Examples are plasmids coding for bacteriocin production or specifying resistance to the host cell, plasmids may also contribute an advantage to part or all of a mixed bacterial population, since many code also for a transfer function that enables the plasmid genes to be passed between strains following cell-to-cell contact.

Recently a new class of plasmids coding for enzymes of catabolic pathways has been described in strains of the catabolically versatile saprophytic bacterium Pseudomonas. The pathways thus encoded are for the degradation of salicylate (Chakrabarty, 1972), camphor (Rheinwald et al., 1973), octane (Chakrabarty et al., 1973), naphthalene (Dunn & Gunsalus, 1973), and toluene and m- and p-xylene (Worsey & Williams, 1975) through benzoate and m- and p-toluates (methylbenzoates) respectively (Williams & Murray, 1974; Wong & Dunn, 1974). By the standards applied to plasmids in the Enterobacteriaceae the criteria for establishing the plasmid-coded nature of these pathways have been somewhat incomplete, namely (a) segregation of viable derivative strains that have lost the plasmid-coded metabolic activity either spontaneously or following the intervention of a curing agent, usually Mitomycin C, and (b) in most, but not all, cases the conjugal transfer of the metabolic activity to the cured derivatives or to other strains. However, more recently, strong confirmatory evidence has been obtained by the isolation of several of these plasmids and the demonstration that they exist as physically...

It is difficult to draw conclusions regarding the environmental significance of plasmid-borne catabolism from the limited data at present available. Certainly the range of organisms in which such plasmids have been isolated is limited, being restricted to either fluorescent *Pseudomonas putida* strains or to non-fluorescent *Pseudomonas* spp. virtually indistinguishable from *Ps. putida* in all nutritional characteristics. The extent to which interstrain conjugational transfer could occur is also difficult to assess. Transfer in the laboratory has been carried out, but all recipient strains have been *Pseudomonas* and the majority were members of the fluorescent species *Ps. putida*, *Ps. fluorescens* or *Ps. aeruginosa*. Whether intergeneric transfer can occur, as has been achieved under laboratory conditions with a drug resistance factor between *Ps. aeruginosa* and *E. coli* (Richmond & Sykes, 1972), or whether such plasmids can be found or exist within genera other than *Pseudomonas* has yet to be determined.

It is likely that a plasmid location for catabolic genes is of much more frequent occurrence than the limited number of reported instances might suggest. In a survey of strains independently isolated from nine different soil samples for their ability to grow on m-toluate, all of 13 strains tested appeared to carry the pathway genes on plasmids (Williams & Worsey, 1976) like the TOL plasmid in *Ps. putida* mt-2 (Worsey & Williams, 1975). Although superficially similar to *Ps. putida* mt-2 these organisms did not constitute a completely homogeneous group with respect to either their plasmid-coded catabolism or their transfer properties. Transmissibility varied considerably; some strains were able to transfer their plasmids into the majority of the other strains, whereas some of the strains appeared unable to transfer at detectable frequencies.

One mechanism by which non-transmissibility can arise has been demonstrated with the plasmid specifying octane degradation (Chakrabarty, 1973a). As isolated, this apparently exists as an aggregate including a non-transmissible plasmid, OCT, specifying the enzymes for octane degradation and a sex factor, K, responsible for its transfer. During curing these two elements can be lost independently thus giving rise to some derivatives that are octane degrading, but non-transmissible. The host cell may also be a factor in determining the transmissibility of a particular plasmid. Three of the isolates harbouring TOL plasmids were unable to transfer either their native plasmids or the otherwise transmissible TOL plasmid from *Ps. putida* mt-2 when it had been introduced by conjugation (Williams & Worsey, 1976).

As well as being a possible means by which genetic exchange may occur, plasmids may also provide a mechanism by which new combinations of genetic information can be achieved. Genetic recombination between plasmid and chromosome can occur since it has been shown that both the camphor degradative plasmid, CAM (Shaham et al., 1973) and the factor K found in association with plasmid OCT can co-transfer chromosomal markers. Recombination between plasmids will depend in part upon their ability to coexist within a bacterial cell, and such compatibility amongst the degradative plasmids has not been comprehensively tested. However, plasmids CAM and OCT appear to be incompatible, and the presence of one within a cell hinders the entry of the other, and yet they can become fused spontaneously or under artificial conditions to give a stable replicon (Chakrabarty, 1973b, Chou et al., 1974). Not only may this represent an important example of the kind of rearrangement that can occur in the environment, it may also be a means by which strains with degradative capabilities of importance in environmental pollution control might be constructed. Another spontaneous change that has been found involves the TOL plasmid from *Ps. putida* MT 20. Strains can be readily selected in which the plasmid appears to have lost part of its function involving both transmissibility and enzyme regulation, thereby losing the ability of the host cell to grow upon m-toluate, but not upon m-xylene, a precursor of m-toluate in the TOL plasmid specified pathway (M. J. Worsey & P. A. Williams, unpublished work). Even though this example represents some loss of function, it may have an adaptive advantage: the plasmid is retained in modified form for the utilization of m-xylene and toluene and yet benzoate, a poor growth substrate in the wild type, is degraded much more rapidly by
the chromosomal ω-oxoadipate pathway. This combination of metabolic possibilities is available to neither the wild type nor the cured strains.

The transmissibility of many plasmids obviously increases the opportunities for genetic rearrangements as outlined above, including recombination with other plasmids or with chromosomal material, and is one way strains may arise from mixed populations with new metabolic capabilities. There are at least two barriers to the spread of an evolving metabolic ability or its advantageous association with a pre-existing ability, both within and between species and genera of bacteria. The first is the transfer of genes between organisms, in which it is clear that plasmids could play a vital role. The second is the lack of genetic homology within the recipient organism that prevents recombination and thus the utilization of the transferred genes. The incorporation of genes in a plasmid could also facilitate the overcoming of this barrier.

Results such as those of Audus (1960) show three features characteristic of many studies on the degradation of xenobiotics: (a) a long lag phase of up to several months before any disappearance of the compound can be detected; (b) the difficulty of isolating pure cultures capable of the complete breakdown even after the onset of disappearance; (c) the inherent instability of the degradative ability in pure strains of competent bacteria, once isolated, when removed from selective media. One possible explanation is that the lag phase represents a period during which processes of plasmid exchange and recombination result in the selection of one or more organisms that finally have a battery of enzymes capable of the complete degradation. The instability of the competent isolates might therefore be a result of the responsible genes being located on an unstable and hence easily lost plasmid.

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Evolution of Catabolic Pathways

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Catabolic pathways are complex units of function mediated by specific sets of enzymes and, in some cases, transport systems. The pathways normally are integrated into cellular processes by control mechanisms that regulate the enzymes or transport systems by modulating the synthesis or the activity of the proteins. Thus the evolution of a physiologically effective catabolic pathway requires the acquisition of a full set of structural and