Effect of Ocean Acidification on Iron Availability to Marine Phytoplankton

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The acidification caused by the dissolution of anthropogenic carbon dioxide (CO_2) in the ocean changes the chemistry and hence the bioavailability of iron (Fe), a limiting nutrient in large oceanic regions. Here, we show that the bioavailability of dissolved Fe may decline because of ocean acidification. Acidification of media containing various Fe compounds decreases the Fe uptake rate of diatoms and coccolithophores to an extent predicted by the changes in Fe chemistry. A slower Fe uptake by a model diatom with decreasing pH is also seen in experiments with Atlantic surface water. The Fe requirement of model phytoplankton remains unchanged with increasing CO_2. The ongoing acidification of seawater is likely to increase the Fe stress of phytoplankton populations in some areas of the ocean.

The dissolution of additional atmospheric carbon dioxide (CO_2) in the ocean will lead to predictable changes in the chemistry of seawater, including an increase in partial pressure of CO_2 (PCO_2), a decrease in pH, and a decrease in the carbonate ion concentration, $[CO_3^{2-}]$. The possible biological consequences of these changes, all described by the term "ocean acidification," are being extensively studied (*1*–*4*). In particular, the effects of increasing PCO_2 and decreasing $[CO_3^{2-}]$ on phytoplankton have received some attention (*1*, *4*–*6*) but not, so far, the potential effects of the decrease in pH, which is nearly 0.3 pH units for a doubling of PCO_2.

Iron (Fe) is the biologically important element whose chemistry is most sensitive to pH. The bulk of Fe(III) in the ocean is known to be chelated by organic compounds (*7*, *8*), and the fraction that is not chelated is present as hydrolyzed species, $Fe(OH)_x^{(3-x)+}$, with the neutral tri-hydroxy species, $Fe(OH)_3$, being very insoluble. As ocean waters acidify, decreasing the hydroxide ion concentration, Fe's speciation and solubility will be altered. A decrease in pH by 0.3 unit should slightly increase iron's solubility in seawater (*9*). The hydroxide ion and organic chelators compete for binding Fe(III) so that a decrease in pH should affect the extent of organic chelation of Fe and hence its availability to ambient organisms. At the same time that a decrease in pH may affect the availability of Fe to phytoplankton, an increase in PCO_2 may change their Fe requirements. For example, increasing the extracellular concentration of CO_2 should decrease the need to operate a carbon-concentrating mechanism (CCM) for CO_2 fixation (*10*, *11*) and hence may allow an economy in the Fe involved in the photosynthetic or respiratory processes that provide energy for the CCM. Through changes in Fe availability and requirements, ocean acidification may affect primary production and the ecology of phytoplankton. Here, we present data on the effect of acidification on Fe availability and requirements in laboratory cultures of diatoms and coccolithophores and on the uptake of Fe bound to natural ligands

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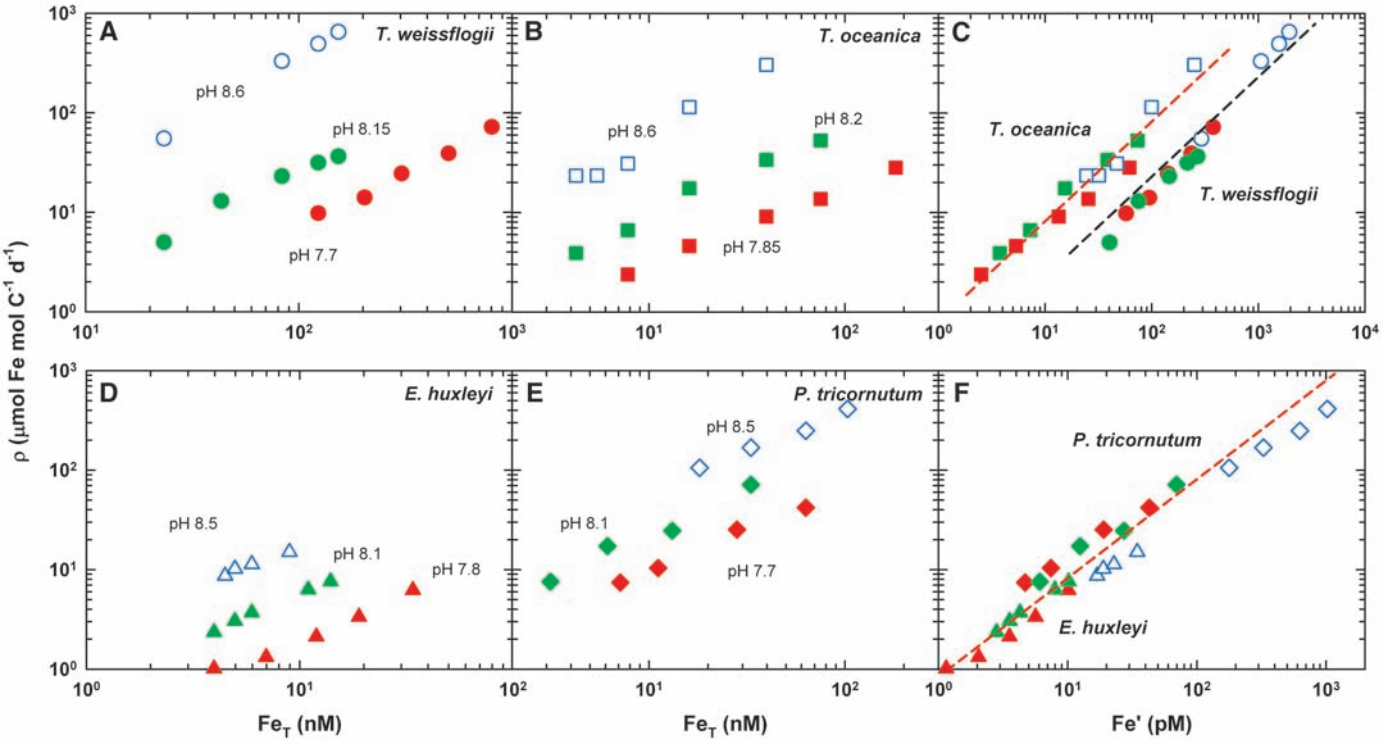

Fig. 1. Steady-state iron uptake rates in cultures of (**A**) *T. weissflogii*, (**B**) *T. oceanica*, (**D**) *E. huxleyi*, and (**E**) *P. tricornutum* as a function of total iron concentration (Fe_T) in EDTA-buffered culture medium over a range of pH/PCO_2. Circles indicate *T. weissflogii*: red, pH 7.7/PCO_2 950 ppm; green, pH 8.15/PCO_2 320 ppm; and blue, pH 8.6/PCO_2 90 ppm. Squares indicate *T. oceanica*: red, pH 7.85/PCO_2 680 ppm; green, pH 8.2/PCO_2 290 ppm; and blue, pH 8.6/PCO_2 100 ppm. Triangles indicate *E. huxleyi*: red, pH 7.8/PCO_2 770 ppm; green, pH 8.1/PCO_2 370 ppm; and blue, pH 8.5/PCO_2 130 ppm. Diamonds indicate *P. tricornutum*: red, pH 7.7/PCO_2 950 ppm; green, pH 8.1/PCO_2 360 ppm; and blue, pH 8.5/PCO_2 130 ppm. (**C**) and (**F**) When plotted as a function of the unchelated iron concentration, Fe′, uptake rates coalesce for each organism following a one-to-one line. The red dashed line in (C) is identical to the one shown in (F). Results of each organism are from a single experiment. Additional experiments yielded results that follow the same lines shown in (C) and (F).

in coastal and open-ocean surface waters of the Atlantic.

Dissolved Fe in surface seawater is strongly complexed by organic ligands (*7*, *8*). Fe uptake by marine phytoplankton depends on the extent of Fe(III) chelation, as well as on the nature of the chelating agent (*12*–*14*). We examined the effect of pH on Fe uptake by four model species—the coastal centric diatom *Thalassiosira weissflogii*, the open ocean–centric diatom *Thalassiosira oceanica*, the pennate diatom *Phaeodactylum tricornutum*, and the coccolithophore *Emiliana huxleyi*—under conditions in which Fe is bound to a variety of organic ligands representing a range of functional binding groups.

The uptake by phytoplankton of Fe(III) chelated by an excess of the tetracarboxylic acid EDTA represents a limiting case in which the uptake rate depends only on the unchelated Fe concentration $Fe' = \sum_x [Fe(OH)_x^{(3-x)+}]$, which can be precisely calculated (*15*). At a given total Fe concentration in an EDTA-buffered medium, we observed a significant decrease in the steady Fe uptake rate of all phytoplankton species with decreasing pH in the range of 8.6 to 7.7 (Fig. 1, A, B, D, and E) (*16*). However, all the data for one species closely follow a single line when plotted as a function of the calculated Fe′ (Fig. 1, C and F). The uptake rates are proportional to Fe′, showing that the effect of pH is due to a change in the chemical speciation of Fe. The oceanic species have a higher Fe uptake rate; this has been shown before to be due to their smaller size (*17*, *18*). In short-term uptake experiments with *T. weissflogii* in the presence of excess EDTA, both the direction and the magnitude of the pH effect on the kinetics of Fe uptake were similar to those observed in the steady-state experiments (Fig. 2A). The data of Fig. 1 and Fig. 2A demonstrate that the effect of pH on the rate of Fe uptake by our model species results from changes in Fe chemistry and not a physiological response of the organisms.

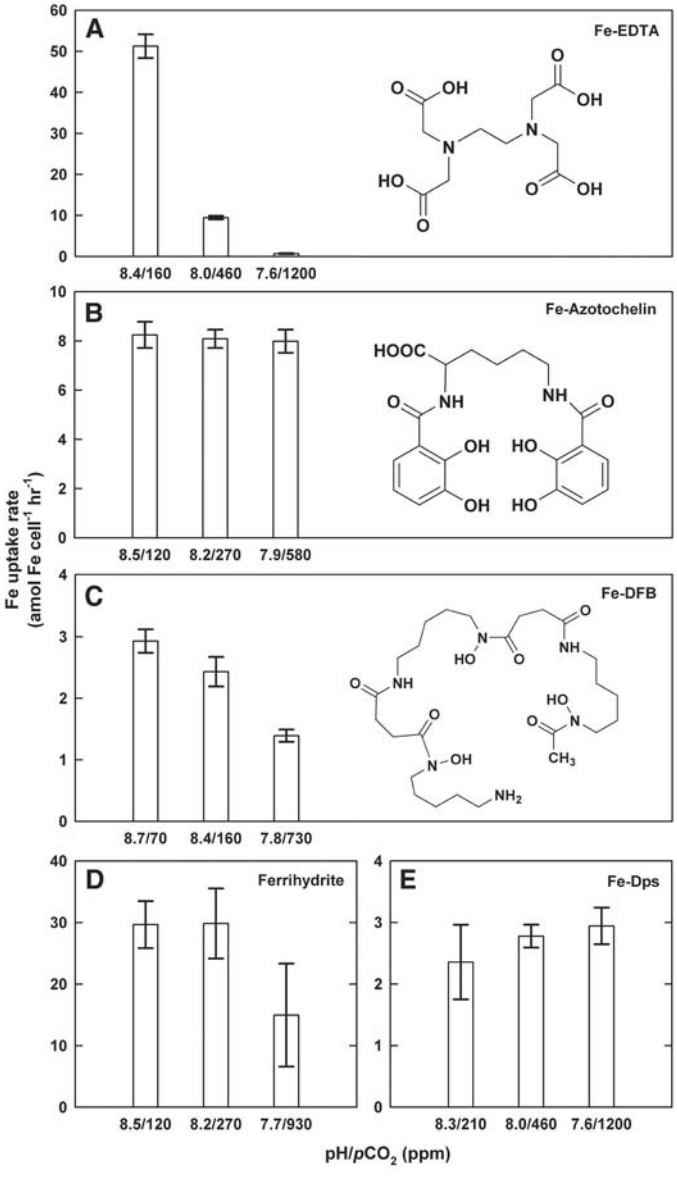

Fig. 2. Short-term Fe uptake by iron-limited *T. weissflogii* from iron bound to three chelators, (**A**) the aminocarboxylate EDTA, (**B**) the biscatecholate siderophore azotochelin, and (**C**) the trihydroxamate siderophore desferriferrioxamine B, and from iron in the forms of (**D**) freshly precipitated ferrihydrite and (**E**) ferrihydrite sequestered in the iron storage protein Dps at three different pH/P_{CO_2}. Error bars represent the SD of biological replicates (n = 2 replicates).

The high pH sensitivity of Fe′ in the presence of excess EDTA results from the fact that the dissociation of Fe-EDTA releases about 2.3 protons in seawater at pH 8.1 [supporting online material (SOM) text] and becomes increasingly less favored at low pH (charges omitted for simplicity):

$$Fe(OH)_{0.6}Y + Ca^{2+} + 2.3\ H_2O = Fe(OH)_{2.9} + CaY + 2.3\ H^+ \quad (1)$$

A qualitatively similar result should obtain for Fe bound to any chelator with acidic binding groups that are not protonated in seawater, whether or not bound to Ca^{2+} or Mg^{2+}. The extent of decrease in Fe′ with decreasing pH depends on the number of protons released upon dissociation of Fe. Therefore, no effect of pH on Fe′ or Fe uptake should be seen when Fe is bound to chelators that are protonated in seawater and whose dissociation from Fe does not release H^+. This is the case for catechols, which are known to serve as strong ligands for Fe(III) binding and have been identified as components of the organic matter presumptively responsible for Fe chelation in the surface ocean (*19*). We thus tested the effect of pH on the uptake of Fe(III) bound to the bis-catecholate azotochelin. The catechol groups of this compound are protonated at the pH of seawater, and the dissociation of the Fe(III)-azotochelin complex thus releases only 0.1 protons at pH 8.1 (SOM text):

$$FeY + 2.9\ H_2O = Fe(OH)_3 + H_{2.8}Y + 0.1\ H^+ \quad (2)$$

We observed, as expected, a negligible effect of pH on Fe uptake by *T. weissflogii* when Fe was bound to azotochelin (Fig. 2B).

Besides carboxylic acids and catechols, hydroxamates are among the principal functional groups involved in Fe(III) binding in strong chelators like siderophores. The uptake of Fe(III) chelated to the model trihydroxamate DFB (desferri-ferrioxamine B) is another limiting case because uptake is mediated by a reduction of the Fe(III)DFB complex at the cell surface catalyzed by transmembrane reductases (*12*, *13*). As a result, the uptake rate is proportional to the concentration of Fe(III)-DFB, which is unaffected by pH. But the uptake depends on the effectiveness of the enzymatic reduction of Fe(III)-DFB, which might decrease with pH because the transfer of electrons from cellular reductants usually liberates protons (*20*). Short-term Fe uptake experiments with *T. weissflogii* in the presence of excess DFB show a significant decrease in uptake rate with decreasing pH, by about a factor of two for a decrease by one pH unit (Fig. 2C).

Phytoplankton can acquire Fe from oxyhydroxide colloids. In this case, the rate of Fe uptake by the organisms is governed by either the solubility of the colloidal Fe or its rate of dissolution, which should increase with its degree of under-saturation (*21*). Because in the pH range of

interest the average number of hydroxyls bound to dissolved Fe(III) is about three (*22*) (SOM text), both the solubility of Fe and its dissolution rate are nearly independent of pH, as can be seen in the dissolution reaction:

$$Fe(OH)_3(s) = Fe(OH)_3(aq) \quad (3)$$

But the uptake of Fe by phytoplankton in the presence of colloidal Fe (and hence in the absence of excess chelator) is inherently difficult to quantify because, in particular, of adsorption of the colloids on the cells and on the walls of the vessel. Our experiments with *T. weisflogii* in the presence of freshly precipitated ferrihydrite yielded noisy data with an unexpected lower uptake rate at low pH, although the trend is not statistically significant [$P = 0.15$, one-way analysis of variance (ANOVA)] (Fig. 2D).

We performed a similar experiment with ferrihydrite sequestered in the iron-storage protein Dps (*23*). It has been shown that phytoplankton can take up Fe from Dps and that the uptake rate depends on the dissolution of the colloidal protein core that releases Fe (*24*). Our experimental results were slightly less noisy than those with ferrihydrite and showed no significant effect of pH on the kinetics of Fe uptake by *T. weissflogii* ($P = 0.43$, one-way ANOVA) (Fig. 2E). Because the Dps we used in this experiment was an overexpressed protein from the abundant marine cyanobacterium *Trichodesmium*, this source of Fe may plausibly be present in ocean waters.

Concomitantly, with our studies of Fe uptake by the phytoplankton, we examined the effect of varying Pco_2/pH on the Fe requirements of our model species. On the basis of simultaneous measurements of carbon and Fe accumulation, we calculated cellular iron to carbon ratios (Fe:C). Under present-day and higher Pco_2, growth rate increased with increasing cellular Fe:C ratios, but the Fe requirement was unaffected by pH/Pco_2 (Fig. 3, A to C). Consistent with these results, proteins representative of the major photosynthetic protein complexes, which constitute the majority of cellular iron under iron limitation (*25*), were unaffected by Pco_2/pH, as illustrated in Fig. 3D for *T. weissflogii*. Although the CCM was up-regulated at low Pco_2 as shown by the large increase in carbonic anhydrase abundance, the cellular content of PsbA (D1 protein of PSII), cytochrome b6, and PsaC (Fe-S binding protein of PSI) remained unchanged. For a given growth rate, we observed a much higher Fe:C ratio at low than at ambient and high Pco_2 in diatoms, but not in the coccolithophore *E. huxleyi* (Fig. 3, A to C). Such low ambient Pco_2 presumably results in a stress in the organism that may need to up-regulate the CCM or detoxification mechanisms for reactive oxygen species, leading to an increase in energy and Fe requirement (*10*, *11*, *26*).

Our laboratory data provide a framework to interpret field experiments on the effects of ocean acidification. On the basis of our data, we expect that the availability of Fe to phytoplankton in surface seawater should decrease with pH to an extent dictated by the acid-base chemistry of the chelating ligands. We conducted experiments with surface seawater collected from the New Jersey coast and the Bermuda Atlantic Time-series Study (BATS) region in June and September 2009 using clean techniques and modulating the pH/Pco_2 by adding ultraclean acid or base. We measured the uptake of Fe bound to natural iron-complexing ligands by *T. weissflogii* after pre-equilibration of a low concentration of Fe radioisotope in 0.22 μm filtered seawater at varying pH/Pco_2. As in most laboratory experiments, the Fe uptake rate decreased systematically with increasing Pco_2/decreasing pH in samples collected near the New Jersey coast and in two samples from the BATS region (Fig. 4). Although the effect of pH in any given experiment was not statistically significant, sta-

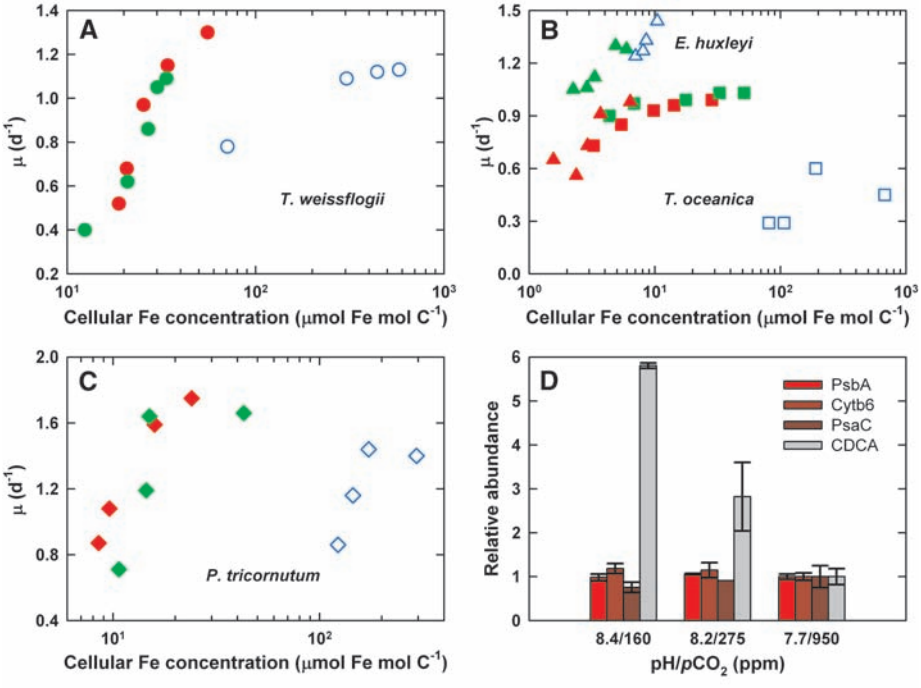

Fig. 3. Specific growth rate of (**A**) *T. weissflogii*, (**B**) *T. oceanica* and *E. huxleyi*, and (**C**) *P. tricornutum* as a function of cellular iron concentration at three different pH/Pco_2 levels in EDTA-buffered culture medium. Symbols correspond to the same pH/Pco_2 values as in Fig. 1; results of each organism are from a single experiment. (**D**) Western blot analyses of the photosynthetic proteins PsbA (the D1 protein of photosystem II), cytochrome b_6 of the cytochrome b_6f complex, PsaC (Fe-S binding protein of PSI), and CDCA (the cadmium carbonic anhydrase) in *T. weissflogii* at three different pH/Pco_2. The values shown are normalized to abundance at pH 7.7/Pco_2 950 ppm. Error bars represent the SD of biological replicates ($n = 2$ to 4 replicates).

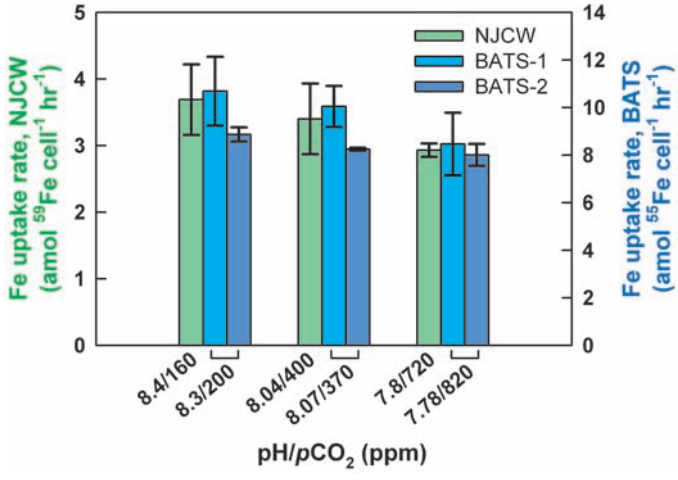

Fig. 4. Short-term Fe uptake by iron-limited *T. weissflogii* from iron bound to natural Fe-binding ligands in surface waters from the New Jersey coast (NJCW) and the Bermuda Atlantic Time-series Study (BATS) region at three different pH/Pco_2. There is no significant difference among treatments in any given experiment ($P > 0.13$, one-way ANOVA). However, statistical analysis on normalized compiled data from all the experiments reveals that both the difference between low pH and ambient pH and that between low pH and high pH ($P = 0.044$ and $P = 0.005$, respectively; *t* test, two-tailed distribution) are significant. Error bars represent the SD of biological replicates ($n = 2$ to 3 replicates).

tistical analysis on normalized compiled data from all the experiments reveals that both the difference between low pH and median pH and that between low pH and high pH are significant ($P = 0.044$ and $P = 0.005$, respectively; t test, two-tailed distribution). Because we know that pH variations in this range have no effect on the Fe uptake capabilities of *T. weissflogii*, the observed decrease in Fe uptake rate is presumably due to a change in the chemical speciation of Fe with pH. On average, the Fe uptake rate decreased by about 10 to 20% from the high-pH (~8.4) to the low-pH (~7.8) conditions in the experiments we conducted, reflecting presumably the acid-base chemistry of the mixture of functional groups responsible for Fe chelation in the various water samples used for the experiments. It would appear from the modest effect of acidification on Fe uptake rates that little of the iron was bound to carboxylic acid moieties in our field samples. The only published experiments on the effect of pH on iron speciation in seawater show that, in a sample of surface water from the North Sea, Fe′ decreased with decreasing pH (*27*), which is consistent with our observations. Those results also imply that the effectiveness of natural ligands in maintaining Fe in solution might be increased at low pH and may result in a slower Fe loss via the formation of Fe oxyhydroxide precipitates. The very low ambient biomass in Sargasso Sea samples and the high concentrations of suspended particulate material in unfiltered New Jersey samples precluded Fe uptake experiments with the ambient phytoplankton populations.

The ongoing increase in atmospheric CO_2 will have many indirect and direct effects on the physics, chemistry, and biology of the ocean. Our laboratory and field results show that one such effect is a decrease in the bioavailability of dissolved Fe caused by the acidification of the water. This effect should be particularly important in areas where the major source of Fe at the surface is from the dissolved Fe in deep waters (*28*). In areas where particulate Fe inputs are important (*29*), this effect may be partially compensated by the increased effectiveness of some chelators in dissolving Fe from oxyhydroxides and/or by enhancing the photo-induced redox cycle of Fe (*30*). We have found so far no evidence that an increase in P_{CO_2} above present-day values results in a lower Fe requirement. It thus seems likely that, unless Fe inputs to surface seawater increase as a result of global change, the net result of seawater acidification should be an increase in the Fe-stress of the phytoplankton in many areas of the oceans.

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Gradual Adaptation Toward a Range-Expansion Phenotype Initiated the Global Radiation of Toads

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Recent studies have identified range expansion as a potential driver of speciation. Yet it remains poorly understood how, under identical extrinsic settings, differential tendencies for geographic movement of taxa originate and subsequently affect diversification. We identified multiple traits that predict large distributional ranges in extant species of toads (Bufonidae) and used statistical methods to define and phylogenetically reconstruct an optimal range-expansion phenotype. Our results indicate that lineage-specific range-shifting abilities increased through an accumulation of adaptive traits that culminated in such a phenotype. This initiated the episode of global colonization and triggered the major radiation of toads. Evolution toward a range-expansion phenotype might be crucial to understanding both ancient widespread radiations and the evolutionary background of contemporary invasive species such as the cane toad.

Bursts of species diversification have played a central role in shaping current biodiversity patterns across the world (*1*). Such periods of accelerated speciation have been typically linked to adaptive radiations, whereby ecological differentiation happens in a group of related sympatric species (*2*). However, recent studies have suggested an important role for range expansion in promoting speciation rates (*3*, *4*), which raises the question of why, under identical extrinsic settings (e.g., land bridges, climate change), some lineages have dispersed while others diversified in situ (*5*). Transferring this notion from ecological to historical biogeography is difficult because of the lack of lineage-specific information on traits promoting range expansion (*3*, *4*, *6*, *7*). We identified such traits in extant toads (Bufonidae) through their present-day correlation with species distribution ranges. Evolutionary reconstructions in a comprehensive phylogenetic, biogeographic, and temporal framework provide a means to elucidate the evolutionary history of these traits and their consequences for speciation in this group.

Toads attained a subcosmopolitan distribution in a very short time frame (*8*, *9*), and the