that results in an increase in the oscillator strength of these excitations, such as we have observed, could then turn on the superconducting phase transition. Even if the mechanism is primarily phonon-mediated, an anharmonic mode such as that described here could have a much reduced isotope effect on T_c than that expected from standard Bardeen-Cooper-Schrieffer phenomenology (*29*), as is found for $YBa_2Cu_3O_7$ (*35*). This same mechanism could be operative in the other cuprate-based, axial oxygen-containing superconductors, and especially in the thallium- and bismuth-based compounds, where these oxygen atoms are also the bridge between the CuO_2 planes and a highly polarizable layer.

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16. For the sake of consistency, we have adopted the same labels for the final states as in (*5*) and (*6*). Although we concur with the assignment of feature C to the Cu1 $4p_x\pi \leftarrow 1s$ transition, to a final state molecular orbital of π symmetry composed of the Cu1 $4p_x$ and O1 and O4 $(3)p_x$ atomic orbitals, we believe that feature D, assigned to the Cu2 $4p_z\pi \leftarrow 1s$ transition, actually involved a final state molecular orbital which is not strictly of π symmetry because it possesses at least some O4 $(3)p_z$ character, analogous to the Cu2 $3d_{z^2}$–O4 $2p_z$ band near the Fermi level. Another issue in these assignments is the choice of the single- or multi-electron model. In the multi-electron model (*10, 11*), used in the assignments for $YBa_2Cu_3O_7$ (*5, 6*), the final state of the C/D transition is the bound Cu $4p\pi$, and the lower energy A/B transition is assigned to the Cu $4p\pi$ + shakedown state. In the single-electron model (*8*), the final state of the C/D transition is the quasi-bound Cu $4p\pi$L (L = ligand), which differs from the lower energy A/B transition to the bound Cu $4p\pi$ final state in that it is more delocalized, with appreciable photoelectron density on ligand-centered orbitals. If the single-electron model is correct, because changes in the intensities of transitions are more significant than changes in their energies, the overlap is apparently affected more than the nature of the $4p\pi$L state. Therefore, our interpretation is based only on the fact that the final states of these transitions have Cu1 $4p_x$ and Cu2 $4p_z$ character, which has been observed experimentally (*5, 6*), and is independent of the exact nature of the final state.
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Honeyguides and Honey Gatherers: Interspecific Communication in a Symbiotic Relationship

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In many parts of Africa, people searching for honey are led to bees' nests by the greater honeyguide (*Indicator indicator* Sparrman). The Boran people of Kenya claim that they can deduce the direction and the distance to the nest as well as their own arrival at the nest from the bird's flight pattern, perching height, and calls. Analyses of the behavior of guiding birds confirmed these claims.

According to rock paintings from the central Sahara, Zimbabwe, and South Africa, man has collected honey in Africa for 20,000 years (*1, 2*). Even today, honey contributes significantly to the diets of many African people (*2–5*). When searching for honey, Africans are often joined by the greater honeyguide (*Indicator indicator*), which leads them to bee colonies (*Apis mellifira*) located in large trees, rock crevices, or termite mounds. After the gatherers have opened and left the nest, the bird feeds on pieces of honeycomb left behind. From these it extracts mainly the larvae and the wax to supplement its normal diet of insects (*5–7*). The earliest written accounts of this bird-man interaction date back to the 17th century (*6*). Because of the anecdotal nature of most of these reports, however,

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many consider this interaction to be a myth. Soon, it may no longer be possible to prove them wrong, because in many areas honey is more and more being obtained from beekeepers or is even being replaced by commercial food, sugar, drugs, and alcohol. In these areas, the birds no longer guide (*5, 6*).

A 3-year field study of the greater honeyguide was carried out near Sololo and Marsabit in northern Kenya (*5*). The area, dry bush country, is the home of the nomadic Boran people who still follow the honeyguide regularly. In unfamiliar areas, their search time per bees' nest was, on average, 8.9 hours when not guided and 3.2 hours when guided (n = 329 hours and 238 hours, respectively). This 64% reduction in time is a conservative estimate, because it includes only those days during which at least one nest was found. Because many days without guiding lead to no nest detection at all, the acutal benefit to the humans is even more substantial (*5*). The honeyguide benefits as well. The Borans' use of smoky fire reduces the bird's risk of being stung, and their use of tools increases the amount of food, because 96% of all nests (n = 186) are accessible to the birds only after humans have opened them.

In view of this mutual benefit, it is not surprising that humans and the honeyguide have developed an elaborate interspecific communication system. To draw the attention of the bird, the Borans use a penetrating whistle that can be heard from a distance of over 1 km on our study sites. This whistle, known in Boran language as "*Fuulido*," is produced by blowing air into clasped fists, modified snail shells, or hollowed-out doum palm nuts (*Hyphaene coria-* wood are also used to draw the bird's attention. Such noise doubles the encounter rate with the bird ($P < 0.02$, Mann-Whitney U test).

The greater honeyguide draws the attention of a human by flying close to him, moving restlessly between perches around him, and emitting a double-noted, persistent call, which sounds like "tirr-tirr-tirr-tirr" (Fig. 1A). Thereafter, it often performs a directional flight above the tree tops and may disappear for a minute or more (here termed first disappearance). Upon returning, the bird perches again on a conspicuous tree or bush. If approached to within 5 to 15 m, the bird takes off, still calling. After a short undulating flight, during which the white outer tail feathers are displayed, it perches again and continues calling. As the Borans follow, they whistle, bang on wood, and talk loudly to the bird to keep it interested in the guiding. When they get close to it, the bird flies to another perch. This pattern of leading and following is repeated until the bee colony is reached.

Professional honey gatherers (interviewed by H.A.I., a Boran) said that, through its guiding pattern, the bird informs them about the direction of, the distance to, and their arrival at the colony.

Direction. The direction in which the bird flies is said to indicate the direction of the colony. We plotted several guiding routes (Fig. 2B) and found a clear directional preference toward the nest (Fig. 2A; $P < 0.01$, Rayleigh test) (*8*). The mean bearing of the closely clustered guiding vectors deviated only 0.5° from the nest direction; the large mean length of the vectors (0.834) indicates that this direction was maintained with a high consistency. As the variance in the direction of the last three sections was lower than in previous ones ($F = 5.476$, $\nu_1 = 38$, $\nu_2 = 56$, $P < 0.001$), the directional precision seems to increase as the colony is approached. Once the location of a colony was known to us, we could elicit repeated guidings to the same nest by not destroying it. When we attracted the bird to the same starting point, we were led via more or less the same route (Fig. 2C). From different compass points, the bird led us via different, but again fairly direct routes (Fig. 2D). When more than one colony was present in an area, the nest to which we were guided first was closer to the point of first encounter than subsequent ones in 88% of the cases (Fig. 2E) (n = 25 trips). These experiments and data suggest that the directionality exhibited by the bird is a reflection of its prior knowledge of the nest locality.

Distance. According to Boran honey collectors, three features decrease with diminishing distance to the nest (Nestdist): the duration for which the bird disappears after the first encounter (Disdur); the distance between the perches where it waits until the follower has caught up (Stopdist) (*9*); and the height of the perch (Perch). During several guiding tours we recorded these four variables plus the number of stops (Stopno) and the distance already covered (Dist-

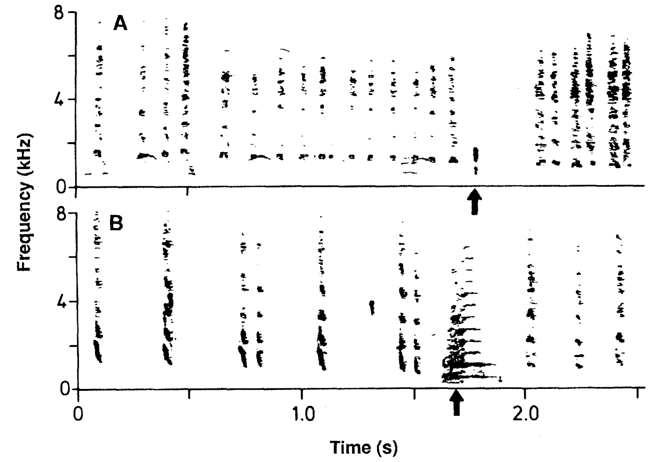

Fig. 1. Sonograms (**A**) of a typical guiding call emitted by the honeyguide on the route and (**B**) of an indication call emitted after the bird has reached the colony. Arrows indicate the input of human sound, which increased the repetition rate markedly in (A) but not in (B).

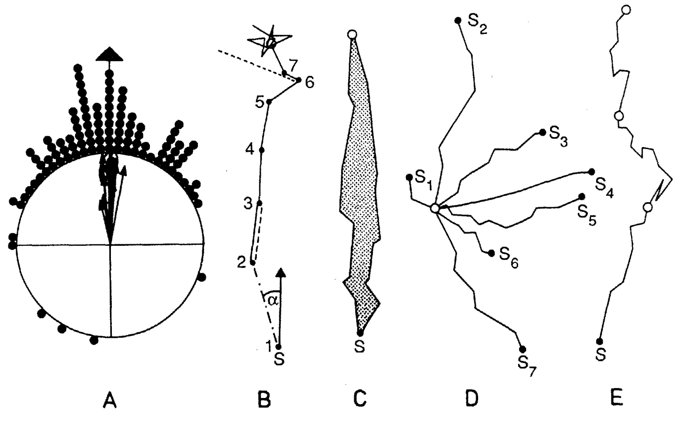

Fig. 2. (**A**) Orientation of 16 guiding routes in relation to nest direction (0°, large arrow). Black dots at the periphery give the pooled bearings of all guiding sections. Arrows originating in the center represent the vectors, calculated as the mean bearing of all sections in a particular guiding route. The overall bivariate means of the single vectors correspond to the centers of the ellipse, which indicates their 99% confidence limits (according to Hotelling's T^2 test) (*8*). (**B–E**) Sketches of guiding patterns from the first encounter (S) to the nest (O): (B) Typical fairly straight course, also showing the variables used in the distance analysis: Stopno (1 through 7), Stopdist (-----) (here between Stopno 2 and 3), and the corresponding Nestdist (———) and Distflown (–·–·–); α is the angle between any one section of the guiding and the straight direction to the nest (arrow). The dotted line originating from Stopno 6 shows an overshooting. (C) Area of five guiding courses between the same starting point and the same nest. (D) Routes to the same nest starting from different points (S_1 through S_7). (E) Consecutive guidings to three nests.

flown) from the start of the tour to the present perch (see Fig. 3A for an illustrative example) (*10*).

We found all three Boran statements to be true. The closer the nest, the shorter the duration of the first disappearance (Fig. 3A). When disappearing, the bird probably flies toward the nest to confirm its position before starting a guiding tour. Whether it covers the whole distance or only flies until it finds a conspicuous landmark, we cannot tell.

Also, the closer we approached the nest, the shorter the distance between stops, especially during the last 200 m (Fig. 3B) ($P = 0.011$, Wilcoxon test; *11*). Stopdist was not significantly related, however, to Distflown and Stopno (both $P > 0.180$). Thus, first disappearance and stopping distance reflect the remaining distance to the nest. Conversely, perching height reflects the number of stops and the distance already covered since the tour started. Perch decreased with Stopno (Fig. 3C) and Distflown (not shown in Fig. 3; $b = -0.002 \pm 0.001, P = 0.015$) (*12*). As guiding is fairly direct (Fig. 2, B through E), an increase in Stopno and Distflown normally leads to a decrease in Nestdist. Thus, our results confirm the honey gatherers' observation that the bird perches lower as it gets closer to the colony. The results do not, however, confirm their interpretation that perching height indicates the distance to the nest; Perch was not significantly related to Nestdist ($P = 0.275$).

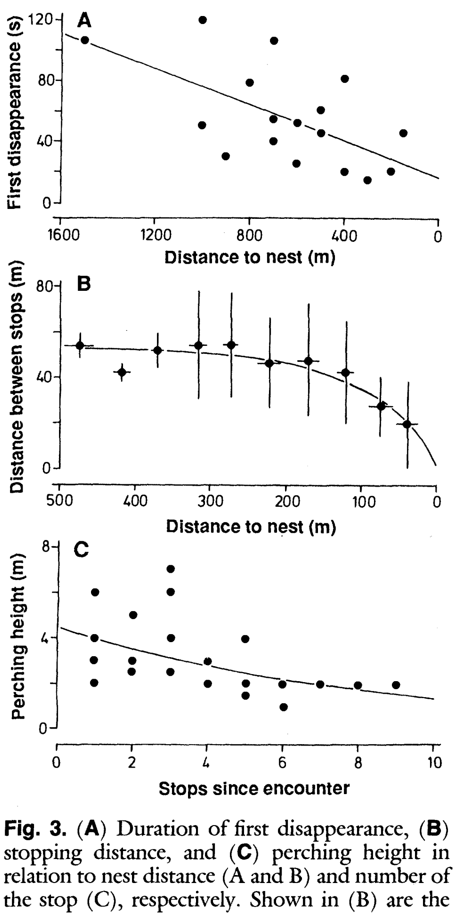

Fig. 3. (**A**) Duration of first disappearance, (**B**) stopping distance, and (**C**) perching height in relation to nest distance (A and B) and number of the stop (C), respectively. Shown in (B) are the means and standard deviations calculated from pooling data within the same 50-m category of nest distance (1 to 50, 51 to 100, . . ., 451 to 500 m). (A) and (C) give the original data together with the respective regression lines. Their slopes ($b \pm$ SE) are 0.059 ± 0.019, $P = 0.007$ [(A), linear model] and -0.115 ± 0.034, $P = 0.002$ [(C), exponential model].

Arrival. Boran honey gatherers maintain that they can tell from changes in the bird's behavior when it has reached its goal. We found two behavioral changes to support this statement; one is related to the call, the other to the flight pattern. On arrival at the nest, the bird perches close to it and emits the "indication call" (Fig. 1B). This call differs from the previous guiding call in that it has a softer tone, with longer intervals between successive notes. There is also a diminished response, if any at all, to whistling and shouting by humans. After a few indication calls, the bird remains silent. When approached by the searching gatherer, it flies to another perch close by, sometimes after circling around the nest. The resulting flight path (Fig. 2B) finally reveals the location of the colony to the gatherer. If the honey collector does not (or pretends not to) detect the nest, the bird gives up after a while. It may then leave the area either silently or start a guiding session to another colony. In the latter case, it switches from the indication call to the guiding call and resumes a fairly direct flight pattern.

Although a few investigators (*7*) have assumed that greater honeyguides know the location of one or more bee colonies in a particular area, the prevailing opinion still is that the bird does not know where it is taking a person but rather "leads in a most erratic course" until the sight and sound of incidentally encountered bees brings the guiding to a halt (*6, 13*). The very first discovery of a colony may indeed depend on such signs; but thereafter the birds (regularly?) monitor the nests even when no guiding is taking place. From camouflaged observing positions occupied before dawn, we observed several marked and unmarked honeyguides visiting a nest. They always appeared singly, stayed for only about a minute, and then flew away. When the bees were still docile, as on cloudy and cool mornings, the bird would fly straight into the entrance of the nest and peer into it.

The information gathered during such visits enables the bird to engage in its goal-oriented guiding behavior. Our finding that native people are able to interpret this pattern reliably is, however, not equivalent to saying that every aspect of the bird's guiding behavior is meant to inform them. The changes in call and flight pattern after the arrival (Figs. 1 and 2B) probably are informative; but there are more parsimonious explanations for other aspects of the bird's behavior. The directional flight (Fig. 2A) and the duration of the first disappearance (Fig. 3A) are inevitable results of a bird flying to a nest that it knows. The reduction in perching height (Fig. 3C) could be due to the honeyguide's gradual loss of fear of the follower. Similar cases of distances decreasing over time are known from mobbing birds and other animals interacting with predators (*14, 15*). Decreasing risk and fear, however, are unlikely to account for the reduced distance between stops (Fig. 3B), because Stopdist decreased neither with Distflown nor with the frequency with which the bird had been approached (Stopno). Also, the bird allows people to approach to within 5 to 15 m of its perch, much closer than even the shortest average stopping distance of 20 m (Fig. 3B).

We suggest that the decreasing Stopdist represents an "area-restricted search" (*16*) that is performed by many animals when close to their goal, be it food, hosts, or homes (*17–19*). One common characteristic of this search pattern is reduced step length and thus speed. Consequently, the animal spends more time scanning the promising area and is more likely to detect signs of the goal, such as swarming bees or specific landmarks. This will enable it to correct directional errors (compare the smaller directional variance toward the end) to avoid an overshooting or even missing (dotted line in Fig. 2B). Farther away from the nest, longer stopping distances may be more economical because they reduce the number of energetically expensive maneuvers associated with takeoff and landing. According to this interpretation, distances between the final stops should decrease when any feature impedes detection of the nest (for example, dense vegetation). Unfortunately, our present data do not allow us to test this prediction, but the high variation in stopping distances may have resulted partly from such differences in visibility. This high variation also makes it unlikely that the bird "deliberately" tells the follower where to look precisely for the nest.

Our data also do not yet allow us to test the following two claims of Boran honey gatherers: (i) that a bird, flying lower than the tree tops, will guide to a colony close to the ground, and (ii) that when nest distances become very long (about 2 km or more), the birds "deceive" the gatherers about the real distance by stopping at shorter intervals. However, having found all the other Boran observations to be true, we see no reason to doubt the statements of these excellent "ethologists."

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9. The observation that stopping distances decrease as the bees' nest is approached was also reported by G. W. Stow [*The Native Races of South Africa* (Swan Sonnenschein, London, 1905)].
10. We measured distances by counting paces and later converting them into meters. Perching heights were estimated to the nearest 0.5 m. In cases of skewed distribution, original data were log-transformed.
11. The relation between Stopdist and Stopno was tested within guidings, yielding one regression coefficient (*b*) for each tour. The relations between Stopdist and Nestdist and Distflown, respectively, were tested across guidings. In the case of Nestdist, separate regressions were calculated for each Stopno, whereas, in the case of Distflown, separate regressions were calculated for each 50-m category of Nestdist. In all cases, the regression coefficients were then tested against the 0 hypothesis of no relation ($b = 0$) by means of a Wilcoxon matched-pairs, signed-ranks test.
12. The small sample size did not allow us to calculate separate regressions as in the case of Stopdist. Therefore, Perch data from all guidings were pooled.
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Structure of Recombinant Human Renin, a Target for Cardiovascular-Active Drugs, at 2.5 Å Resolution

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The x-ray crystal structure of recombinant human renin has been determined. Molecular dynamics techniques that included crystallographic data as a restraint were used to improve an initial model based on porcine pepsinogen. The present agreement factor for data from 8.0 to 2.5 angstroms (Å) is 0.236. Some of the surface loops are poorly determined, and these disordered regions border a 30 Å wide solvent channel. Comparison of renin with other aspartyl proteinases shows that, although the structural cores and active sites are highly conserved, surface residues, some of which are critical for specificity, vary greatly (up to 10 Å). Knowledge of the actual structure, as opposed to the use of models based on related enzymes, should facilitate the design of renin inhibitors.

RENIN (E.C. 3.4.23.15) IS A HIGHLY specific aspartyl proteinase with only one known substrate, angiotensinogen. In humans, a decapeptide, angiotensin I, is released from angiotensinogen by the catalytic hydrolysis of the Leu^{10}–Val^{11} bond. Angiotensin I is processed by the angiotensin-converting enzyme (ACE) to angiotensin II, a potent vasoconstrictor involved in regulating blood pressure and fluid balance. However, presently available therapeutic agents for reducing blood pressure target ACE (*1*) and not renin.

Because only very small quantities of kidney renin have been available, much of the biochemical characterization of renin has been done on the mouse submaxillary gland enzyme. Mouse renin has been protein- (*2*) and cDNA-sequenced (*3*), and crystals of it have been reported by several groups (*4, 5*). In the five crystal forms of mouse renin obtained, the corresponding asymmetric units contain multiple copies of the molecules (*5*), which complicates the crystallographic problem enormously. No further progress in this analysis has been reported.

The primary structure of the human enzyme has been deduced solely from cDNA (*6*) and gene sequences (*7*). We converted secreted human prorenin (*8*) from transfected Chinese hamster ovary cells (*8, 9*) to active renin by cleavage with immobilized trypsin. The purified recombinant human (rh) renin (*10*) was treated with endoglycosidase F to remove attached carbohydrates without affecting the specific activity of the final product (*11*).

Sample homogeneity from batch to batch was difficult to regulate. Most preparations exhibited three to five bands on isoelectric focusing gels. From ~1500 different crytallization trials, the optimal conditions were 5 to 6% polyethylene glycol 600 buffered with 50 m*M* NaH_2PO_4-K_2HPO_4 to *p*H 4.7. The resulting crystals exhibited tetragonal symmetry, space group *I*4, with unit-cell dimensions, $a = b = 133.5$ Å, $c = 41.7$ Å, with one renin molecule per asymmetric unit, $V_M = 2.53$ Å^3 per dalton.

Intensity data were collected on a twin multiwire detector system (*12*). A total of 60,512 measurements (13,343 unique data) were measured from a single renin crystal. The overall symmetry agreement factor [$= \Sigma(I_i - \langle I \rangle)/\Sigma\langle I \rangle$, where I are the net intensities] was 0.09. The structure was solved by the molecular replacement method (*13*). Several renin models based on the known structures of three fungal aspartic proteinases have been built (*14*). The more extensive homology among mammalian species led us to construct a search model for renin based on the molecular structure of porcine pepsinogen (*15, 16*). The correlation coefficient between observed and calculated structure factors based on the oriented and translated model was 0.39 (9.5 σ above the mean) for the data in the 6 to 4 Å resolution shell. The corresponding R factor [$= \Sigma||F_o| - |F_c||/\Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are observed and calculated structure factor amplitudes]

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