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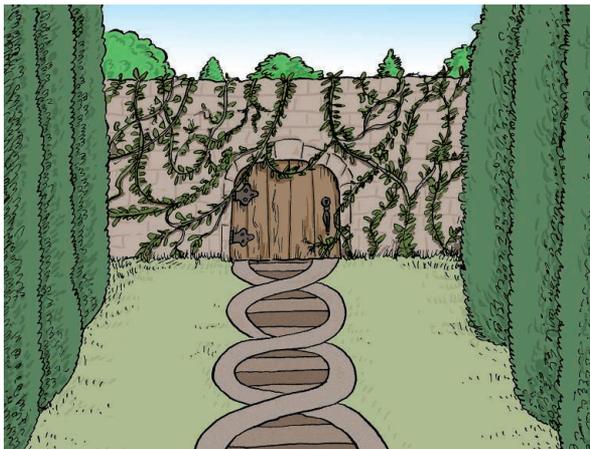
The Secret Garden—Epigenetic Alleles Underlie Complex Traits

Robert J. Schmitz

Numerous studies are investigating the basis of complex traits in a wide range of species (1). However, largely absent are efforts aimed at examining the possible contribution of natural epigenetic variation to heritable phenotypic diversity. Epigenetics is the study of heritable phenotypic variation that is not due to changes in the DNA sequence. Epigenetic variation is often overlooked because most populations used to analyze the basis of complex traits contain abundant DNA sequence variation—the major driver of phenotypic variation—and disentangling epigenetic variants from these sequence variants is a challenging task (2). On page 1145 of this issue, Cortijo *et al.* (3) demonstrate that epialleles (epigenetic alleles; alleles with the same DNA sequence but different DNA methylation patterns) are associated with heritable variation for two complex traits in the plant *Arabidopsis thaliana*. These results provide strong evidence that epialleles contribute to the heritability of complex traits and therefore provide a substrate on which Darwinian evolution may act.

Cortijo *et al.* analyzed a unique population of experimentally generated epigenetic recombinant inbred lines (epiRILs) derived from a cross between a mutant defective in DNA methylation, *ddm1* (*decreased in dna methylation 1*) (4), and its respective wild-type plant. In contrast to the widespread, albeit incomplete (5), erasure and reprogramming that happens in animals, DNA methylation is inherited across generations in plant genomes. Thus, mosaic epigenomes that are homozygous for methylation states originating from either the wild-type parent or the mutant parent could be generated: Only plants containing the wild-type *DDM1* allele were retained for further analysis (6).

Previous analyses of epiRIL populations have revealed widespread morphological variation for traits such as plant height, biomass, fruit size, number of fruits, time to flowering, germination rates, and response



to bacterial infection (6–8). Furthermore, the heritability associated with the phenotypic variance of these traits is comparable to the heritability estimates found in natural accessions of *A. thaliana* (7), suggesting that perturbation of DNA methylomes can generate heritable phenotypic diversity similar to that observed in the wild.

Whether perturbation-induced epiallelic states [observed as differentially methylated regions (DMRs)] in the epiRILs are stable enough to become targets of either artificial or natural selection has been unclear. The authors previously used the methylation states of DMRs that segregate in a Mendelian manner as physical markers to generate a genetic map (9). That such a map can even be made indicates that many of the newly induced methylation states are stable, at least over the number of generations used to create the epiRIL population. Cortijo *et al.* successfully used this genetic map for linkage mapping of two highly heritable complex traits: time to flowering (FT) and primary root length (RL). The linkage analysis revealed three quantitative trait loci (QTLs) for FT and three different QTLs for RL, and the combined additive effects of these QTLs explained large proportions of total phenotypic variation associated with either FT or RL. Therefore, this study clearly establishes that many of the epialleles derived from the creation of the epiRIL population are stable over at least eight generations and that these epialleles affect observed phenotypic variation. Identification of the causal epiallelic variants underlying each QTL is the

In the plant *Arabidopsis thaliana*, epigenetic variation, like genetic change, is potentially a substrate for Darwinian evolution.

next major challenge to demonstrate that epialleles contribute to the phenotypic variation observed in the epiRIL population.

Cortijo *et al.* relied on experimentally induced epiallele formation in artificial populations. Presently, this is the only method capable of disentangling the link between natural genetic and epigenetic variation. It raises the question, however, of whether these results are applicable to populations of *A. thaliana* accessions found in the wild. To address this limitation, Cortijo *et al.* compared the DMRs present in the epiRIL population with DMRs identified

from whole-genome bisulfite sequencing of 138 natural accessions of *A. thaliana* (10) and discovered that ~30% of the DMRs in their data set are also present in the natural accessions. This overlap between experimentally induced and naturally occurring epialleles suggests that these loci are highly relevant and could contribute to at least some proportion of the often discussed “missing heritability” of complex traits (11, 12).

Cortijo *et al.* also uncovered a sizable number of allelic variants in natural populations that are silenced by DNA methylation. The method used to create the epiRIL population led to the reactivation of some of these alleles, which resulted in heritable phenotypic variation. The appreciable overlap between experimentally induced and naturally occurring epialleles could result from natural loss-of-function mutations of *ddm1* in the wild. There is also evidence in *A. thaliana* for spontaneous epiallele formation between generations (13, 14), some of which are recurrent, in the absence of *ddm1* mutations.

The detected epialleles from all of these populations, whether experimental or natural, arose in the absence of experimental environmental perturbation. The possibility of environmentally induced epiallele formation and its role in local adaptation to changing environments has generated considerable interest, but there is currently limited evidence to support the existence of these environmentally induced transgenerationally stable epialleles. An important next step will be to determine if the environment can

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affect the rate at which these spontaneous epialleles appear.

Cortijo *et al.* demonstrate that heritable phenotypic variation can be achieved by perturbing DNA methylomes and reactivating silenced DNA sequences. The implications for crop systems are exciting, suggesting that heritable phenotypic variation can be rapidly generated that is linked with previously unstudied natural genetic variants. Developing techniques to precisely engineer DNA methylation sites without affecting the underlying DNA sequence will allow testing of

candidate epigenetic QTLs and examination of the potential to select for previously epigenetically silenced alleles in a wide range of plant populations. Future studies will determine the potential of these epialleles to rapidly generate heritable phenotypic variation upon which natural selection can act to alter the evolutionary trajectory of a species.

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CHEMISTRY

CO Meets CO, One at a Time

Miquel Salmeron

In his 1959 lecture, “There is plenty of room at the bottom” (1), Richard Feynman challenged scientists to build microscopes that could be used to manipulate atoms one by one. Twenty-five years later, the scanning tunneling microscope (STM) was invented (2), enabling individual atoms to be dragged to specific locations in a smooth surface (3). The atomic force microscope (AFM) (4) allowed even more sophisticated manipulations of atoms and molecules. At first sight, the importance of these feats may appear to be of an academic nature, but the wider implications in nanotechnology, which aims to manipulate matter at the atomic level to produce new materials, soon became clear. On page 1120 of this issue, Weymouth *et al.* (5) measure the forces between two single CO molecules, an example of the type of fundamental understanding that can be obtained with atomic force microscopy.

In an AFM, a sharp tip is mounted on a vibrating cantilever like that of the tuning fork used in the humble wrist watch. When the AFM is operated in noncontact mode (in which the sharp tip does not directly touch the surface), the last atom in its tip feels the tiny forces associated with attraction to and repulsion from other nearby atoms or molecules. These forces are the same as those between two atoms that are in the process of forming a chemical bond; their strength decays exponentially at distances comparable to the size of the atom.

With the noncontact AFM, one can obtain images of individual atoms and of atoms

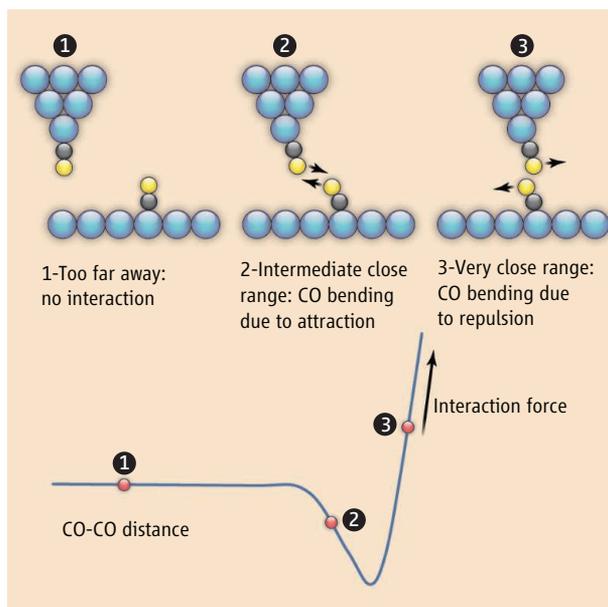
within molecules with picometer resolution (10^{-12} m). It can also distinguish the component atoms inside the molecules by mapping the electron clouds of the chemical bonds, as shown recently in detailed images of pentacene, a planar molecule with five benzene-like rings (6). In this work, Gross *et al.* followed Eigler’s pioneering work (7) by attaching an atom or molecule to the end of the tip; this atom or molecule is then used to image the surface. This tip engineering removes the uncertainty of the atomic structure of the tip apex, a problem that plagues more standard STM and AFM imaging.

An atomic force microscope is used to measure the molecular forces between two carbon monoxide (CO) molecules.

The high spatial resolution achieved in noncontact AFM results from the exquisite sensitivity of the vibration frequency of the quartz tuning fork, the same reason that quartz watches keep time so accurately. Tiny changes in this frequency due to atom-atom interactions can then be used to obtain atomically resolved images when the tip is rastered over a surface. More important, the measured frequency shifts can be used for a quantitative determination of the forces at play.

Weymouth *et al.* do precisely this. They measure the shifts in the tuning-fork frequency when a CO molecule attached to the tip end is brought close to another CO molecule adsorbed on the surface of a copper crystal. As the oxygen atoms of the molecules approach each other, they first attract and later repel when their electron clouds overlap too much (see the figure). From the frequency shifts, the authors obtain a map of interaction energy between the oxygen atoms in the molecules. The departure of the repulsive part of the force from the expected curve shows that at close contacts, the molecules bend to avoid each other (see the figure).

To investigate the stiffness of this bending motion in detail, the authors next measured the frequency shift when the tuning fork and its



Close approach. Weymouth *et al.* attached a CO molecule to the AFM tip and then measured the changes in interaction energy as the tip came near a surface-bound CO molecule.

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