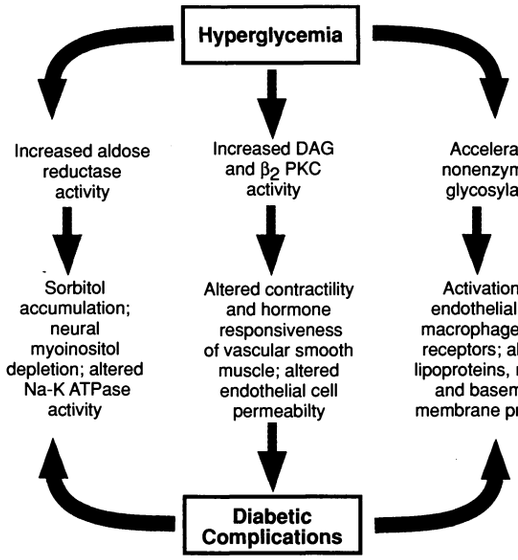

How too much glucose may lead to the long-term complications of diabetes. AGE, advanced glycosylation endproducts; DAG, diacylglycerol; PKC, protein kinase C.

appropriate. The connection between the endpoints measured by Ishii *et al.*—hyperfusion of the kidney and albuminuria—and the permanently impaired renal function of diabetics remains a reasonable but unproven hypothesis. The link between increased retinal blood flow and the eventual retinal hypoxia and capillary closure in diabetics is also tenuous. Nevertheless, the potential development of a specific enzyme inhibitor with minimal short-term toxicity in vivo opens up many possibilities for future, longer term study.

But the pathway from discovery to application in patients can be long and tortuous. Before the new inhibitor can be used therapeutically, some hurdles must be overcome. First is the issue of toxicity. There are many isoforms of PKC, and it must be determined that the new drug does not produce toxic side effects by its action on other forms of PKC. In addition, the β_2 isoform is heavily expressed in the central nervous system (CNS) (*3*). Because the inhibitor reduces retinal PKC activity to a value below normal, it is possible that the normal CNS function of PKC β might be impaired by the inhibitor and that such effects might be difficult to discern or require a longer time frame to be recognized. Second, the drug may not inhibit all of the complications of diabetes. PKC and its endogenous activator, diacylglycerol (DAG), are decreased, not increased, in peripheral nerve from diabetic animal models or after hyperglycemic exposure of normal tissues in vitro (*8*). The specific isoform affected is not yet known, but PKC activity may not be equally critical in all diabetic complications. Complete treatment of diabetic complications may therefore require additional drugs. Finally, other metabolic pathways that have been implicated in the toxic effects of hyperglycemia need to be considered, such as activation of the aldose-reductase pathway (*8*) or enhanced protein glycosylation (*9*) (see the figure). Both of these pathways contribute to potentially harmful effects of hyperglycemia that can be reversed by inhibitors of these pathways. Aldose reductase inhibitors have been under clinical testing for 20 years (*10*), but they remain to be proven effective enough to merit Food and Drug Administration approval (*11*). A potent inhibitor of protein glycation is also under clinical trial after a similar report of its efficacy in a diabetic animal model, published in *Science* 10 years ago (*12*), but its promise similarly awaits fulfillment.

Glucose is a molecule essential for life (particularly for function of the nervous system), but its concentration must be carefully controlled because of the powerful adverse effects of both too much and too little glucose. The manifestations of this toxicity are legion, and maintaining the concentration of glucose within a narrow window is extraordinarily difficult for many individuals (diabetes affects approximately 5% of adults). Alternate approaches, such as those based on the new information reported by Ishii *et al.*, are to be applauded, but the obstacles to the complete understanding of glucose toxicity and its prevention are formidable.

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Speciation in Action

Jerry Coyne

In trying to understand the two great engines of biological diversity—adaptation and speciation—evolutionary biologists occupy an uncomfortable niche. Blessed with the immense challenge of reconstructing and understanding the evolution of organisms, we are cursed by the historical aspect of this enterprise, which regularly denies us the crucial experiments and observations to test our theories. All too often our questions are addressed rather than answered, and plausibility arguments must do in place of facts. Reconstructing the past is of course a perfectly valid way of doing science—if it were not, cosmologists and geologists would be out of business. Nevertheless, evolutionists often suffer from "molecular biology envy," the fear that we are not as scientifically rigorous as our colleagues down the hall with their big grants and decisive experiments.

To allay this insecurity, we search for evolution in real time and treasure our examples of adaptation in action, such as the case of melanism in the pepper moth that graces every general biology text. It is much rarer, however, to see speciation in action, because that process requires not just evolution at a single locus (as in the moth) but more extensive genetic divergence that makes populations reproductively incompatible. In a report in this issue of *Science*, Rieseberg and co-workers (*1*) have reproduced in the greenhouse the genetic changes leading to the formation of a naturally occurring species of sunflower and have shown that these changes are repeatable across independent experiments. This unique study bridges the gap between the experimental and historical aspects of our field.

The species in question is *Helianthus anomalus*, an outcrossing diploid restricted to swales and sand dunes in Arizona and Utah. (This species has long provided the Hopi Indians with food and pigment for facial decoration.) Molecular evidence (*2*) indicates that *H. anomalus* arose by recombinational speciation, a process in which two distinct species hybridize, and the mixed genome of the hybrid becomes a third species that is reproductively isolated from its ancestors.

The putative ancestors of *H. anomalus* are *H. annuus* and *H. petiolaris* (see figure), which occur widely in the western United

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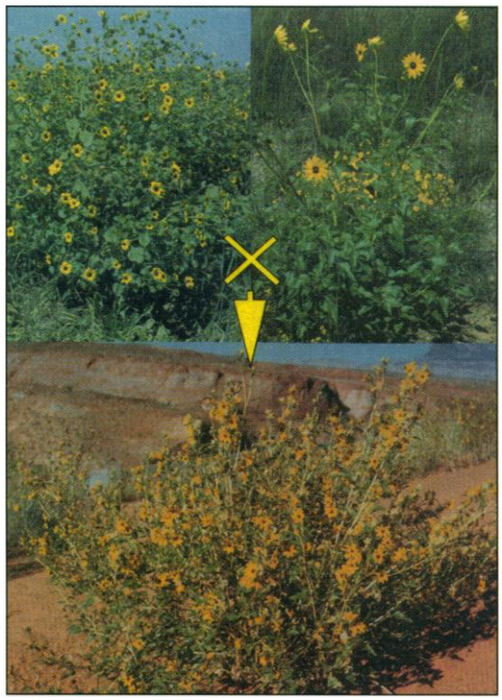
States and often form hybrid swarms. These offspring are largely sterile, because the species differ by fixed chromosome arrangements that cause meiotic difficulties in the heterozygous hybrids. Over several generations, however, these arrangements can sort themselves out into a new genome that is perfectly compatible with others of its type but incompatible with the genomes of its ancestors. In *H. anomalus*, this chromosomal sorting was supplemented by the fixation of new rearrangements as well as more conventional genetic changes, some of which must involve adaptation to its peculiar habitat.

In the present experiment, Rieseberg and co-workers reconstructed the first steps in the origin of *H. anomalus* by hybridizing *H. annuus* and *H. petiolaris* and then producing three independent hybrid lines undergoing different regimes of sibmating and backcrossing to *H. annuus*. After five generations, plants in these lines were assayed with 197 randomly amplified polymorphic DNA (RAPD) markers to determine which combination of ancestral genes persisted in the hybrids. Remarkably, despite the different crossing schemes, plants in all three lines converged to nearly identical gene combinations. Many of the segments that were lost were, of course, the rearranged regions of the *H. petiolaris* genome that are sterile when heterozygous. However, parallel changes also occurred in the non-rearranged portions of chromosomes, representing fitness effects of genes themselves. Statistical analysis gave the unexpected result that some combinations of *H. petiolaris* genes were actually favorable in the hybrid background deriving largely from *H. annuus*. Although negative interactions are expected among genes from reproductively isolated species, the basis for positive interactions is not yet clear. What is clear is that a complex network of genetic interactions was involved in this example of microevolution, and that despite this complexity the path of evolutionary change was repeatable. The authors may eventually be able to use such an analysis to map the "sterility genes" themselves, data that are now available from only a handful of *Drosophila* species.

Even more remarkably, the authors found that the gene combinations evolving in the experimental lines were similar to those seen in the naturally occurring *H. anomalus*, which probably arose more than 100,000 years ago. This similarity between artificial and natural evolution probably indicates that many genes were selected more for their effects on fertility than for their adaptations to a xeric habitat.



Making a new species. The two parental species of sunflower (*Helianthus annuus*, upper left, and *H. petiolarus*, upper right) and their hybrid derivative species (*H. anomalus*, lower). [Photographs by L. Rieseberg and G. Seiler]

It is intriguing that selection after hybridization has operated to favor the incorporation of genes from another species. This finding should not, however, be taken as support for the oft-heard view that plant hybridization is itself an adaptive phenomenon, selecting for a propensity to mate with members of other species. If this were generally true, we would see a profusion of hybrid swarms instead of distinct plant species. Moreover, many plant hybrid zones are limited in extent, implying that the hybridization is often deleterious.

Much has recently been made of the unpredictability of evolution and the effect of historical contingency on the origin of species. Gould, for example, has repeatedly asserted that "rewinding the tape of life" would always produce a different array of evolutionary end products (3). In *Helianthus*, however, the sequence of evolutionary change is largely repeatable over both the long and short term: When this tape is rewound, it plays pretty much the same program.

Recombinational speciation is, of course, only one of several paths leading to the formation of a new species. Hybridizing plants can also form new species by allopolyploidy, a process that eliminates the semisterility of hybrids by increasing chromosome number and is probably responsible for the formation of many new species of ferns and angiosperms. This process has also been duplicated in the laboratory (4), although it involves only changes in the level of ploidy and not in the sequences of genes. There is also the "classical" mechanism of speciation in which geographically isolated populations diverge genetically, but this process has not been duplicated experimentally. It is not yet clear whether recombinational speciation is as frequent as these other processes because, as in *H. anomalous*, it can usually be detected only by tedious molecular study.

The findings of Reiseberg *et al.* are also important as an example of how work on speciation has been revitalized by reductionist approaches that use both molecular tools and population-genetics theory. Early evolutionists like Dobzhansky and Muller (5) made a good start in understanding speciation through classical genetic analysis, but this approach was sidetracked when evolutionary geneticists discovered the joys of gel electrophoresis and DNA sequencing. The study of speciation then experienced a decline, its progress checked by a surfeit of verbal theorizing and a paucity of experimentation.

We have recently realized, however, that the molecular variants that were once the sole objects of study can be used as markers of the genome to attack many older questions about speciation, including estimating the number of genes involved in reproductive isolation (6), understanding how they interact (7), and determining how these interactions produce evolutionary patterns that hold across many taxa (8). These approaches are bolstered by new and explicit theoretical models showing how genetic change and interaction can produce reproductive isolation among populations (9).

Some researchers have insisted that speciation is an emergent macroevolutionary phenomenon that is refractory to the tools of evolutionary genetics and requires us to consider concepts like self-organization, hierarchical selection, and the emergent properties of organic matter. Such nebulous holism has, however, led nowhere. It is likely that painstaking studies such as that of Rieseberg and his colleagues will be far more useful in understanding the origin of species.

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