Medfly Transformed—Official!

Michael Ashburner

Transformation—the stable introduction of DNA into the germ line of a species—is an essential component of a researcher's armory. For over 10 years entomologists have looked with jealousy at *Drosophila* biologists (whom they do not regard as entomologists) for their ability to transform *Drosophila* at will. They—the entomologists—have rightly seen transformation as a necessary tool for both fundamental and applied research. In this issue of *Science* a team from the Institute of Molecular Biology and Biotechnology in Crete and the European Molecular Biology Laboratory in Heidelberg announce success in transforming *Ceratitis capitata*, the Mediterranean fruit fly (medfly) (*1*, *2*). Not only will this be good news to the inhabitants of Los Angeles, tired of being sprayed with insecticide, but it will also be a boost to the morale of those attempting to transform other insects of immediate importance to human welfare (not least the mosquitos) and to the many interested in basic aspects of insect science .

It would be hard to overestimate the effect of the announcement in the spring of 1982 by Rubin and Spradling of *P*-element–mediated transformation of *Drosophila* (*3*). The *P* element is a small transposable element that has invaded populations of *D. melanogaster* within the last 50 years or so. It encodes its own transposase and can hop around the *Drosophila* genome, often causing mutations, at a remarkably high frequency. Rubin and Spradling showed that if two *P* elements, each carried on a plasmid, were injected into *Drosophila* embryos then one—coding for an intact *P*-element transposase—catalyzed the genomic integration of the other, which could contain almost any piece of DNA the researcher wished. Within months of the announcement of this method, scores of laboratories had confirmed the technique. By now tens of thousands of independent transformations of *Drosophila* must have been achieved; the technique is robust. It is also very productive and has been used for gene cloning (by *P*-element tagging), for genetic mapping (both by chromosomal in situ localization of inserted *P* elements and by the imprecise excision of *P* elements to generate deletions), for gene replacement, for gene identification by the pattern of gene expression (with enhancer trap elements), to study the consequences of ectopic gene expression, to characterize promoter and enhancer elements of genes, and to ablate cells or organs (by the expression of a toxin gene carried by the inserted element).

It is not surprising, therefore, that others would like to be able to manipulate their own pets in the same way. In December 1985 a group of "experts" gathered in Vienna. Rather than search for the ghost of Harry Lime (or the grave of Mozart) in the St. Marxer Friedhof, they discussed the prospects for the transformation of the medfly. Why the medfly? A native of eastern Africa, this beautiful fly has invaded most of the warmer parts of the world, causing immense devastation to coffee and fruit crops. To see how serious a problem the medfly can be to the commercial fruit grower, try to carry a bag of oranges from Hawaii through customs into California (*4*). Worldwide, the economic cost of this insect is estimated in hundreds of millions of dollars.

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Making medflies. A facility in Metapa, Mexico, that can produce 600 million sterile medflies each week.

An effective method to control medfly is SIT (sterile insect technique); natural populations are flooded with large numbers of factory grown males, sterilized by irradiation. Large factories (see the figure) can make millions of sterile flies a day and these can be released from the air. This method has indeed eradicated the medfly from Mexico and northern Guatemala (*5*). But there is a problem: the factories produce equal numbers of males and females. Although sterile, the females are worse than useless; they cost millions of dollars to grow and they interfere with the mating between the sterile males and wild females (*6*). Worse still, they damage the fruit with their ovipositors, allowing infection of the fruit by bacteria and fungi. Eliminate the unwanted females from the production line? Easy, said the experts in Vienna, once you can transform this insect. As for that possibility: "The recommendations for research that we have made will, *if well executed*, provide a system for the genetic transformation of medfly within 1 to 2 years..." (emphasis mine). Of course the experts covered themselves (as experienced experts always do): "...*unless* *P*-element mediated transformation fails for unforeseen and fundamental biological reasons" [their emphasis]. It is almost 10 years to the day since I helped my distinguished colleagues draft that report (*6*). Meanwhile, those at the sharp end got fed up with waiting and have used classical genetics to construct strains that produce only males under factory conditions and have shown these to be better for control in the field (*7*).

We still do not understand the fundamental biological reasons for the failure of *P*-element transformation of medfly, or of any other non-drosophilid insect. But the reasons for the success of Savakis and colleagues are clear and deserve emphasis (*1*). To transform, three obstacles must be overcome: introducing the DNA into the germ line, recognizing transformants, and finding an efficient vector. The characteristics of the insect species will determine how to introduce the DNA. The simplest method (which works for the medfly) is injection of embryos; but for other species biolistics or infection with a retroviral vector (*8*) may be better. Early attempts at transformation of insects often used drug resistance as a marker. This may appear to be an attractive method, but the selection conditions are empirical and the false positive rate is high. Insecticide resistance [for example, to dieldrin or parathion (*9*)] genes may be better. But, best of all would be a mutant in the target insect that has a phenotype which can be complemented by a transformed gene. In a companion paper, Kafatos and colleagues cloned the *white* gene from the medfly (*2*). They showed that it worked by its ability to rescue a *white* mutation in *Drosophila*. Armed with this gene and a mutant white-eyed strain of the medfly, Savakis and colleagues had the ideal tools. Finally, the vector. *Drosophila* researchers now have, in addition to the *P* element, four other transformation vectors: *hobo*, *mariner*, *Minos*, and *Hermes*; at least eight

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elements of similar structure are known in *Drosophila*, but are untested. For some years it has seemed inconceivable that *Drosophila* is unique among insects in its ability to be transformed. One obvious rule is to choose an element absent from the target species; if the element is present, its transposition may be repressed. Savakis and colleagues chose the *Minos* element (*10*), which they had isolated from *D. hydei* and which had been shown to work in *D. melanogaster* (*11*). A similar strategy is being used by others; for example the *Hermes* element from the housefly will transform *Drosophila* (*12*) and is now being tested for the transformation of the Queensland fruit fly, *Bactrocera tryoni* (*13*). Many elements of similar structure, some known to be mobile, are being identified in a wide range of other insect species. Several, like *Minos*, are members of the Tc1 family of elements; others, such as *hobo* and *Hermes*, belong to the hAT family. Their characterization should now be seen as a top priority for research. The importance of using *Drosophila* as a test-bed, both for testing possible vectors and for testing possible marker genes, should not escape attention.

Will the successful transformation of the medfly result in better methods to control this pest? Readers should be wary of my predictive powers, but yes, in the long run. Certainly, it will allow us to learn much more about the basic biology of this beast. But the result has greater import; it should relieve the frustration of those trying to transform other insects; it should, in Voltaire's immortal words (writing, I admit, about the English habit of killing off the odd Admiral), be an example "pour encourager les autres."

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Ages of the Oldest Clusters and the Age of the Universe

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One of the most heated debates in the history of astronomy focuses on the numerical value of the Hubble parameter H_o. This parameter is of fundamental importance because it gives the scale-size of the universe and provides constraints on world models and the age of the universe. The most direct path to the determination of the extragalactic distance scale is through the Cepheids in the Virgo cluster. The relative merits of other techniques for determining H_o have recently been reviewed in great detail by Jacoby *et al.* (*1*), van den Bergh (*2*, *3*), and Kennicutt *et al.* (*4*).

Distance moduli of spirals in Virgo region

Galaxy	m_0	D (Mpc)	Telescope	Reference
NGC 4321 (M100)	31.00 ± 0.20	15.8	HST	Farrarese *et al.* (*9*)
NGC 4496	31.10 ± 0.15	16.6	HST	Saha *et al.* (*10*)
NGC 4536	31.05 ± 0.15	16.2	HST	Saha *et al.* (*10*)
NGC 4571	30.91 ± 0.15	15.2	CFHT	Pierce *et al.* (*11*)

HST, Hubble Space Telescope
CFHT, Canada-France-Hawaii Telescope

The table lists the true distance moduli μ_o, which is the apparent magnitude corrected for absorption that a star of absolute magnitude M = 0.0 would have, in four spiral galaxies in the Virgo region in which Cepheid variables have been observed so far. The distances of all four of these spirals are in excellent agreement. The data in this table yield a formal weighted mean distance modulus $\langle\mu_o\rangle$ = 31.02 ± 0.08 (mean error) for the Virgo cluster. To this quoted mean error should be added a 0.1-magnitude (mag) systematic uncertainty resulting from possible errors in the calibration of the zero-point of the Hubble Space Telescope (HST) photometry and an uncertainty of ~0.1 mag in the distance modulus of the Large Magellanic Cloud relative to which the Virgo distances were determined. In the subsequent discussion, it will be assumed that the true distance modulus of the Virgo cluster is μ_o(Virgo) = 31.02 ± 0.2 (D = 16.0 ± 1.5 Mpc). This distance modulus for four Virgo spiral galaxies is consistent with the value μ_o(Virgo) = 31.12 ± 0.26 that Whitmore *et al.* (*5*) have recently determined with HST by comparing the luminosity function of globular clusters in the Virgo elliptical galaxy M87 with that for globular clusters in the Milky Way system and in the nearby Andromeda galaxy. Because M87 lies at the center of the Virgo cluster, this observation appears to rule out the possibility that the spirals listed in the table lie a significant distance in front of the core of the Virgo cluster.

Tanvir *et al.* (*6*) have used HST observations of Cepheids in NGC 3368 to derive a distance modulus μ_o = 30.32 ± 0.16 for the Leo I cluster. In conjunction with a difference $\Delta\mu_o$ = 0.99 ± 0.15 between the distance moduli of the Virgo and Leo I clusters this yields μ_o(Virgo) = 31.31 ± 0.22. This indirect distance determination is also consistent with, but slightly larger than, the value μ_o(Virgo) = 31.02 ± 0.20 derived above from Cepheids observed in four Virgo spirals. It is concluded that the distance of the Virgo cluster is now well determined.

Because both the peculiar motion of the Virgo cluster and the magnitude of the retardation of the Local Group by the Virgo supercluster remain controversial, it is safest to determine the Hubble parameter from the Coma/Virgo distance ratio and the Coma velocity relative to the microwave background. The difference in the distance moduli of the Virgo and Coma clusters is well determined. From 12 concordant determinations, van den Bergh (*2*) finds $\Delta\mu_o$ = 3.71 ± 0.05. Adopting $\Delta\mu_o$ = 3.71 ± 0.05, in conjunction with a distance modulus μ_o(Virgo) = 31.02 ± 0.20, yields μ_o(Coma) = 34.75 ± 0.21, corresponding to a distance D(Coma) = 89 ± 9 Mpc. Durret *et al.* (*7*) found a mean redshift $\langle V\rangle$ = 6901 ± 72 km s^{-1} for the Coma cluster. With a correction of +258 ± 10 km s^{-1} to place Coma in the cosmic microwave background frame, this yields a true velocity V(Coma) = 7159 ± 73 km s^{-1}. From these values, one obtains H_o =

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