it must have a somewhat different appearance and function. Since caterpillars are ectothermic, the major source of heat available to caterpillars is solar radiation. Therefore, evolving an insulation that reduces the rate of solar input would seem counterproductive. Also, since there is no large thermal gradient from one body region to another, thermal windows would appear to be useless to the caterpillar.

Nevertheless, the caterpillar setae serve as insulation by reducing the rate of convective heat exchange (Fig. 2A). Unlike insulation in endotherms, the capacity for radiant heat uptake is not affected by the setae. Therefore, a caterpillar having setae should absorb as much solar radiation as a smooth-skinned caterpillar of similar mass and body diameter, but should have lower rates of heat loss due to lower rates of convective heat exchange (Fig. 2D). Furthermore, the caterpillar can maintain a wide range of body temperatures simply by changing its orientation from parallel to perpendicular to solar radiation (Fig. 2C).

Why then, are not all terrestrial ectotherms "furry"? First, and perhaps most important, is the matter of size. The elongate shape of the caterpillar and its small body diameter relative to the size of the setae should maximize the effect of setae on heat exchange. For reptiles, which also control T_b by behavioral thermoregulation, the relative increase in body diameter caused by scales is small compared to the body diameter. Second, caterpillars are relatively sedentary and slow-moving, and therefore projections from the body surface should not interfere with movement. This may not be the case for relatively fast-moving terrestrial insects or reptiles. Other functions that setae perform in caterpillars might explain a selective advantage for their development before they had any significance in thermal balance. For example, setae are important for providing sensory information through tactile stimulation (*13*). In addition, since many setae are barbed (*7*), they serve as predator deterrents. Birds often refuse to feed on tent caterpillars (*Malacosoma disstria*), which have a similar distribution of setae as *L. dispar*, despite the fact that these caterpillars routinely feed in full sunlight and are more conspicuous than smooth-skinned caterpillars (*14*).

The contributing role of leaf boundary layers to convection needs to be assessed before a full understanding of steady-state T_b of caterpillars in the field is achieved. Nevertheless, regardless of their primary function and the selective pressures that caused them to arise, setae coupled with behavioral thermoregulation should be beneficial to caterpillars in maximizing T_b.

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Plasmid-Assisted Molecular Breeding: New Technique for Enhanced Biodegradation of Persistent Toxic Chemicals

Abstract. *The persistence of synthetic herbicides such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its release in massive amounts as a herbicide (Agent Orange) have created toxicological problems in many countries. In nature, 2,4,5-T is slowly degraded by cooxidation and is not utilized as a sole source of carbon and energy. The technique of plasmid-assisted molecular breeding has led to the development of bacterial strains capable of totally degrading 2,4,5-T by using it as their sole source of carbon at high concentrations (greater than 1 mg/ml). Spectrophotometry and gas chromatography reveal various intermediates during growth of the culture with 2,4,5-T.*

During the past several decades, the release of various synthetic chemicals—mostly chlorinated aromatics—into the environment has resulted in serious environmental pollution (*1*). The problem is not only the toxicity of the chemicals, but their persistence, so that they ultimately contaminate human bodies (*2*). An example of such a toxic chemical is the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which is often suspected to exert genotoxic effects; in particular, it is suspected of causing certain birth malformations in humans (*3*). Natural microflora degrade 2,4,5-T very slowly by cooxidative metabolism (*4*). Bacteria metabolizing 2,4,5-T and other recalcitrant compounds do not increase in cell number and seldom incorporate the carbon derived from the cooxidative metabolism of these compounds into their cell mass (*5*). The persistence of these compounds is therefore due to an inability of natural microbial flora to degrade them totally in order to derive their carbon and energy from the process.

We have recently reported the occurrence of plasmids that specify total degradation of chlorinated aromatic compounds such as 3-chloro- or 4-chlorobenzoic acid (*6*). We have demonstrated that the plasmid pAC25, which encodes a complete 3-chlorobenzoate degradative pathway, does not allow the host cells to utilize 4-chlorobenzoate; however, introduction of the TOL plasmid (specifying xylene and toluene degradation) into such a cell provides a broad substrate-specific benzoate oxygenase, which helps in the conversion of 4-chlorobenzoate to 4-chlorocatechol (*7*). 4-Chlorocatechol can then be completely metabolized by the pAC25-specified enzymes. Similarly, introduction of the TOL plasmid into the cells harboring the pAC25 plasmid and continued selection on 3,5-dichlorobenzoate allow the emergence of cells that can also utilize 3,5-dichloro-

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benzoate (*6*). The interaction of these two plasmids in extending the substrate ranges of the pAC25-coded enzymes is an example of the participation of various plasmids in the total degradation of xenobiotic compounds.

Various degradative plasmids not only demonstrate considerable homology with one another, but also with antibiotic-resistance plasmids belonging to the same incompatibility group (*8*). Degradative plasmids appear to evolve by recruitment of various genes from other plasmids (*9*). In order to see how much homology pAC25 has with other degradative plasmids such as TOL and SAL (specifying salicylate degradation) or with antibiotic-resistance plasmids such as pAC30 (specifying resistance to tetracycline, carbenicillin, and streptomycin), we have hybridized ^{32}P-labeled nick-translated pAC25 DNA with Eco RI restriction fragments of SAL, TOL, and pAC30 plasmids. The results clearly demonstrate that pAC25 has considerable homology not only with SAL and TOL, but also to some extent with pAC30 (Fig. 1). There is one band, common to SAL, TOL, and pAC30, that hybridizes with ^{32}P-pAC25 DNA, perhaps indicating an evolutionary relationship among these plasmids.

If plasmids evolve in nature by recruitment of genes from other plasmids and interact among themselves to extend the substrate range of novel xenobiotic compounds, it may be possible to help the evolution of new degradative plasmids in the laboratory under strong selective conditions where a particular persistent xenobiotic compound is the only carbon source available. A case in point is the degradation of chlorinated phenoxyacetic acids, which have been widely used as herbicides. Although several types of plasmids appear to have evolved for the degradation of 2,4-dichlorophenoxyacetic acid (2,4-D), continued searches for the isolation of microorganisms capable of utilizing 2,4,5-T as a sole carbon source have been unsuccessful (*4*, *5*, *10*). Our hypothesis is that because of the low concentration of 2,4,5-T in the environment (usually 10 to 500 parts per million in contaminated areas), the microorganisms have no need to evolve a 2,4,5-T degradative pathway. In addition, while such concentrations of 2,4,5-T may be toxic toward human beings, they cause little toxicity toward microorganisms.

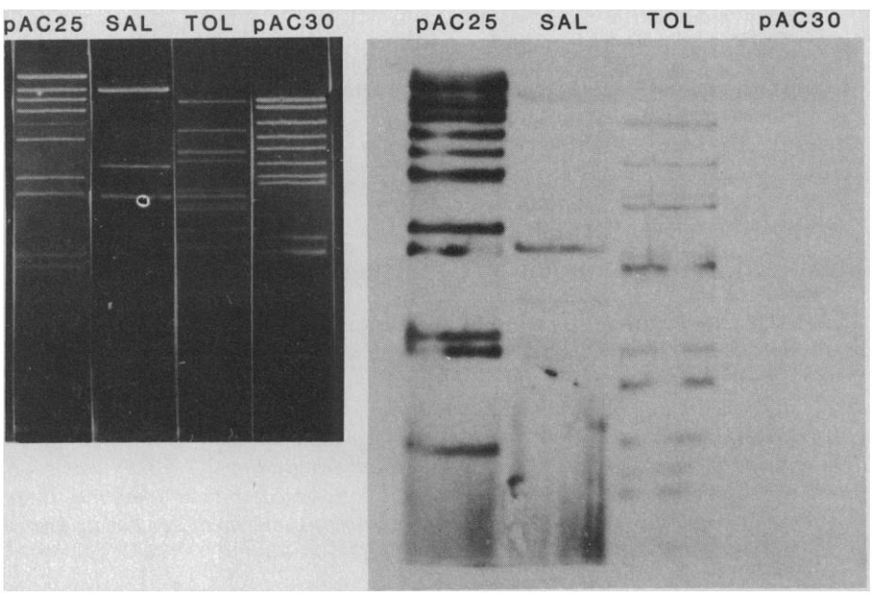

Fig. 1. Genetic homologies among pAC25, SAL, TOL, and pAC30 DNA's as determined by restriction hybridization. (Left) Separation of Eco RI restriction fragments for these plasmids. (Right) Extent of restriction hybridization, with ^{32}P-labeled pAC25 DNA as a probe. One band is common to SAL, TOL, and pAC30.

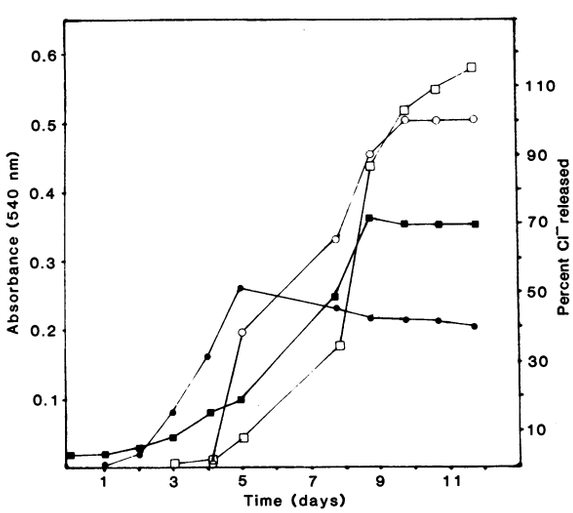

Fig. 2. Growth kinetics of the mixed culture on 2,4,5-T as a sole carbon source at 500 and 800 μg/ml. Chloride release is expressed as a percentage of total available chlorine. (■) Growth and (□) chloride release with 2,4,5-T at 800 μg/ml; (●) growth and (○) chloride release with 2,4,5-T at 500 μg/ml.

In order to breed microorganisms capable of utilizing 2,4,5-T as sole source of carbon and energy, we inoculated into a chemostat microorganisms from various waste-dumping sites (Love Canal, New York; Eglin Air Force Base, Florida; and an Arkansas dump site), along with microorganisms harboring a variety of plasmids such as CAM, TOL, SAL, pAC21, and pAC25 (*6*, *9*) to provide appropriate genes for the evolution of a degradative pathway. The chemostat was started with low concentrations of 2,4,5-T (50 μg/ml) and higher concentrations of plasmid substrates (toluate, salicylate, or chlorobenzoate at 250 μg/ml). Microorganisms from waste-dumping sites were inoculated separately into enrichment flasks with 2,4,5-T as the sole carbon source to determine whether they already harbored cells capable of growing with 2,4,5-T. Even on prolonged incubation in the enrichment flasks, we have not detected any culture from waste-dumping sites that can utilize 2,4,5-T. In some cases, increasing the 2,4,5-T concentration in the chemostat led to a washout of the culture. Under such conditions, the cultures were allowed to grow with the lower 2,4,5-T concentration as a batch culture, and then 2,4,5-T concentration was increased gradually. After 8 to 10 months of cultivation under these conditions, the chemostat started to develop higher turbidity and a brownish coloration, with 2,4,5-T (500 μg/ml) as a sole source of carbon. Streaking of the culture in nutrient agar plates demonstrated the presence of several types of bacterial colonies. Continued subculturing of this population in flasks with higher concentrations of 2,4,5-T demonstrated the ability of this population to utilize 2,4,5-T at 1.5

to 2.0 mg/ml (Fig. 2). At 500 μg/ml, the maximum growth is attained in 5 days. At 800 μg/ml, there is a lag of about 5 days, after which rapid growth ensues. About 50 to 70 percent of the chlorine is released during this period, and the rest is released in another 3 or 4 days. Continued subculturing also greatly shortens the growth lag, so that the microorganisms can utilize more than 70 percent of the 2,4,5,-T (1.5 mg/ml) in about 7 days. Incubation of the resting 2,4,5-T–grown cells with 2,4,5-T and subsequent ultraviolet and gas chromatographic examination of the supernatant fluids has demonstrated a gradual loss of 2,4,5-T with appearance of newer peaks on the gas chromatograms and shifts in the absorption maxima. More than 68 percent of chloride release occurs in about 11 hours under the incubation conditions with resting cells.

Our data demonstrate the possibility of breeding, in the laboratory, specific cultures that can utilize a persistent compound such as 2,4,5-T as a sole source of carbon. Continued subculturing with higher concentrations of 2,4,5-T has resulted in a significant reduction of the growth lag (maximal growth in 5 days with 2,4,5-T at 1.5 mg/ml), and a reduction in the number of colony types (three or four, as compared to seven or eight initially). Continued subculturing may allow the emergence of a single strain, perhaps with a 2,4,5-T–degradative plasmid.

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Extrinsic Microbial Degradation of the Alligator Eggshell

Abstract. *The outer, densely calcified layer of the alligator eggshell shows progressive crystal dissolution, with the production of concentrically stepped erosion craters, as incubation progresses. This dissolution is caused by the acidic metabolic by-products of nest bacteria. Extrinsic degradation serves to gradually increase the porosity and decrease the strength of the eggshell.*

Calculations to determine the gas and water conductances of any calcified vertebrate eggshell have been based on the assumption that the porosity of the latter remains constant throughout incubation (*1*). However, in considering animals *(Alligator mississippiensis)* with a long incubation period (65 days), it is difficult to equate this concept with the need to prevent dehydration of the recently laid egg and to provide efficient respiratory gas exchange for the late embryo. Moreover, if alligator eggs are artificially incubated without nesting media—as in experimental embryological investigations (*2*)—the embryos develop fairly normally but fail to hatch because of an abnormally tough eggshell. In order to investigate these phenomena, I studied the structure and chemical composition of the alligator eggshell in 396 specimens removed from eggs at daily intervals throughout the 65-day incubation period (*3*).

The alligator eggshell consists, from the surface inward, of an outer densely calcified zone (about 100 to 200 μm thick), a honeycomb zone (about 300 to 400 μm thick), and a mammillary zone (about 20 to 30 μm thick) to which is attached the eggshell membrane (about 150 to 250 μm thick) (*3*). The entire eggshell is composed of small rhombohedral crystals of calcite (Fig. 1A) interspersed with a variable amount of organic matrix. In the outer densely calcified zone there is no detectable organic matrix, and the calcite crystals are regularly stacked in vertical layers on their *a* faces (Fig. 1, A and C to J; Figs. 3 and 4) with their *c* axes at right angles to a tangent to the shell surface at any point (Fig. 1, C to J; Figs. 3 and 4). In contrast, in the honeycomb zone there is a higher percentage of organic matrix, which creates a meshwork of vesicular holes between the calcite crystals. These holes serve as interconnections with the egg contents via spaces between the mammillae and pores in the eggshell membrane. Furthermore, in the honeycomb zone, the calcite crystals are regularly stacked in horizontal layers on their *b* faces (Fig. 1, A and C to J), with their *a* axes at right angles to a tangent to the shell surface at any point.

At the time of egg laying, numerous small surface defects are present in the outer densely calcified layer, where crystals have either failed to form or been dislodged as the eggs clink together when they are deposited in the nest one after the other. As incubation proceeds, these small defects increase in size and depth to produce erosion craters with characteristic stepped concentric rings (Figs. 2 and 3). Each of these rings is one crystal high (Figs. 3 and 4) and corresponds to a layer of calcite crystals in the outer densely calcified zone (Fig. 1, C to J; Figs. 3 and 4). Numerous microorganisms are present around the surface defects and erosion craters (Fig. 2). These microorganisms produce acidic metabolites as a fermentation product of the decaying nest vegetation; and these acids in combination with carbonic acid (generated by the hydration of expired carbon dioxide) dissolve the calcite crystals in the outer densely calcified layers, enlarging the surface defects into erosion craters (Figs. 2 and 3) in much the same way that acidogenic oral bacteria decalcify tooth enamel to produce dental caries (*4*). The method of formation of the erosion craters is shown in Fig. 1, C to J. The stepped pattern of concentric rings is caused by the regular stacking of the calcite crystals (Figs. 3 and 4) and their anisotropic properties; that is, the *c* faces of the crystals dissolve faster than either the *a* or *b* faces (*5, 6*) (Fig. 1, C to J). The dissolution of the outer layers of calcite crystals is under kinetic control (Fig. 1J; Figs. 3 and 5) since the dissolved minerals can diffuse into the adjacent nesting media while the acid is replenished at the shell surface (Fig. 1J).

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