Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

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Discovery of the Transposable Element *Mariner*

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"THERE is nothing like looking, if you want to find something," said the enormously important dwarf, Thorin Oakenshield, to the young dwarves, "You certainly usually find something, if you look, but it is not always quite the something you were after" (TOLKIEN 1937). So with this quotation, mindful of its implied promise of unimagined possibilities and great adventure, did Millard Susman inspire students at the University of Wisconsin in Madison, where as a graduate student I took his course in microbial genetics in 1967. Thorin's sly invitation to explore the world for its own sake is an apt epigraph for this reminiscence, for it was by looking for something—and finding not quite the something we were after—that the transposable element *mariner* was discovered, exactly 20 years ago (HARTL *et al.* 1997a).

The key Drosophila mutant that led to the discovery emerged in a half-pint milk bottle inside a moving van, somewhere between West Lafayette, Indiana, and St. Louis, Missouri, in August of 1981. Everything in the Purdue laboratory, including the occasional dust bunny and 6000 individually wrapped half-pint glass milk bottles, had been carefully packed for the move to Washington University. Not wanting to lose time from experiments, we had set up crosses that could be trucked along as well, the progeny of which were to be examined immediately upon arrival.

Laurel Mapes found the original mutant. She was a recent graduate of Purdue who had found a summer job bartending and who, out of sheer love of genetics, had volunteered to work in the daytime hours without pay in the fly lab. She proved to be so sharp-eyed and enthusiastic that after 2 weeks I hired her as a full-time technician, and she gave up her nighttime job. Fortunately for me, she was willing to relocate to St. Louis, along with colleague Daniel E. Dykhuizen and graduate students David S. Haymer and James W. Jacobson.

The "something" that was found: At that time there was great interest in the possible role of transposable elements in species formation, occasioned by the discovery of a type of nonreciprocal hybrid sterility in *Drosophila melanogaster* (KIDWELL and KIDWELL 1975). This phenomenon was later called hybrid dysgenesis and was shown to be due to the mobilization of the transposable element *P* (BINGHAM *et al.* 1982). We decided to test the Kidwells' hypothesis by making reciprocal interspecific crosses between *D. simulans* and *D. mauritiana*, which yield fertile female hybrids; these hybrids were backcrossed to search among the progeny for new *X*-linked mutations resulting from transposable-element mobilization in the female parent.

The experimental crosses yielded no new mutations, but the control intraspecific crosses did. One of these was found in *D. mauritiana* and had peach-colored eyes. It later proved to be an allele of *white* and was named *white-peach* (w^{pch}). At the time, this and the few other new mutants seemed to be of secondary interest, but, rather than being discarded, they were added to the laboratory stock collection.

Genetic instability: Haymer was the first to notice that w^{pch} was unstable. Approximately 1 per 1000 progeny carried a mutant w^{pch} allele yielding either a wild-type or white-eye phenotype, indicating instability of w^{pch} in germline cells. Somatic instability could be detected directly, because many animals had a mosaic eye color consisting of one or more small patches of wild-type tissue appearing in an otherwise peach-colored eye (Figure 1A). He was at that time well launched on his thesis project comparing experimental measures of fitness, and he soon finished and went off to San Diego as a postdoctoral student but maintained an interest in the mutant (HAYMER and MARSH 1986). Jacobson also took an interest. In certain genetic backgrounds he had noticed the appearance of flies with lemon-colored eyes, but to his great surprise he discovered that they were double mutants of w^{pch} along with another *X*-linked eye-

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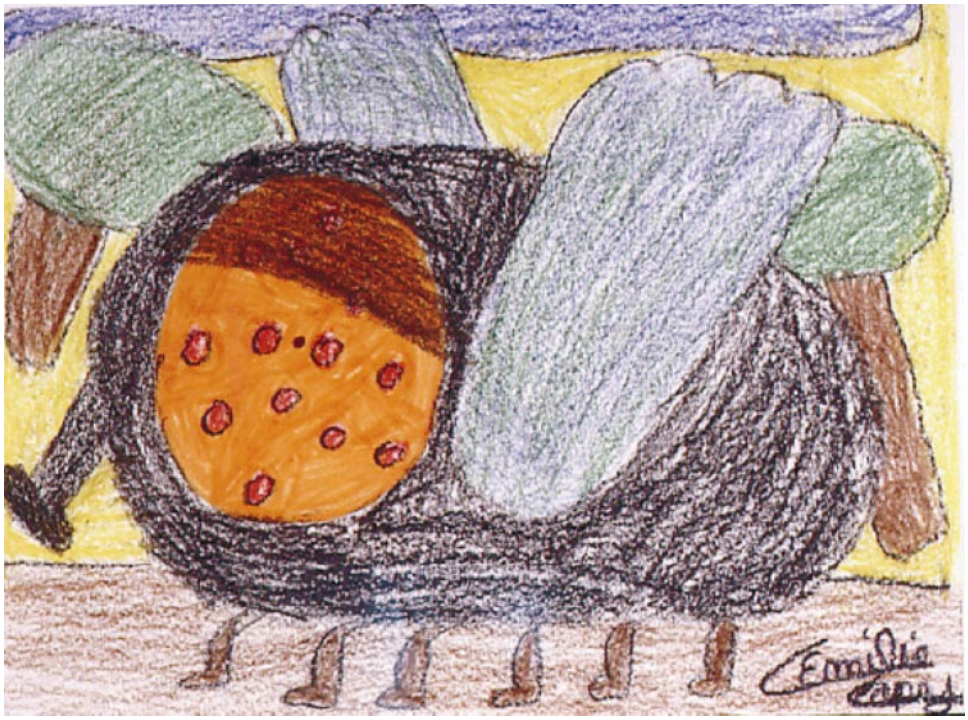

The mosaic eye color associated with active *mariner* elements was a captivating phenotype for everyone who passed through the fly lab, including Emilie Capy, who was 8 years old at the time. Note the mosaic red spots on the peach-colored background. (Crayola on paper, 1991.)

color mutation, which by itself yielded a plum-colored eye, located 40 or more map units from *white* (Jacobson and Hartl 1985). Later studies showed that the second locus is orthologous to *garnet* in *D. melanogaster*. Although *garnet* is a hotspot of *mariner* insertion in at least some strains of *D. mauritiana*, it does not appear to be in *D. melanogaster*.

While the genetic studies were proceeding, Jacobson cloned the w^{bch} allele to identify the molecular basis of the mutation, using a *white* probe from *D. melanogaster* (Bingham *et al.* 1981). It was already evident that w^{bch} was probably due to the insertion of a transposable element, but the situation seemed especially interesting owing to the instability in somatic cells as well as in germline cells. The element proved to be a 1286-bp sequence, terminated by 28-bp imperfect inverted repeats with four mismatches and including one long open reading frame encoding a putative polypeptide of 345 amino acids. The insertion was at position 7555/7556 in *white*, numbering as in the current version of FlyBase, 131 bp upstream from the transcription start site, flanked by a TA duplication, and so oriented as to be transcribed in the opposite direction from *white* (Jacobson *et al.* 1986). We did not know at the time that this element, later denoted the *peach* element, is a nonautonomous element incapable of catalyzing its own transposition. When the w^{bch} allele was later introduced into strains of either *D. simulans* (Capy *et al.* 1990) or *D. melanogaster* (Garza *et al.* 1991) lacking autonomous, transposase-producing copies of *mariner*, the w^{bch} allele was found to be completely stable with no evidence of either somatic excision (Figure 1B) or germline excision. Having cloned and characterized the transposon, Jacobson was invited to name it. He chose *mariner*—not, as usually conjectured, after Samuel Taylor Coleridge's 1798 poem, *The Rime of the Ancient Mariner*, although the eponymy is apt—but in honor of his newborn daughter, Marin.

Mechanism of transposition: We now know that *mariner* and Tc*1*, a transposon discovered in *Caenorhabditis elegans* at about the same time (Emmons *et al.* 1983), are eukaryotic members of a large superfamily of transposable elements whose transposase proteins contain a so-called D,D(35)E motif (Doak *et al.* 1994). (The number denotes the typical spacing between the second D and the final E residue.) This motif serves as a binding domain for a divalent cation (Mg^{2+} or Mn^{2+}) necessary for catalysis (Kulkosky *et al.* 1992; Mizuuchi 1992). The D,D(35)E superfamily includes such prokaryotic elements as the bacteriophage Mu, the transposon Tn*7*, and many bacterial insertion sequences, including the *Escherichia coli* elements IS*2*, IS*3*, IS*4*, and IS*30* (Doak *et al.* 1994). It is related to a still larger assemblage of sequences that includes human immunodeficiency virus (HIV) and the copia and gypsy families of retrotransposons having long terminal repeats (Capy *et al.* 1996).

Proteins with the D,D(35)E motif can create a single-strand scission in a duplex DNA molecule that exposes a reactive 3′ hydroxyl (Craig 1995). In *mariner*-like elements (MLEs) and Tc*1*-like elements (TLEs), the trans-

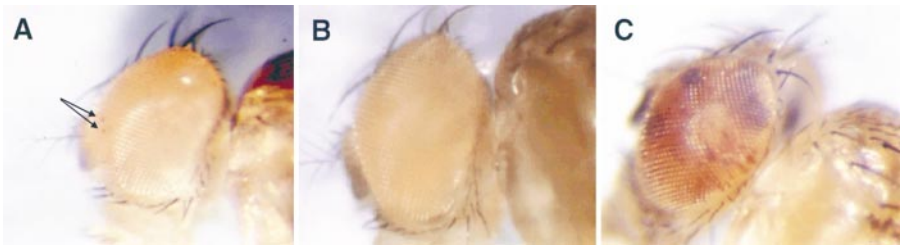

Figure 1.—(A) Typical low-level somatic mosaicism observed in the w^{pch} mutant of *D. mauritiana*; note the small pigmented spots (arrows). (B) The w^{pch} phenotype in the absence of autonomous *mariner* elements in the genetic background, in this case in a strain of *D. simulans*. (C) The heavily mosaic phenotype of w^{pch} in the presence of the *Mos1* mosaic factor.

position reaction is a cut-and-paste mechanism in which a staggered double-strand scission releases the entire element from the donor molecule prior to its being ligated into a staggered cut at the target site. The cut-and-paste mechanism, first established for Tn*7* (Craig 1989) and Tn*10* (Kleckner 1989), is also the mode of transposition of TLEs (van Luenen *et al.* 1994; Vos *et al.* 1996) and MLEs (Lampe *et al.* 1996, 1998). One diagnostic difference between MLEs and TLEs is that the cation-binding signature in TLEs actually has the formula D,D(34)E, whereas in the MLEs it is D,D(34)D. The difference turns out to be important, as demonstrated emphatically by the finding that a site-directed mutation that converts the *mariner* D,D(34)D into D,D(34)E results in a completely inactive transposase (Lohe *et al.* 1997). Genetic evidence based on abortive excision reactions suggests that strand scission in *mariner* occurs first at the junction of the 5′ inverted repeat and only later at the 3′ end (Lohe *et al.* 2000).

Finding an autonomous element: Most strains of *D. mauritiana* contain 10–20 copies of *mariner*, and when w^{pch} is present they yield a low level of somatic mosaicism like that shown in Figure 1A. Occasionally an animal that has exceptionally strong somatic mosaicism arises (Figure 1C), indicating the presence of one or more active autonomous elements that cause the *peach* element inserted in w^{pch} to undergo excision at a high rate (Bryan *et al.* 1987; Bryan and Hartl 1988). Although excision is imperfect, usually leaving a characteristic footprint consisting of the TA duplication and three nucleotides from either the 5′ inverted repeat (TACCATA) or the 3′ inverted repeat (TATGATA), the resulting *white* allele is functional and yields wild-type eye pigmentation (Bryan *et al.* 1990). The spontaneous origin of these active elements (called *Mos*, or *mosaic* elements, *Mos1* being the first discovered) is still unclear. They arise much too frequently to be accounted for by new nucleotide substitutions. One possibility is that they result from the escape of a preexisting element from some silencing mechanism (such as being embedded in heterochromatin); another is that they result from recombination or gene conversion. However they arise, once activated they remain active through successive generations (Bryan *et al.* 1987). The recovery of numerous new visible mutations due to *mariner* insertions in *Mos1*-containing strains demonstrated the utility of *mariner* for transposon tagging (Bryan *et al.* 1990).

Molecular isolation and analysis of the *Mos1* element showed that it differed from *peach* in 11 nucleotide sites, including 4 amino replacements, 5 substitutions at either synonymous or noncoding sites, and 2 single-nucleotide indels (Medhora *et al.* 1988, 1991). The transpositional inactivity of the *peach* element appears to be due primarily to an F344L amino acid replacement at the penultimate position in the polypeptide chain (Maruyama *et al.* 1991; Medhora *et al.* 1991).

An important advance in studies of *mariner* was spearheaded by Dan Garza, who introduced both a *wpch* transgene and the *Mos1* element into *D. melanogaster*, thereby opening the door to genetic studies. The *wpch* transgene is a chimeric gene in which a *Bam*HI fragment containing the *peach* element from w^{pch} in *D. mauritiana* was used to replace a corresponding *Bam*HI fragment in the wild-type *D. melanogaster white* gene. Using *P*-element germline transformation, the chimeric gene became inserted into the *D. melanogaster X* chromosome at map position 27.0 (Garza *et al.* 1991). Phenotypically, the *wpch* transgene is indistinguishable from w^{pch} in *D. mauritiana*, and it is stable in the genetic background of *D. melanogaster* owing to the lack of autonomous *mariner* elements in this species (Maruyama and Hartl 1991b). Single *Mos1* elements were also introduced into *D. melanogaster*, but the copy number increased quite rapidly (Garza *et al.* 1991). This problem was later solved by generating immobile *Mos1* elements lacking the 5′ inverted repeat. Most of the genetic experiments with a transpositionally competent transposase used a transgene called *Mr182*, which is a *P[hsp70::Mos1, ry*$^{+}$*]-182* construct inserted in chromosome 2; the *hsp70::Mos1* sequence has a dual promoter in which the *heat shock 70* promoter (*hsp70*) is fused to the *Mos1* promoter at nucleotide position 58–59 (Lohe *et al.* 1995a). The dual promoter has high activity even in the absence of heat shock (Lohe *et al.* 1995a).

Horizontal transmission: Simultaneous with the genetic studies, Kyoko Maruyama began investigating the evolutionary biology of *mariner* in species related to *D. melanogaster* (Maruyama and Hartl 1991b). These studies led to a detailed analysis in the *D. melanogaster* species subgroup (Capy *et al.* 1992a) and more generally in the Drosophilidae (Brunet *et al.* 1994). In some species, such as *D. simulans*, autonomous *mariner* elements related to *Mos1* are found segregating in natural populations (Capy *et al.* 1990, 1992b; Giraud and Capy

1996). In other species, such as the sibling species *D. sechellia*, all copies are inactive, and in this case the insertion sites are fixed in the genome (Capy *et al.* 1991). In still other species, such as *D. teissieri*, characteristic deletions are found at high frequency (Maruyama and Hartl 1991b; Brunet *et al.* 1996).

One of the most interesting of Maruyama's findings was that *mariner* elements in the subgenus Zaprionus were very closely related to those in the *melanogaster* species subgroup, even though the relationship between the species themselves is very distant (Maruyama and Hartl 1991a). Horizontal transmission seemed the likely explanation, although such a hypothesis is difficult to prove on the basis of sequence similarity alone. The problem is that a sequence may become so constrained in evolution that similarity is maintained between species even while less constrained sequences diverge. In the case of *mariner* in Zaprionus, however, even synonymous codon positions were very similar. The issue was finally resolved in favor of horizontal transmission by showing that the molecular phylogeny of *alcohol dehydrogenase* among the species was incompatible with that of *mariner* elements (Maruyama and Hartl 1991a; Lawrence and Hartl 1992).

Shortly thereafter, evidence that MLEs are extremely widespread and seem to perpetuate themselves by horizontal transmission began to accumulate. The initial discovery was an MLE present in 1000 or more copies in the genome of the silk moth *Hyalophora cecropia* (Lidholm *et al.* 1991), which was only distantly related to those we had been studying in Drosophila. The breakthrough was Hugh Robertson's comparison of the fruit fly and silk moth sequences to design primers for the polymerase chain reaction that would amplify nucleotides 544–996 of both MLEs (Robertson 1993). About 15% of insect species were found to contain one or more subfamilies of diverse MLEs (Robertson 1993; Robertson and MacLeod 1993). In several cases, close sequence similarity between MLEs from distantly related species provided *prima facie* evidence for horizontal transmission (Robertson and MacLeod 1993; Robertson and Lampe 1995b). Owing in part to horizontal transmission (Kidwell 1993), MLEs are now known to be present in a wide range of eukaryotic genomes (Robertson 1995; Robertson and Lampe 1995a; Arkhipova and Meselson 2000), including plant genomes (Jarvik and Lark 1998) and the human genome (Augegouillou *et al.* 1995; Morgan 1995; Oosumi *et al.* 1995; Hartl 1996; Robertson *et al.* 1996; Smit and Riggs 1996).

Vertical inactivation: A mechanism of regulation? An unexpected finding is that the vast majority of naturally occurring MLEs are defective. Many are inactive because they contain multiple chain-termination, deletion, or frameshift mutations that disrupt the open reading frame (Robertson 1993; Robertson and MacLeod 1993). A surprisingly large number have an open reading frame with only missense replacements, but they produce a transpositionally inactive protein (Maruyama *et al.* 1991).

Why are most MLEs inactive? One possibility is mutation pressure: MLEs that are not selected for transposase function may accumulate mutations by chance alone. Another possibility is that transpositionally inactive elements are positively selected because they reduce the fitness cost of transpositions. MLEs are active in the soma as well as in the germline, and the presence of active elements in a genome is associated with reduced life span (Nikitin and Woodruff 1995). A third possibility is that some transpositionally inactive elements reduce the net transposase activity of active elements, a form of downregulation.

Downregulation of transposition by mutant transposase proteins was first observed in certain chemically induced mutations (Lohe and Hartl 1996a), including an E345K replacement at the carboxyl terminal. The E345K mutant transposase was inactive in transposition, but its presence in the genome resulted in partial inhibition of the activity of *Mos1* (Lohe *et al.* 1997). This was the first indication that mutant transposase proteins might play a role in the regulation of transposition, most likely through the formation of heteromultimers, with wild-type subunits having reduced activity (Lohe *et al.* 1996). The E345K replacement was of some interest because, as noted, the inactivating change in the *peach* element is F344L. Sure enough, when present in the genome with an autonomous *Mos1* element, the net level of transposition is decreased from that found in controls (De Aguiar and Hartl 1999). This effect had not been noticed previously because it had been swamped by the strong *hsp70::Mos1* promoter used in most experiments.

Transposition of *mariner* is regulated in other ways as well, including an unusual phenomenon in which increased production of wild-type transposase downregulates the net level of transposition (Lohe and Hartl 1996a; Hartl *et al.* 1997b,c). The molecular mechanism of this type of regulation is still unclear (Townsend and Hartl 2001).

***Mariner* as a transformation vector:** From the very beginning there has been great interest in *mariner* as a vector for germline transformation, which was further intensified by its seemingly unrestricted host range (Warren and Crampton 1994; Sentry and Kaiser 1995; O'brochta and Atkinson 1996; Ashburner *et al.* 1998; Plasterk *et al.* 1999). The *Mos1* element from *D. mauritiana* has been implemented as a transformation vector in *D. melanogaster* (Lidholm *et al.* 1993), *D. virilis* (Lohe and Hartl 1996b), and many other species of insects (Berghammer *et al.* 1999), including the housefly (Yoshiyama *et al.* 2000), silkworm (Wang *et al.* 2000), and mosquito (Coates *et al.* 1998). Applications of *Mos1 mariner* transformation have so far spanned the range of protozoans (Gueiros-Filho and Beverley 1997) to

vertebrates (Fadool *et al.* 1998). Another MLE, *Himar1*, derived from the hornfly *Haematobia irritans* (Robertson and Lampe 1995b), has been used for transformation in cells of bacteria (Pelicic *et al.* 2000) and archaea (Zhang *et al.* 2000).

Persistence and prevalence of *mariner*: Why is *mariner* so prevalent among organisms? At one level the answer is that the ability to be horizontally transmitted and to transpose in newly affected genomes more than compensates for any deleterious effect on fitness (Lohe *et al.* 1995b). At this level, *mariner* is an example of selfish DNA. But is it necessarily? Recent evidence indicates that ancient asexual lineages of bdelloid rotifers have *mariner* in their genomes, even though virtually all other types of transposons have been eliminated (Arkhipova and Meselson 2000). Does this result imply that *mariner* elements undergo such a high rate of horizontal transmission that they continually reinfect even asexual organisms? Or could it indicate that the presence of *mariner* can confer a selective advantage, at least in some kinds of organisms?

There is, indeed, nothing like looking, if you want to find something. And much more experimental looking will be required to find the practical limits of *mariner* as a transformation vector, to define its molecular mechanisms of self-regulation, to discover the unknown processes of horizontal dissemination, and to explore the possibility that the presence of *mariner* might be beneficial in some organisms.

I am grateful to numerous students, postdocs, and colleagues who worked on *mariner* in my laboratory, including (in alphabetical order) Isabel Beerman, Nathan Blow, Glenn Bryan, Pierre Capy, Dan De Aguiar, Dan Garza, David Haymer, Jim Jacobson, Akihiko Koga, Jeff Lawrence, Danne Lidholm, Allan Lohe, Elena Lozovsky, Laurel Mapes, Kyoko Maruyama, Meetha Medhora, Javare Nagaraju, Yunsun Nam, Dmitry Nurminsky, Dave Sullivan, Courtney Timmons, and Jeff Townsend. Many others, too numerous to name here, also contributed their ideas and comments. The work was made possible by grants from the National Institute of General Medical Sciences.

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