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$J_{X-X})^2\}$ . We then expect the singlet lifetime lengthening to be on the order of  $[(J_{A-A} - J_{X-X})/(J - J)]^2$ . However, the importance of disconnected eigenstates was apparently not appreciated, in part because, in the absence of hyperpolarization and a mechanism to populate the state, the associated state has no obvious applications.

The perturbation calculation is easily extended to diacetyl, where the ratio  $(J \pm J)/(J_{A-A} - J_{X-X})$  is also small (the minus sign gives the larger value in cases such as ours, where the couplings have opposite signs). Although the spectra in Fig. 4, C and F, are quite complex, assuming the couplings have the same value as in the hydrate (which gives the excellent spectral fit in Fig. 4F) shows that the overlap of the singlet state with an eigenstate is better than 0.96. This result is also readily verified by precise numerical analysis of this eight-spin system, and thus we predict more than an order-of-magnitude lengthening of the spin lifetime. In effect, the strong coupling between the two carbons quenches communication with other spins—virtually all the spectral complexity comes from the other three carbon states, and singlet-to-triplet interconversion is slow. Of course, perdeuteration dramatically reduces even this limited singlet-triplet mixing and further increases the lifetime.

The advantage of the perturbation theory calculation is that it lets us discuss the generic case. The generalization is more subtle than one might expect. The common case of magnetic equivalence [where the two spins have the same resonance frequency and each of the two spins is coupled identically to every other spin (*12*)] does not necessarily produce a disconnected eigenstate. For example, any two of the spins in a freely rotating methyl group are magnetically equivalent, but the energy level diagram for three equivalent spins produces no fully disconnected states, so the immunity to environmental perturbations is not present. The only possible disconnected eigenstate for two spins is the singlet; for a larger even number of spins with enough symmetry [e.g., benzene (*29*)] other disconnected states exist, although they might be difficult to access in practice.

The critical constraint for producing a disconnected eigenstate is that the coupling between two spins substantially exceeds both the couplings to other spins and the resonance frequency difference between the spins. Systems of interest as hyperpolarized contrast agents have two nearby H,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{19}\text{F}$ , or  $^{31}\text{P}$  atoms that satisfy this constraint. They have a precursor where the two atoms are inequivalent (hence permitting hyperpolarization of the  $\alpha_1\beta_2$  population), which can be converted to the contrast agent by chemical manipulation in a time that is short compared with the normal  $T_1$ . Finally, they have a biological pathway that again makes them inequivalent, permitting detection of the hyperpolarization. Diacetyl in vivo satisfies these conditions. Partition in vivo between hydrophobic and hydrophilic phases would modulate the exchange rate (drastically reducing the water concentration and, hence, lengthening the time to convert from singlet); even ignoring hydration, the first

metabolite is acetoin with inequivalent carbons. More generally, the simplest case is two equivalent adjacent carbons or nitrogens without directly bonded hydrogens, as in diacetyl, naphthalene, and oxolin (an antiviral compound) or in many derivatives of pyridazine or phthalazine, which have recently been shown to have vascular endothelial growth factor receptor-2 inhibitory activity (*30*). In other cases, deuteration can essentially eliminate the coupling to outside nuclei, as could very weak irradiation (far less than is necessary when the spin systems differ in their chemical shift frequency). At moderate fields, even molecules with not-quite-equivalent spins (such as the  $3,4\text{-}^{13}\text{C}$  versions of L-dopa or dopamine) might be usable, as the degradation pathway leads to compounds such as HVA with substantial asymmetry.

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## Greatly Expanded Tropical Warm Pool and Weakened Hadley Circulation in the Early Pliocene

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The Pliocene warm interval has been difficult to explain. We reconstructed the latitudinal distribution of sea surface temperature around 4 million years ago, during the early Pliocene. Our reconstruction shows that the meridional temperature gradient between the equator and subtropics was greatly reduced, implying a vast poleward expansion of the ocean tropical warm pool. Corroborating evidence indicates that the Pacific temperature contrast between the equator and 32°N has evolved from ~2°C 4 million years ago to ~8°C today. The meridional warm pool expansion evidently had enormous impacts on the Pliocene climate, including a slowdown of the atmospheric Hadley circulation and El Niño-like conditions in the equatorial region. Ultimately, sustaining a climate state with weak tropical sea surface temperature gradients may require additional mechanisms of ocean heat uptake (such as enhanced ocean vertical mixing).

The early Pliocene [~5 to 3 million years ago (Ma)] is often considered the closest analog to the effects of contemporary global warming on Earth's climate. Indeed, the external factors that control the climate system—the intensity of sunlight incident on Earth's surface,

global geography (*1, 2*), and, most important, the atmospheric concentration of CO<sub>2</sub> (*3*)—were similar to present-day conditions. However, high latitudes were warmer, continental ice sheets were largely absent from the Northern Hemisphere, and the sea level was ~25 m higher than today (*4, 5*).

The climate of the tropics was also quite different. The sea surface temperature (SST) contrast between the eastern and western equatorial Pacific was very small, and cold surface waters were almost absent from coastal upwelling zones off the western coasts of Africa and the Americas (6–10). These climate conditions are often called a “permanent El Niño” or permanent El Niño-like state (11). This term describes the long-term mean state of the ocean-atmosphere system. In contrast, the modern, intermittent El Niño is the warm phase of a quasi-periodic climate oscillation, which affects weather and climate patterns worldwide every 4 to 5 years (12, 13). Interannual climate variability may have also existed in the Pliocene (14), but only during times when the equatorial SST gradient exceeded some critical value (12).

This study focuses on the meridional distribution of SST and its effects on the early Pliocene climate. Understanding changes in this distribution, including variations in the meridional extent of the ocean tropical warm pool, is essential for understanding mechanisms responsible for the gradual transition from the warmer Pliocene to the cooler Pleistocene. It has been hypothesized that the early Pliocene had a relatively deep ocean thermocline in the tropics and that the subsequent shoaling of the thermocline signaled the transition to a colder climate (6). A reduced meridional gradient is required to sustain a deeper tropical thermocline (see below).

We first examined the evolution of the meridional SST gradient over the past 4 million years in the eastern tropical Pacific (Fig. 1, A and C). We used orbitally resolved records from two Ocean Drilling Program (ODP) sites: Site 1012 (32°N, 118°W) from the California margin, and Site 846 (3°S, 91°W) from the eastern Pacific cold tongue just south of the equator (Fig. 1B). Both data sets are based on alkenone-derived estimates of SST (15). The new data from ODP Site 1012 agree overall with previously published results from ODP Site 1014 (8); however, the present data set has much higher resolution, allowing precise temporal alignment of equatorial and subtropical records.

According to these data, the mean temperature contrast between the two sites evolved from a very small value of ~2°C at 4 Ma to 7°C by 2 Ma, and then remained fairly constant at nearly present-day levels (Fig. 1C). Superimposed on the gradual trends at each site are Milankovitch cycles driven by variations in Earth’s orbital parameters (1). On orbital time scales, these cycles can produce changes in the SST contrast between the two sites as large as the underlying long-term trends.

The temporal development of the zonal SST gradient along the equator (7) generally mirrors that

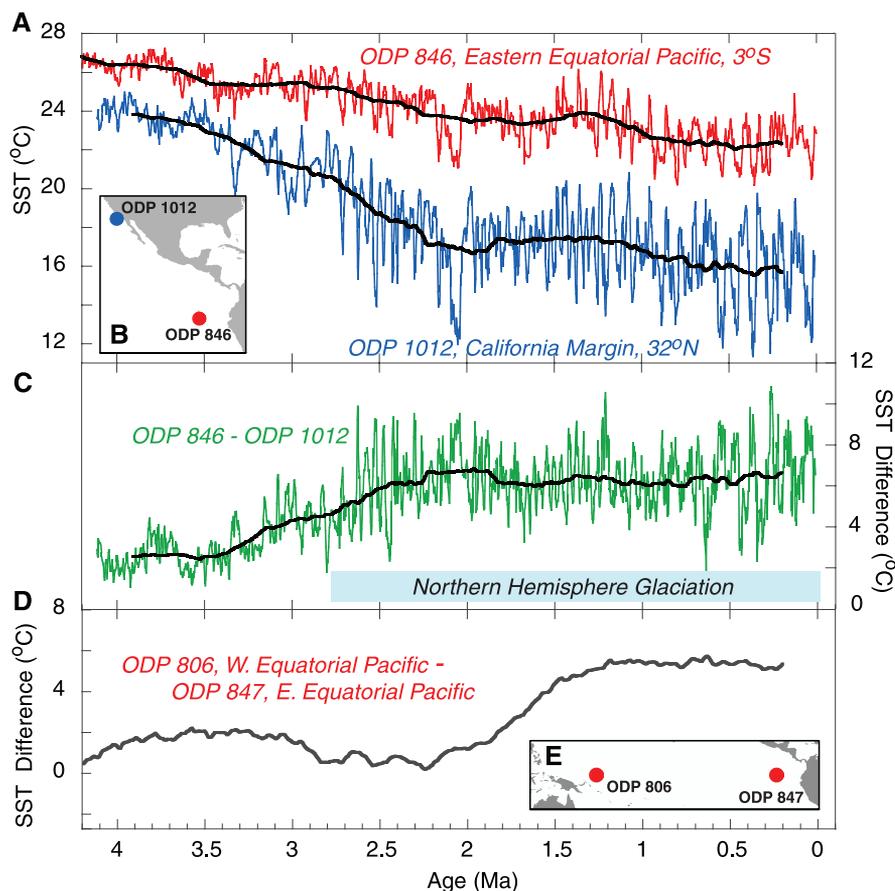
of the meridional SST gradient in the eastern Pacific (Fig. 1D), suggesting a strong link between extratropical and tropical ocean conditions. The zonal SST gradient reaching its modern value later in the record indicates that meridional temperature changes precondition zonal changes along the equator.

To show that the strong reduction in the meridional temperature gradient in the early Pliocene is a robust result, we reconstructed the meridional SST distribution in the mid-Pacific roughly along the dateline (Fig. 2). The period of this reconstruction is ~4 to 4.2 Ma, sometimes called the Pliocene thermal optimum (16). This period coincides with an almost complete collapse of the east-west temperature gradient along the equator (6–8) (Fig. 1D); it also follows the closure of the Central America Seaway (2) but precedes climate cooling and the development of large Northern Hemisphere ice sheets (1).

The data in Fig. 2 are based on alkenone and Mg/Ca temperature proxies. Alkenone-derived records for ODP Sites 982, 1012, and 1090 and for Deep Sea Drilling Project Site 607 were produced specifically for this study; the original

temperature records for other sites were published elsewhere (6–10, 17–21). Adjustments were applied to some of the SST data to represent the mid-Pacific in the early Pliocene (15). For example, when using temperatures from the Atlantic to reconstruct Pacific SSTs, one must consider that temperatures in the North Atlantic are typically 2° to 7°C higher than in the North Pacific; when using SSTs from the eastern boundary of the basin to reconstruct temperatures in the mid-Pacific, one should correct for the effect of coastal upwelling. The overall temperature distribution in Fig. 2 is robust and does not depend on any individual data point.

Our analysis indicates a considerably warmer Pacific climate in the early Pliocene than that determined by the PRISM project in its mid-Pliocene SST reconstructions (5, 22, 23). Furthermore, a strong reduction in the SST gradient from the equator to the subtropics described in our study implies a vast poleward expansion of the ocean low-latitude warm pool (fig. S1), whereas the maximum tropical temperatures remained close to present-day values (28° to 29°C).



**Fig. 1.** (A) The evolution of SSTs, as derived from alkenones (15), in two locations: ODP Site 1012 (blue) off the coast of California (32°N, 118°W) and ODP Site 846 (red) in the eastern Pacific just south of the equator (3°S, 91°W). Note the cooling trends over the past 4 million years, shown here as 400,000-year running means (black lines). (B) Locations of the sites in (A). (C) SST difference between the two locations in (B). The meridional SST gradient is minimal at ~4 Ma (2°C) and then increases gradually to modern values (~7.5°C). (D) The zonal SST gradient along the equator (7) from Mg/Ca paleothermometry between ODP Site 806 in the western equatorial Pacific (0°N, 159°E) and ODP Site 847 in the eastern equatorial Pacific (0°N, 95°W). The strength of this temperature gradient varies from 0° to 2°C between 5 Ma and 2 Ma and then increases gradually to modern values (~5.5°C). (E) Locations of the sites in (D).

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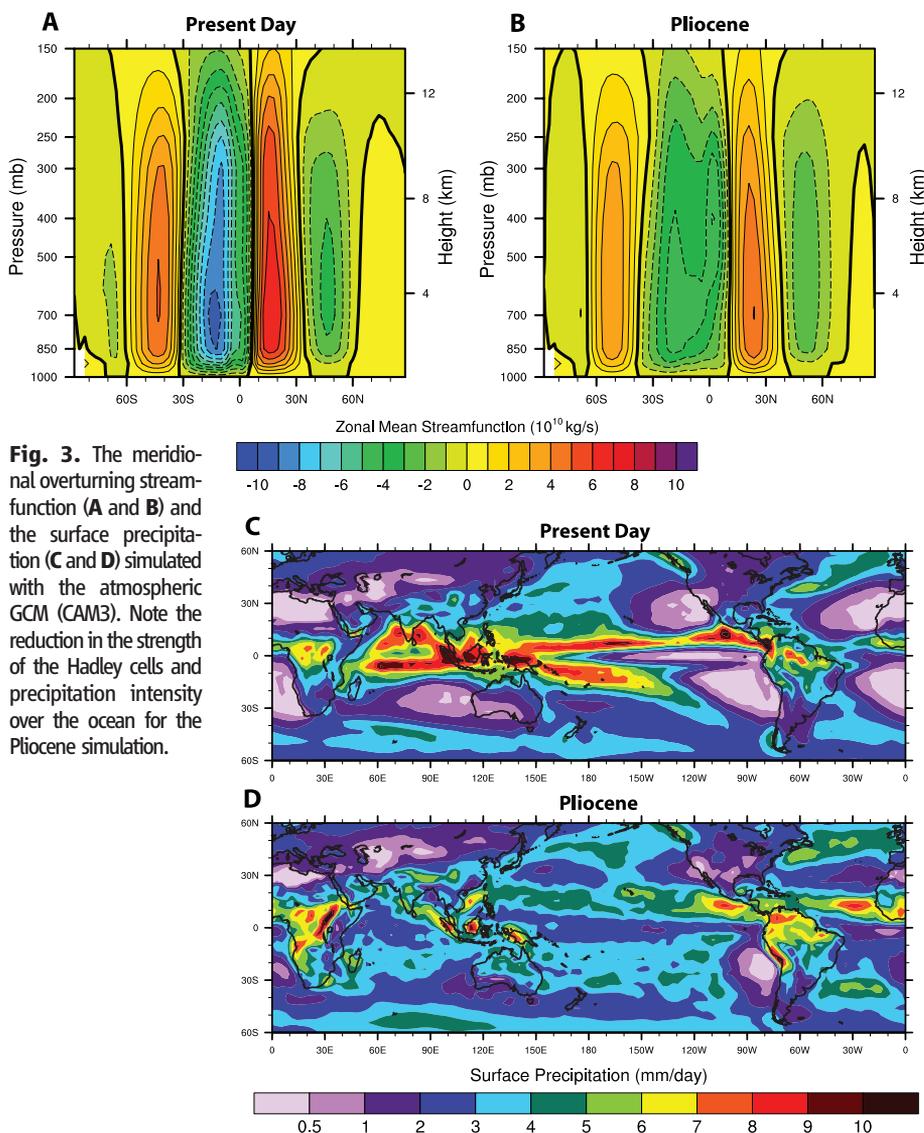
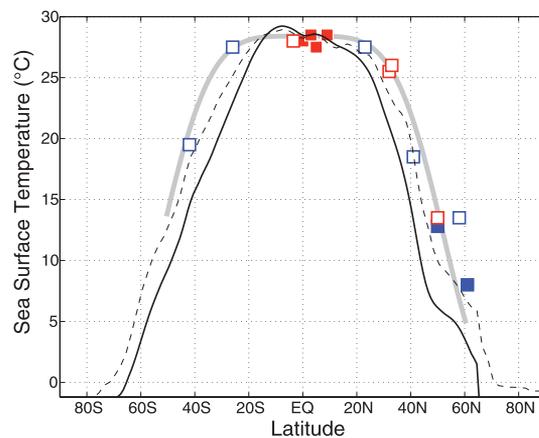
The ocean tropical warm pool (currently confined mainly to the western equatorial Pacific) is a critical component of the climate system (24), and changes in its meridional extent can have large implications for climate. In particular, meridional expansion of the warm pool provides a crucial mechanism for maintaining permanent El Niño conditions in the equatorial region, because cold water in the modern eastern equatorial Pacific is sourced from the subtropical subduction zones. Poleward expansion of the warm pool into the subduction regions would lead to warmer waters upwelling in the eastern equatorial Pacific, a deeper ocean thermocline, and consequently a substantial reduction of the SST gradient along the equator. Apparently, such a climate state prevailed at ~4 Ma (Fig. 1, C and D). Thus, to explain the permanent El Niño conditions during the early Pliocene, it is necessary to understand the meridional expansion of the ocean warm pool.

To quantify the atmospheric response to the expanded tropical warm pool and reduced meridional temperature gradient, we fit a hypothetical SST profile to the data in Fig. 2, which was then used as a boundary condition for an atmospheric general circulation model (GCM) (15). In the absence of reliable simulations of the Pliocene climate with coupled models, simulations with atmospheric GCMs forced with ocean surface boundary conditions arguably remain the best way to assess climate conditions. Previous studies of the Pliocene with atmospheric GCMs used either earlier PRISM SST reconstructions (5), which lacked adequate data for the tropics and subtropics, or the modern SST profile taken from the dateline and extended zonally (25).

Our numerical calculations confirm that the SST changes in Fig. 2 strongly affect the global climate and atmospheric circulation. As expected, the lack of an SST gradient along the equator acts to eliminate the atmospheric zonal circulation [the Walker cell (13)] (fig. S2). However, if the meridional SST gradient were kept at present-day values as in previous studies, the atmospheric meridional circulation [the Hadley cells (26)] would compensate by strengthening substantially (25). In contrast, in calculations using our Pliocene SST reconstruction, the Hadley circulation weakens (Fig. 3, A and B).

The Northern Hemisphere branch of the Hadley circulation weakens on average by roughly 30%. During boreal winter, the reduction reaches nearly 40%. The center of the Hadley cell moves northward by ~7°, whereas the cell's latitudinal extent (26) increases by 3° to 4°. These changes of the Northern Hemisphere circulation are a robust response to changes in meridional SST gradient as evinced by additional sensitivity calculations (table S2). The volume transport of the circulation's southern branch nearly halves, making the southern Hadley cell even weaker than the northern cell. Whether this is a genuine feature of the early Pliocene climate remains to be seen, because there are only a few SST data points currently available in the Southern Hemisphere. Note that coupled models simulating global warming also show a weakening and poleward expansion of the Hadley circulation. However, those effects

**Fig. 2.** Estimated distribution of SST with latitude in the middle of the Pacific (roughly along the dateline) at ~4 Ma. This temperature reconstruction uses data from the Pacific and Indian oceans (red boxes) and adjusted data from the Atlantic (blue boxes). Open boxes indicate alkenone-based data; solid boxes indicate Mg/Ca-based data. Thin solid line: SST from the dateline for the modern climate. Dashed line: latest estimates of the mid-Pliocene temperatures (~3 Ma) along the dateline from the PRISM project (22, 23). Thick gray line: a portion of the hypothetical SST profile used as a boundary condition for numerical simulations. The original data and the temperature adjustments are given in table S1.

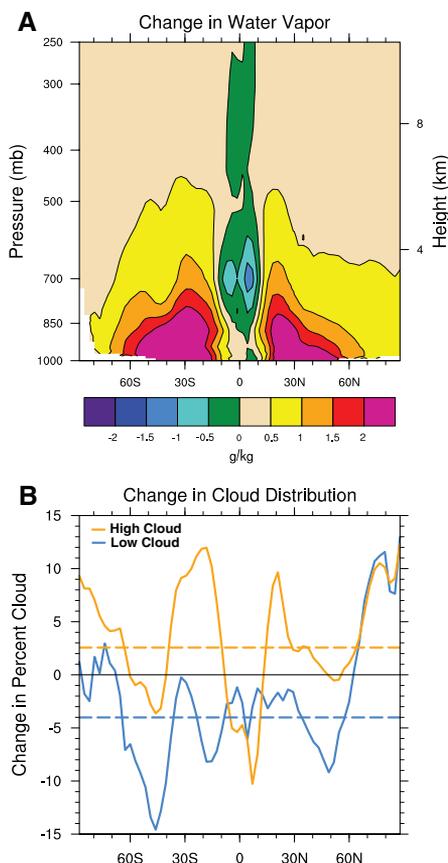


**Fig. 3.** The meridional overturning streamfunction (A and B) and the surface precipitation (C and D) simulated with the atmospheric GCM (CAM3). Note the reduction in the strength of the Hadley cells and precipitation intensity over the ocean for the Pliocene simulation.

are rather modest—roughly 4% and 1° of latitude, respectively, by the end of the 21st century (26).

In our calculations, the reduced meridional temperature gradient also widens the Intertropical Convergence Zone [ITCZ (13)] and decreases its

precipitation intensity (Fig. 3, C and D). A second, weaker ITCZ develops in the Southern Hemisphere, caused by the use of a completely symmetrical SST profile. The spatial structure of Pliocene precipitation indicates that the strongest air uplift occurs over

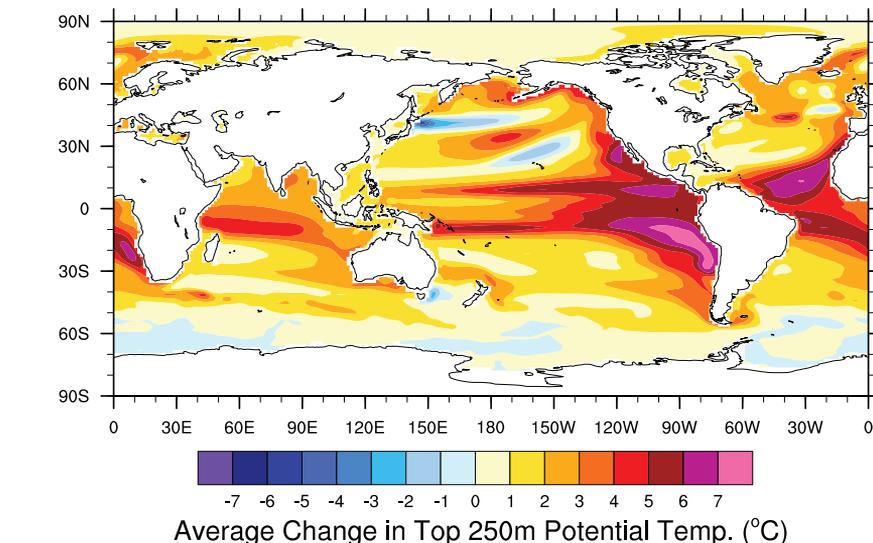


**Fig. 4.** Factors critical for maintaining a warm Pliocene climate. **(A)** The increase in zonal-mean specific humidity (or water vapor content) from the present day to the early Pliocene (in g/kg). The only reduction in water vapor occurs in a narrow region above the modern ITCZ location, which indicates a weakening of the deep convection in the tropics. **(B)** Changes in cloud distribution with latitude (solid lines) and their globally averaged values (dashed lines). There is a net increase in cloud fraction for “greenhouse” high clouds and a net decrease for highly reflective low clouds; both effects tend to warm Earth’s surface.

orographic features (such as the East African Highlands) rather than over the ocean. Weak SST gradients, and hence a lack of localized wind convergence, over the ocean cause orographic air uplift and ocean-land temperature contrasts to become essential for triggering tropical precipitation.

Terrestrial paleodata provide verification of gross changes in precipitation anticipated over land by our calculations. For example, the model predicts stronger precipitation over southwest and east Africa, the Sahara desert, and Australia, consistent with pollen-based precipitation data (table S3). Comparison of changes over North America is inconclusive because of large error bars in the paleodata (27) and large natural variability in precipitation intensity in that region.

The water vapor content of the atmosphere increases significantly (Fig. 4A) as a consequence of the Clausius-Clapeyron relationship (28), despite the substantial reduction in the strength of atmo-



**Fig. 5.** Difference in ocean temperatures (averaged over the top 250 m) between two greenhouse-warming simulations: a simulation for which we increased the background vertical diffusivity in the upper 400 m of the ocean by an order of magnitude between 40°N and 40°S, and a control simulation. In each case, the CO<sub>2</sub> concentration was increased instantaneously from preindustrial levels to 355 ppm, and the coupled model (CCSM3) has been integrated for 180 years (the plot is obtained from a 30-year average at the end of the simulations). A gradual ocean adjustment will continue after 180 years, but we do not expect large qualitative changes in the warming pattern. The actual values of the ocean vertical diffusivity in the Pliocene are highly uncertain, so these experiments serve only to demonstrate the proposed mechanism.

spheric convection over the ITCZ in the Pacific. The increase in water vapor (a potent greenhouse gas) is a major factor in sustaining the warm Pliocene climate. It causes a reduction of  $14.6 \text{ W m}^{-2}$  in outgoing long-wave radiation at the top of the atmosphere (relative to the present); variations in cloud distribution (Fig. 4B) account for a further reduction of  $5.6 \text{ W m}^{-2}$ . Because the net increase in atmospheric water vapor (by roughly 30%) is largely a consequence of the expanded ocean warm pool, a climate model will not be able to capture the full extent of the Pliocene warmth unless it can simulate SST patterns in the tropics and subtropics correctly.

Further, our calculations show a slight increase in total poleward heat transport from low to high latitudes (fig. S3). However, the partitioning of the heat transport between the ocean and atmosphere poses the following problem: We find a significant decrease in the heat transport by the atmosphere and an implied increase in ocean heat transport (fig. S3). This result contradicts studies using ocean GCMs with low vertical diffusion, which suggest that a permanent El Niño should be associated with reduced heat transport by the ocean (29). According to those studies, the ocean typically gains a large amount of heat over the tropical Pacific cold tongue; warmer conditions in that region would entail a reduction in ocean heat uptake there, and hence a smaller ocean heat transport. Attempts to simulate the Pliocene climate with coupled atmosphere-ocean GCMs (14) have not yet succeeded in replicating the collapse of the SST gradient along the equator, presumably because of this issue.

One way to resolve the heat transport problem in such a climate state with weak SST gradients is to allow the ocean to gain heat over a much broader

region of the tropics than just the equatorial cold tongue. This would necessitate a substantial increase in ocean vertical mixing rates (or vertical diffusion), which may result from weaker ocean stratification (20) and/or enhanced mixing of the upper ocean by hurricanes (30). Should such an increase in ocean mixing occur, it would lead to larger heat uptake and greater poleward heat transport by the ocean, even with a weak or absent equatorial cold tongue.

To test this hypothesis, we ran a coupled GCM with elevated atmospheric CO<sub>2</sub> concentrations and vertical diffusion uniformly increased in the upper tropical ocean. Preliminary results with enhanced mixing produce a mean state much closer to a permanent El Niño (Fig. 5). There is a much greater warming in the eastern equatorial Pacific than in the west, a warming of the upwelling regions, and a deeper ocean thermocline. At the same time, interannual variability becomes substantially weaker.

Thus, it may be necessary to incorporate additional mechanisms for increased ocean heat uptake when simulating the early Pliocene climate and, potentially, the response of the tropics to contemporary global warming. The enormous impacts of changes in the warm pool (such as shifts in global precipitation patterns and cloud cover), as well as tentative evidence that the tropical belt has been expanding poleward over the past few decades (31), make our findings especially relevant to current discussions about global warming.

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### Supporting Online Material

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SOM Text

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References

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# Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding

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P-glycoprotein (P-gp) detoxifies cells by exporting hundreds of chemically unrelated toxins but has been implicated in multidrug resistance (MDR) in the treatment of cancers. Substrate promiscuity is a hallmark of P-gp activity, thus a structural description of poly-specific drug-binding is important for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo P-gp at 3.8 angstroms reveals an internal cavity of ~6000 angstroms cubed with a 30 angstrom separation of the two nucleotide-binding domains. Two additional P-gp structures with cyclic peptide inhibitors demonstrate distinct drug-binding sites in the internal cavity capable of stereoselectivity that is based on hydrophobic and aromatic interactions. Apo and drug-bound P-gp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding.

The American Cancer Society reported over 12 million new cancer cases and 7.6 million cancer deaths worldwide in 2007 (1). Many cancers fail to respond to chemotherapy by acquiring MDR, to which has been attributed the failure of treatment in over 90% of patients with metastatic cancer (2). Although MDR can have several causes, one major form of resistance to chemotherapy has been correlated with the pres-

ence of at least three molecular “pumps” that actively transport drugs out of the cell (3). The most prevalent of these MDR transporters is P-gp, a member of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily (4). P-gp has unusually broad poly-specificity, recognizing hundreds of compounds as small as 330 daltons up to 4000 daltons (5, 6). Most P-gp substrates are hydrophobic and partition into the lipid bilayer (7, 8). Thus, P-gp has been likened to a molecular “hydrophobic vacuum cleaner” (9), pulling substrates from the membrane and expelling them to promote MDR.

Although the structures of bacterial ABC importers and exporters have been established (10–15) and P-gp has been characterized at low resolution by electron microscopy (16, 17), obtaining an x-ray structure of P-gp is of particular interest because of its clinical relevance. We describe the structure of mouse P-gp (ABC1), which has 87% sequence

identity to human P-gp (fig. S1), in a drug-binding-competent state (18, 19). We also determined cocrystal structures of P-gp in complex with two stereoisomers of cyclic hexapeptide inhibitors, cyclic-*tris*-(*R*)-valineselenazole (QZ59-RRR) and cyclic-*tris*-(*S*)-valineselenazole (QZ59-SSS), revealing a molecular basis for poly-specificity.

Mouse P-gp protein exhibited typical basal adenosine triphosphatase (ATPase) activity that was stimulated by drugs like verapamil and colchicine (fig. S2A) (20). P-gp recovered from washed crystals retained near-full ATPase activity (fig. S3). Both QZ59 compounds inhibited the verapamil-stimulated ATPase activity in a concentration-dependent manner (fig. S2B). Both stereoisomers inhibited calcein-AM export with median inhibitory concentration (IC<sub>50</sub>) values in the low micromolar range (fig. S4) and increasing doses of QZ59 compounds resulted in greater colchicine sensitivity in P-gp-overexpressing cells (fig. S5).

The structure of P-gp (Fig. 1) represents a nucleotide-free inward-facing conformation arranged as two “halves” with pseudo two-fold molecular symmetry spanning ~136 Å perpendicular to and ~70 Å in the plane of the bilayer. The nucleotide-binding domains (NBDs) are separated by ~30 Å. The inward-facing conformation, formed from two bundles of six transmembrane helices (TMs 1 to 3, 6, 10, 11 and TMs 4, 5, 7 to 9, 12), results in a large internal cavity open to both the cytoplasm and the inner leaflet. The model was obtained as described in (18) by using experimental electron density maps (figs. S6 and S7 and table S1), verified by multiple  $F_{\text{obs}} - F_{\text{calc}}$  maps (figs. S8 to S10), with the topology confirmed by (2-hydroxy-5-nitrophenyl)mercury(II) chloride (CMNP)-labeled cysteines (figs. S6, B to D; S7C, and S11, and table S2). Two portals (fig. S12) allow access for entry of hydrophobic molecules directly from the membrane. The portals are formed by TMs 4 and 6 and TMs 10 and 12, each of which have smaller side chains that could allow tight packing during NBD dimeriza-

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