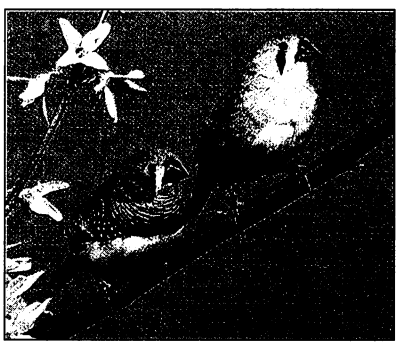
that the birds failed to make any attempts at copying and practicing their tutor's song on the first day, although this might be difficult to detect with the relatively small sample of songs that were analyzed. If the birds did practice on the first day, they would have had the opportunity to rehearse their earliest attempts at imitation in their first period of sleep. The importance of sleep for learning could be tested by presenting the tutor material early in the day (giving birds considerable time to practice on the first day) or late in the day (when birds have had no time to practice before sleep). The ability to control the onset of rapid vocal learning with the Tchernichovski *et al.* protocol now makes this experiment feasible.



The zebra finch (*Taeniopygia guttata*).

In swamp sparrows (*10*) and indigo buntings (*11*), song learning has been described as following sequential modification of repeated structured syllables on the basis of acoustic similarity, and sometimes combining repeated structures (prototypes) to produce new syllables. The process described by Tchernichovski *et al.* is quite different. Young zebra finches often repeat a syllable prototype two or more times. The investigators observed that sequences of new syllables often developed from sequential repetitions of prototypes, a process they termed "in situ" differentiation. Remarkably, the prototypes could differ wildly from the target syllables of the tutor's song, so that sometimes in situ differentiation required major modifications of the leading or following components of the sequence. This is particularly surprising as better material (closer matches) was available to the birds in their untutored songs, although not in the proper sequence. How prevalent is this counterintuitive process? Here some caution is required. Because the experimental design delayed vocal learning, the young zebra finches had an unusually long time to practice in the early phases of song development before they were exposed to a tutor model. Nevertheless, in situ differentiation reinforces the conclusion that acoustic similarity is an insufficient predictor of vocal learning. Why might vocal learning follow such a seemingly difficult trajectory? The neural vocal pathway is hierarchically organized, with syllables and sequences of syllables programmed at higher levels than the RA (*3*, *4*, *12*). The dynamics of learning might be expressed differently at each level of the hierarchy. Once song sequences have developed, they may be relatively invariant, forming the scaffolding for subsequent morphological changes in individual components.

Behavioral analysis alone cannot solve the riddle of how birds learn to sing and neurobiological approaches alone cannot explain behavior. The Tchernichovski *et al.* study lays the groundwork for combining behavior and neurobiology to describe the mechanisms underpinning vocal learning in young birds. If we want to understand normal and abnormal vocal development, especially in inaccessible human brains, we must be prepared to take an integrative and comparative approach.

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Published online 15 March 2001;
10.1126/science.1060338
Include this information when citing this paper.

PERSPECTIVES: ATOMIC FORCE MICROSCOPY

You May Squeeze the Atoms But Don't Mangle the Surface!

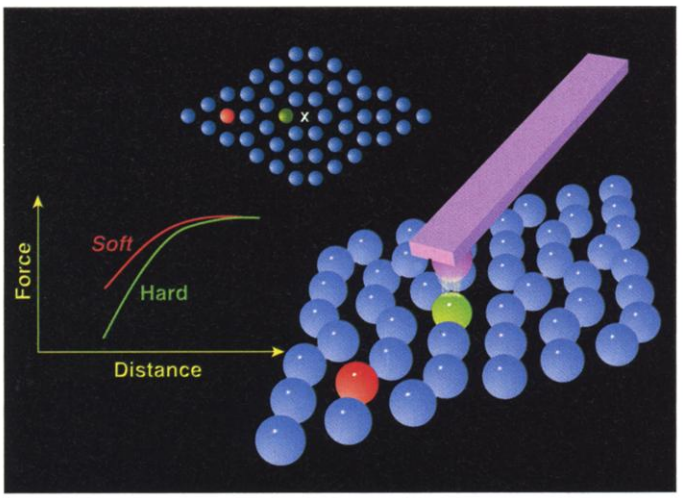
Alex de Lozanne

Imagine yourself at a fruit stand, with a blindfold over your eyes. You could probably identify various fruits by touching them, even using only one hand. Now imagine that you have a boxing glove over this hand and that you can only move it up and down with your arm outstretched. Identifying fruit is now far more difficult, but you may still be able to distinguish between a crisp apple and a ripe persimmon (without using your sense of smell!). You would do so by pressing on the fruit and feeling the reaction force on your glove. A hard fruit responds with a large force within a short distance, whereas a soft fruit gives in and responds with a smaller force.

On page 2580 of this issue, Lantz *et al.* (*1*) report an equivalent experiment at the atomic scale, performed by gently pressing the tip of an atomic force microscope (AFM) on the atoms of a silicon surface. There is one added complication, however: The tip is attracted to the surface at close range (think of sticky fruit). One therefore has to bring the tip to the surface and then quickly snap it back to prevent damage to the tip and/or surface should they stick together. Repetition of this motion produces an oscillatory movement, which is easier to control than a single dip. The oscillation of the AFM cantilever (your arm) changes frequency as the tip (your glove) interacts with the surface. Theoretical modeling is then used to extract information about the stiffness of the surface. This method enables Lantz *et al.* to measure the force resulting from an incipient bond between the tip atom and the surface atom and to distinguish between different types of silicon atoms (see the figure on the next page). For the first time, atomic resolution and a detailed measurement of these forces are obtained on the same surface.

The AFM was born in 1986, when a quintessential "back of the envelope" calculation by Binnig, Quate, and Gerber showed that it should be possible to measure the force between the last atom on a tip and individual atoms on the surface (*2*). Their first prototype showed a lateral resolution of 3 nm (*2*). Soon, beautiful AFM images with atomic resolution graced the covers of prestigious journals. It turned out, however, that these images never showed atomic-scale defects, such as a missing atom. It became clear that the images resulted from the interaction between many atoms on the tip with those on the sample. If the tip has a structure that is congruent with the surface structure,

The author is in the Department of Physics, University of Texas, Austin, TX 78712, USA. E-mail: lozanne@physics.utexas.edu



Gently does it. In their AFM experiments, Lantz *et al.* can distinguish between silicon atoms at different surface locations on the basis of their force response curves (**left**). Shown here are four unit cells of the silicon 7 × 7 surface, with a corner hole at the center (marked with an x). This site serves as a reference to measure background forces. The corner adatom (green) and the center adatom (red) have different stiffnesses, as indicated by the curves of the same color in the graph. This difference is exaggerated for clarity. The tip is shown at the point of closest approach, where the chemical bond (yellow cloud) starts to form.

which is likely if the tip picks up some of the material from the surface, a perfect image showing the periodicity of the sample is obtained, but local defects do not show up. This can be easily understood by taking two washboards and sliding one on top of the other. The resulting up/down motion will be the same even if you remove one of the ridges from one washboard.

"True" atomic resolution was not achieved until 1995, when the 7 × 7 reconstruction of silicon (a beautiful rearrangement of the surface atoms) was imaged with an AFM (*3*, *4*). It was the atomic-scale imaging of this surface that gave the scanning tunneling microscope (STM), the older sibling of the AFM, instant fame in 1983 (*5*). Lantz *et al.* chose this classic surface for their experiment. It is ideal for this first demonstration because the atoms are widely spaced and each has a "lone" atomic orbital sticking out of the surface. All the atoms are silicon, but their stiffness differs nevertheless depending on their position, which determines the way they bond to their neighbors underneath. This type of force spectroscopy has been done on surfaces for some time (*6*), but this is the first time that the surface is also imaged with atomic resolution in the same experiment (after all, you have to take the blindfold off to check that your identification of the fruit is correct!).

In a recent related study, Foster *et al.* identified the fluorine atoms in CaF_2 on the basis of the shape of the features in the AFM image, aided by theoretical modeling (*7*). The observed triangular shape of the surface fluorine atoms is caused by the contribution to the image from fluorine atoms just below. This result is important because this type of sample, being an insulator, cannot be imaged with STM. Giessibl *et al.* have also shown that under special conditions, it is possible to image individual orbitals such as those of an atom on the tip (*8*) This work has elicited criticism (*9*) and a technically compelling response (*10*, *11*). The idea of squeezing microscopic samples with an AFM tip is also being applied to biological specimens. Mahaffy *et al.* have recently made quantitative viscoelastic measurements on fibroblasts at the submicrometer level and concluded that the response is dominated by the cytoskeleton (*12*).

The AFM studies discussed here were performed in the so-called noncontact mode, which uses the weak attractive forces between tip and sample for imaging. The area of noncontact AFM with true atomic resolution is starting to blossom (*13*, *14*). Over the short term, we can expect improvements through the use of stiffer cantilevers and smaller oscillation amplitudes (*8*). The use of carbon nanotubes as tips (*15*) promises to improve the weakest link in these experiments. Once we have identified surface atoms and their bonding strengths, we will be able to do more atomic manipulation (*16*), even at room temperature (*17*). The use of sophisticated tactile ("haptic") interfaces (*18*, *19*) will allow us to "feel" the atoms in real time and gently push them to desired locations. We may at last be able to take off the boxing glove.

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PERSPECTIVES: CARBON CYCLE

Discriminating Plants

F. I. Woodward

The increasing concentration of carbon dioxide (CO_2) in the atmosphere (*1*) is the surest evidence that humans are changing the global environment. Atmospheric CO_2 would accumulate even faster if the oceans and the terrestrial biosphere did not absorb about half the CO_2 emissions from burning fossil fuels and deforestation. The amounts of CO_2 absorbed by these sinks are difficult to quantify, however, and this is hampering a detailed understanding of the carbon cycle.

Recent studies suggest that plants discriminate in a systematic way against CO_2 containing the stable isotope ^{18}O, offering an exciting opportunity to differentiate the terrestrial from the oceanic sink (*2*, *3*); the greater the terrestrial sink the lower the ^{18}O content of atmospheric CO_2 (*4*). However, Gillon and Yakir (*5*) suggest on page 2584 of this issue that current understanding of this discrimination may be incomplete, resulting in incorrect sink estimates.

Both transpiration and photosynthesis are involved in the discrimination of plants against CO_2 containing ^{18}O ($C^{18}O^{16}O$) (see the figure). $H_2{}^{16}O$ transpires more rapidly from leaves than the heavier $H_2{}^{18}O$. The site of evaporation therefore becomes enriched in the heavier isotope (*3*). This

The author is in the Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK. E-mail: F.I.Woodward@Sheffield.ac.uk