reproduction is characteristic of Northern Hemisphere conifers on a large geographic scale. Furthermore, in conifers, mast-seeding is detectable both between species and between genera, at least when restricted to genera requiring a single year to mature cone crops. They also show that patterns of seed production are sufficiently broad, both taxonomically and geographically, to affect the populations of seed predators over large areas. For example, our results support the hypothesis that the synchronized invasion of boreal seed-eating birds into southern latitudes could be the result of geographically widespread seed-crop failures<sup>10</sup>. Similarly, resident populations of birds and mammals that depend on conifer seeds for food are likely to be affected synchronously over large geographic areas by both bumper crops and widespread crop failures.

The many potential effects of such large-scale ecological phenomena on ecosystem function and biodiversity<sup>11</sup> remain to be investigated. Furthermore, because broad

climatic patterns almost certainly correlate with the large-scale synchrony, global climate change<sup>12,13</sup> may affect ecosystems in previously unanticipated ways.

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## Niche adaptation in ocean cyanobacteria

Small unicellular cyanobacteria (*Synechococcus* and *Prochlorococcus*) are the most abundant photosynthetic microorganisms in the world's oceans, yet we know little of the genetic structure of these populations. Here we show that distinct clades of *Prochlorococcus*, and possibly of *Synechococcus*, are adapted to their specific surface and deep oceanic niches.

Genetic and physiological studies of cultivated *Prochlorococcus*<sup>1–5</sup>, together with the discovery that organisms living at different depths have differing cellular properties (such as their divinyl chlorophyll *b:a* ratios)<sup>6,7</sup>, have led to the hypothesis that the widespread abundance of *Prochlorococcus* can be explained by the ability of surface- and deep-adapted clades to use different niches. However, cultivated isolates and bulk properties such as pigments are inadequate as descriptions of natural cyanobacterial communities<sup>8</sup>.

We used the polymerase chain reaction (PCR) as previously described<sup>9</sup> to amplify a fragment of the cyanobacterial RNA polymerase C1 gene (*rpoC1*) from communities of bacteria inhabiting oligotrophic sites in the Pacific Ocean, off the coast of California. We then used sequences from the generated clone libraries to survey marine *Synechococcus* and *Prochlorococcus*