dialects (17); (iii) skills, involving rare innovations (including tool use), whose complexity depends on the nature of socially biased learning [which affects the degree of ratcheting (6)]; and (iv) symbols, probably derived from signal variants that became membership badges of the social unit or population (6, 15).

Species are expected to vary in the kinds of cultural elements they display. Only humans have all four kinds of cultural elements, whereas, unique among nonhuman primates, chimpanzees and orangutans show the first three (2, 3, 8–11), which are made possible by innovative abilities and sophisticated forms of socially biased learning (24–26). Human cultures, therefore, differ from those of great apes in having unambiguously symbolic elements (6, 27), far more complex skills, and far greater repertoire sizes, made possible by cognitive differences affecting innovation or observational learning (1, 5, 6). The presence in orangutans of humanlike skill (material) culture pushes back its origin in the hominoid lineage to about 14 million years ago, when the orangutan and African ape clades last shared a common ancestor (28), rather than to the last common ancestor of chimpanzees and humans.

Important tasks for the future include documenting the possible interdependence among these different kinds of cultural elements, identifying the conditions favoring their evolution, and assessing whether they all show the geographic and social correlates known for humans and demonstrated here for great apes.

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12. After compiling a preliminary list of candidate cultural variants, representatives of all sites with long-term data on wild orangutans convened in San Anselmo, California, from 14 to 17 February 2002 to discuss these variants and to identify new ones through plenary discussion of site descriptions and video footage from multiple sites. We used the same criteria as employed in the chimpanzee comparison, including those for prevalence at a given site (2, 3).
13. Included sites had more than 4 years of intensive observations of at least 25 individual orangutans and 10,000 contact hours. Observation intensity is based on numbers of observation hours and total duration of the study as (i) less than 25,000 hours or (ii) more than 25,000 hours. We excluded (i) universals, which are behavior patterns that were found at all sites or were absent for obvious ecological reasons; (ii) variants with localized distributions that most likely reflect ecological conditions (e.g., lathering of fruit pulp or seeds in the fur of arms, drinking water from natural containers such as pitcher plants, making ground nests, wading through standing water, etc.).
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33. Sponsored by the L.S.B. Leakey Foundation. For contributions to the database, we thank D. Agee, M. Brown, I. Foitova, E. Fox, N. Ghaffar, I. bin'Muhammad, A. Johnson, T. Laman, J. Mitani, T. M. Setia, H. Peters, D. Priatna, and S. Wich. We thank the Indonesian Institute of Sciences (LIPI) and the directorate general of Forest Protection and Nature Conservation Service in the Ministry of Forestry for permission for the various projects. For substantial or long-term support of orangutan field work, we thank the Wildlife Conservation Society; the National Geographic Society; NSF; the Netherlands Organization for Tropical Research; the L.S.B. Leakey Foundation; the Wenner-Gren Foundation for Anthropological Research; the Pittsburgh Zoo; the Conservation, Food, and Health Foundation; the Great Ape Conservation Fund; and the United States Fish and Wildlife Service. We thank C. Boesch, D. Brockman, R. Deaner, J. Galef, M. Huffman, J. Mitani, A. Russon, M. van Noordwijk, and C. Vinyard for help with this paper.

Single-Gene Greenbeard Effects in the Social Amoeba *Dictyostelium discoideum*

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Selection can favor reproductive altruism if an altruism allele aids copies of itself by helping relatives. The alternative "greenbeard" mechanism, in which an allele directly recognizes and aids copies of itself in others, is generally thought to be too complex for a single gene to carry out. The *csA* gene in *Dictyostelium discoideum* acts as a single-gene greenbeard. When wild-type cells are mixed with *csA*-knockout cells, the wild type is more altruistic, but is also able preferentially to direct the benefits to other wild-type cells. Both properties derive directly from homophilic cell adhesion of the protein encoded by *csA*.

Selection can favor an allele that causes self-sacrifice if it enhances the fitness of others who bear the allele (1). Generally, individuals recognize other bearers—relatives—by some combination of social context and learning (2). Alternatively, alleles might directly recognize copies of themselves, regardless of average relatedness (3). These so-called greenbeard alleles, the term originally coined by Dawkins (4), are generally thought to be rare because they must cause a complex of three effects: a perceptible trait (the hypothetical green beard), recognition of this trait in others, and preferential treatment of those recognized (3, 4). The few known examples—poison-antidote systems like bacteriocins (5, 6) and the fire ant gp9 locus (7, 8)—involve or are thought to involve multiple tightly linked genes. However, Haig has suggested that a single homophilic cell adhesion gene could cause all three effects (9). Here, we show that this is true for the *csA* (*contact site A*) gene of the slime mold, *Dictyostelium discoideum*.
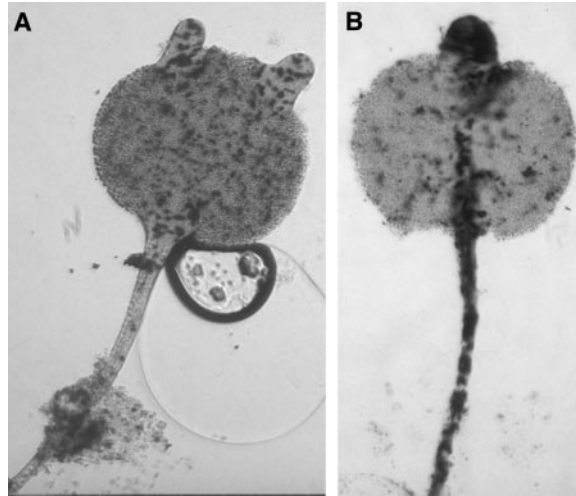
D. discoideum is a highly social eukaryotic microorganism (10). Most of the time,

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Fig. 1. In binary mixtures with *csA*-knockout cells, *Dictyostelium* wild-type cells sort out preferentially in the stalk area. We made 1:1 mixtures of wild-type AX2 cells expressing the *lacZ* gene (AX2/β-gal) under the control of the actin 15 promoter with either (**A**) wild-type AX2 or (**B**) *csA*-knockout T10 cells (*15*) at the beginning of development. Then we plated them on nonnutrient agar for fruiting body formation. Staining with β-gal appears to be homogeneously distributed in control mixtures of wild-type cells (A), whereas it is concentrated in the stalk area in mixtures with knockout cells (B).

Table 1. Percentage of knockout spores from mixtures with wild type. Knockout cells are more likely to become spores; the observed excess was matched in only 5 of 10,000 randomization tests and closely matches the expectation if the stalks consisted entirely of wild-type cells (if one assumes stalk cells are 20% of the fruiting body). Wild-type AX2 (β-gal) and *csA*-knockout cells were mixed in the given proportions and incubated on agar without food. Fruiting bodies were collected randomly and treated with 0.5% SDS to kill undifferentiated cells (*15*). To obtain separate colonies from individual spores, spores washed free of SDS were plated on *E. coli B/2* at a density of 50 spores per plate. When single colonies started forming fruiting bodies, pieces of a 24 colonies (per experiment) were transferred to a 24-well plate for X-gal labeling.

Cells and spores	Percentage for experiment		
	1	2	3
Knockout cells at start of experiment	50	50	80
Knockout spores	62.5	66.7	100
Expected knockout spores if stalk is all wild type	62.5	62.5	100

the single-celled amoebae forage for bacteria in the forest soil. The social phase occurs only when the food runs out. Amoebae use a cyclic AMP signal relay to stream into an aggregation of thousands of cells. Here, roughly 20% of the cells altruistically die in the process of forming a long rigid stalk that supports the other cells, which differentiate into a cluster of spores. Cells from different clones readily form chimeric fruiting bodies in the laboratory (*11*), and they probably do so in the field, as judged from the fact that 19 of 26 small ($\bar{x} = 0.2$ g) field soil samples containing *D. discoideum* had multiple clones (*12*).

The *csA* gene encodes a cell adhesion protein anchored in the cell membrane. The most distal globular domain, with some homology to the immunoglobulin domain**,** interacts by homophilic binding to the identical domain of gp80 proteins anchored in other cells (*13*).

The effects of the *csA* gene have been explored with a knockout mutant lacking functional gp80 protein (*14*). There are two pertinent effects, one a greenbeard effect and another with the opposite effect. The greenbeard effect has been shown in studies in which equal mixtures of wild-type and knockout cells were developed on soil plates (*15*). Spores from the resulting chimeric fruiting bodies were 82% wild type, because their homophilic binding allowed them to adhere in aggregation streams and to pull each other into aggregates, whereas most knockout cells were left behind.

This exclusion of the knockout from aggregates on soil is particularly important because it thwarts the other, anti-greenbeard, effect. Mixed aggregations can form on the less natural substrates of agar and nitrocellulose filters, where streaming is presumably easier for the knockout cells (*15*). In these mixtures, the greater adhesion of the wild-type cells causes them to sort preferentially into the stalk (Fig. 1), as predicted by models of physical interactions (*16, 17*). Typing of

spores grown from these fruiting bodies confirms an excess of knockout cells (Table 1).

As the wild type is more altruistic in mixed aggregates, it would lose out to the knockouts if that were the only effect. But, on the more natural soil substrate, this sacrifice actually benefits mainly other wild-type cells, because the earlier greenbeard effect ensures that few knockout cells enter the aggregate. The single property of homophilic adhesion confers all of the required greenbeard traits: a surface molecule, recognition and adhesion to the same molecule in others, and cooperative streaming from which the knockouts are largely excluded.

As *D. discoideum* normally lives in soil, it is probable that natural selection favors the wild type that does well on this substrate. The greenbeard effects are clearly selectively relevant, because null mutants are certain to appear repeatedly in nature. However, phylogenetic studies are needed to determine whether detailed sequence evolution has been driven by greenbeard effects.

The *csA*-gp80 system shows several novel features. First, gp80 is closer to the original conception of greenbeards because it results in preferential altruism, rather than preferential killing of nonidentical types. Second, it is the common, wild-type allele, whereas other greenbeards halt at lower frequencies because of significant costs that select for cheaters (*18*) or pathological side effects (*7*). Third, its mechanism is unusually well understood and confirms the prediction that homophilic adhesion proteins could be greenbeards (*9*). Fourth, gp80 creates two distinct and opposite greenbeard effects, whose net effect varies with the substrate. Finally, *csA* shows that all the components of the greenbeard—trait, recognition, and action—can result from a single protein coded by a single gene.

Our results, together with those on bacteriocins, suggest that greenbeard effects may be more common in microorganisms than in the animals usually studied by behavioral

ecologists. In animals, the greenbeard gene or complex must correctly orchestrate signal production, reception, integration, and the resulting behavior in different pathways, tissues, and organs. In contrast, in a simple organism like *D. discoideum*, all these functions can take place at the level of individual cells interacting with their neighbors.

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19. Supported by U.S. NSF grant DEB-0108478. Work in Bozzaro's laboratory was supported by the Italian Ministry for the University. We thank N. Medhiabadi and K. R. Foster for comments.

26 August 2002; accepted 30 October 2002