

## A REVIEW OF MARKING TECHNIQUES IN ARTHROPODS AND AN INTRODUCTION TO ELEMENTAL MARKING

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### ABSTRACT

Ecological studies often investigate questions regarding population dispersal, density, and longevity by using a capture-mark-release-recapture method or some variation of it. Marking has been an integral part of such studies and can be divided into six categories: coding, dyes, dusts, radioisotopes, genetic, and elemental enrichment. Elemental composition can also be included in a broad definition. These methods are reviewed here. This paper is an introduction to a conference that focused on elemental enrichment as a marking technique. Elemental marking is defined as the establishment of an elemental concentration or content, in an individual, significantly higher than that carried by the indigenous population. The origin of the technique, history, and numerous questions regarding its appropriate application are discussed. The topics covered range from movement and dispersion studies to the complexities of trophic interactions across species, such as host to parasite.

### INTRODUCTION AND DISCUSSION

Ecological studies with animals have often sought to answer questions regarding population dispersal, density, and longevity. Such studies frequently apply some variation of a capture-mark-release-recapture system to the population being studied (see reviews by Poole 1974, Southwood 1978, and Begon 1979). Arthropods have long been the subject of such endeavors. Studies using marking have investigated a wide range of subjects including individual flight, locomotion, habitat movement, reproduction, number of ecdyses, flight range, inter-niche movement, population density, age grouping, survivorship, dispersal, and migrations (Gangwere et al. 1964, Bartlett 1982). Though the ingenuity employed by investigators in devising marking systems is quite striking, it has been moderated by the recognition that certain criteria must be met for a marker to be useful. Markers should be easy to apply, require minimal manipulation, should be easily recognized, persist with certainty, and be without deleterious biological effects to the recipient (Gangwere et al. 1964, Bartlett 1982).

Based on the intrinsic nature of the marker itself, marking methods can be divided into six categories: external coding, dyes, dusts, radioisotopes, genetic, and elemental enrichment. A seventh category, elemental composition, can be included

as a "chemoprinting" method in a broad definition even though actual marking is not employed.

External coding is one of the earliest marking methods and ranges from simple marks to complex codes. It is usually restricted to one life stage, arthropods large enough to handle, and those with hard integuments. Many coding methods are confined to small rather than large population studies since they often require individuals to be handled. Coding schemes have used series of dots (Elkinton et al. 1987) or color patterns (Opp and Prokopy 1987) applied by dabbing or spraying paints, waxes, or inks. Mutilation is a second coding technique and examples include notches on the thorax (Gangwere et al. 1964), holes in elytra (Weseloh 1987), and wing amputation (Querci 1936). A third coding technique has been attachment of foreign objects (paper, wire, or thread) to body parts such as the thorax, leg, wing, or abdomen (Klock et al. 1953).

Dyes have been applied internally (Kay and Mottram 1986) as well as externally (Dudley and Searles 1923) and often have been used successfully to mark multiple life stages. Their use parallels the historic development of histologic stains that used both water and oil-soluble dyes. Some of the first marker dyes were natural organic dyestuffs as carmine; later use included synthetic organics as anilines (Van Leeuwen 1940). The use of fluorescent dyes followed (Pal 1947, Reeves et al. 1948). Dyes are much in use today. Calco Red<sup>tm</sup> is used in the USDA, APHIS, sterile pink bollworm release program (Stewart 1984, Graham and Mangum 1971, Hendricks 1971). A number of oil-soluble dyes have recently been applied to foliage by Bell (1988a, 1988b) in successful efforts to mark adult *Heliothis spp.* that fed on dyed foliage as larvae. All markers must be evaluated in respect to toxicity and retention (as discussed above, Gangwere et al. 1964), but this is especially true with dyes. Detection is usually made by visual means often aided with microscopy and by eluting with a solvent. Thus, detection is a more limiting parameter for this technique than persistence of the dye. Spectroscopy (UV-visible) could possibly be used to extend dye detection.

Dusts used as markers are almost always external and restricted to one life stage. The particle size is important in getting dusts to adhere (Stern and Mueller 1986). Adhesion compounds such as gum arabic are sometimes added to enhance adhesion (Zukel 1945). Dust types include chalks, metallics, fluorescents, and luminescents. Of these, fluorescent powders of micronized dusts (very small particles) are the most popular method in use and are detected with long wave ultraviolet light. They are not removed with water or alcohol and three to five colors of ten available are usually used. Self-marking devices for treating populations have been developed for both dyes (Joslyn and Fish 1986) and dusts (Hogsette 1983, Price and Slosser 1983). A recent innovation has been the aerial application of fluorescent dusts (Meek et al. 1987). One problem with dusts has been a difficulty in applying them to scaly lepidoptera and getting lasting adherence, but success with the technique has been reported by Raulston (1979). Also, disruption of normal flight activity is possible if fluorescent particles are coated too heavily on the integument of small or delicate arthropods such as mosquitoes or whiteflies.

Radioisotopes have proven useful as markers for single and multiple life stages and also for multigenerational studies. They are commonly used with laboratory-reared arthropods. There have been some studies with labeled plants or habitats. Radioisotopes have been applied internally in diet or holding media or by injection, and externally by topical application (O'Brien and Wolfe 1964). Attachment has also been used (e.g., iridium wire, Sanders and Baldwin 1969, Seely et al. 1985). The

history of radioisotopes as markers is interesting and shows a rapid increase in the range of isotopes used. Polonium (radium A) was used in 1935 to trace aphid saliva (Hamilton 1935), and in 1945 phosphorus<sup>32</sup> (P<sup>32</sup>) was used to trace mealybug saliva (Carter 1945). Three studies in 1949 used P<sup>32</sup> to mark mosquitoes (Bugher and Taylor 1949, Hassett and Jenkins 1949, Jenkins 1949), and the use of other isotopes "exploded" in the 1950's. By 1959, over 45 isotopes had been used for marking; today P<sup>32</sup> is still the most popular isotope used. The use of radioisotope markers produced profound changes in mark-release-recapture studies. Insect and other arthropods too small or difficult to use successfully with earlier techniques became tractable to study. This had an effect of opening marking studies to a wider group of investigators with more divergent interests and contributed to the diverse application of marking techniques discussed earlier. Radioisotope markers must be used with great care to insure the safety of personnel and avoid environmental contamination and these concerns pose the greatest drawback to the method (see Stimmann, this supplement).

Genetic markers, particularly eye or body color mutations, have been applied in marking studies for many years; and these markers are noted for the ease of detection since a visual inspection is usually all that is required (Bartlett 1967, see Bartlett 1982 for summary and review). Bartlett and Lingren (1984) studied population densities of the pink bollworm, *Pectinophora gossypiella* (Saunders) with a genetic marker "Sooty" that was successfully moved into larval F<sub>1</sub> populations. A description of genetic markers for a mosquito was reported by Craig and Hickey (1967), and an application of the technique was made by Hausermann et al. (1971). However, most studies have been limited to laboratory-reared colonies as source material. But as the 1950's were themselves marked by the wide use of radioisotopes, in the mid-1960's, geneticists turned to the use of allozymes (electromorphs) for marking or as investigative probes, and these techniques were extensively developed in the 1970's. Their primary use has been to trace population origins and relationships for studies in population ecology (Selander 1976, Pashley and Bush 1979, Stock 1979). Genetic markers require careful and extensive investigative and preparatory work before field experiments can be conducted with them. This has limited the number of species studied and field experiments conducted with this method.

For the sake of completeness, elemental composition is another related technique that should be mentioned also. This "chemoprinting" technique differs from elemental enrichment in that no marker is applied. The inherent chemical composition of the arthropod is related through cluster analysis to its site of origin or host. Its effectiveness has been limited, perhaps due to the assumption of proportional uptake of elements through the food chain, the need for high heterogeneity of elements among host resources (McLean and Laks 1985), the logistic difficulties associated with the need to analyze several elements, and the extensive statistical treatment needed to ascertain their distribution patterns. Nevertheless, a large body of literature has begun to accumulate that profiles the elemental composition of several arthropods (Bowden et al. 1979, McLean et al. 1979, Dempster et al. 1986, Sherlock et al. 1986). In some instances, analysis has been conducted to determine population origins or characteristics such as geographic sources of populations (Burns et al. 1985).

All of the methods discussed so far satisfy some of the criteria of a "good" marker but usually fail in some aspect relevant to field studies on populations. What was needed was a method that met the "good" marker criteria (Gangwere et al. 1964, Bartlett 1982) and additionally met needs to use field populations. Arthropod

ecologists recognized that populations from laboratory colonies usually did not have the same behavior when released in the field as native populations (see Stimmann, this supplement). Also, it was important that such a method, 1) could be used with native (wild) material without disturbing the members of that population, 2) did not have the safety concerns associated with radioisotopes, and 3) had a greater effectiveness and ease of use than that of elemental composition. This conference addressed the development and application of elemental marking as a technique that most often meets those needs.

Elemental marking is defined as the establishment of an elemental content or concentration significantly higher than that carried by the indigenous population through artificial introduction followed by natural movement (translocation and transfer). The marker has the dynamics associated with these elements in natural systems which can include toxicity at sufficiently high doses. Thus, its concentration is influenced by nutrient uptake, allocation, and utilization and has the potential for ecological transfer within and among trophic levels such as through life stages, generations, and between organisms [this definition contributed by the editors]. The use of trace elements as markers has been fairly easy to implement, and researchers have found ingenious methods to capitalize on the biological transfer of the marker in order to introduce and deliver it to the desired site.

Use of elemental markers has increased rapidly in the last 3 years. At the last four National Meetings of the Entomological Society of America, papers were presented on this subject in Sections C, (Ecology, Behavior and Bionomics) D, (Medical and Veterinary) and F, (Crop Protection). In entomology, the technique originated in the early 1960's with analysis by neutron activation (Jahn et al. 1966, see discussion of neutron activation by Akey and Burns, this supplement). The technique as most frequently applied today began in the early 1970's as a proposal in what is now a classic paper by Berry et al. (1972). The proposal developed from techniques that used rubidium as a surrogate for potassium in studies of potassium distribution and movement (see Stimmann, this supplement). Importantly, rubidium could be analyzed relatively easily by atomic absorption spectroscopy that used much simpler and more available instrumentation than did neutron activation. The use of rubidium as a marker was developed further in laboratory studies by M. W. Stimmann and coworkers (Stimmann et al. 1973, Stimmann 1974) and was moved from the laboratory to the field by Stimmann (1974) and Van Steenwyk et al. (1978a, 1978b). Virtually every recent paper published in this field cites work by the above groups of investigators. Simply put, in the above field studies, rubidium was applied as a spray on the target crop. Later, moths in, around, and away from the rubidium-treated crop, were trapped and analyzed for rubidium. If they were positive for rubidium at a statistically significant level above background (see statement on "uniformity", Fliesher et al., this supplement) it was evidence that they developed as larvae in the treated crop.

Rubidium, strontium, and cesium have been used most extensively as elemental markers associated with detection by atomic absorption spectroscopy (see Akey and Burns, this supplement, for a discussion of detection methods). The use of the technique has grown as investigators have seen the broad applications possible with it. Today it is being used to investigate movement and dispersal of numerous insects and is not restricted to particular orders or ecological niches. Also it is being used in increasingly diverse ways to study a myriad of problems beyond that of movement alone. The technique has proven to be a valuable tool for the study of population dynamics in respect to trophic interactions. For example, detection of marked eggs

that originate from marked mothers are multi-generational studies that yield information on oviposition as well as movement, survival, and dispersal (works of Hayes, and of Hopper, this supplement). Another example of trophic interaction is the use of the technique to detect parasites that have acquired an elemental marker from their insect host (work of Jackson, this supplement). Also, the technique has been used to study viruses in aphids, and blood meal host sources for mosquitoes (work of Kimsey et al. this supplement).

It has been used for basic as well as applied problems. Prior to the conference reported here, this topic had not yet had a public forum at any national meeting. The genesis of this conference began when we (the comoderators-editors) discovered we had each been asked by several investigators "How does one get started in this area?" or "Could a particular subject be investigated with this method?" Most other workers in this field that we talked to reported the same experience to us. Also, a number of questions were in need of discussion. Which markers are best to use?

What are the pros and cons of various detection instrumentation? How much marker is required to be detected before classifying an insect as marked? How do we achieve uniformity of statistical reporting so that the work of various investigators can be compared? How can one determine if the technique is applicable to a particular project? What are the costs? How much of a chemist does one need to be? What are ecological problems that may be tractable for study with this approach? What pitfalls should be avoided in experimental design, experimental work, and conclusions?

We have responded by organizing this conference to draw together what is known relative to the questions posed above, particularly in respect to the development and application of this relatively new technique. We have attempted to include all major areas of investigation and to have them presented by the principle workers, either as speakers or co-authors. The conference report begins with two papers that cover the history of the technique as presently most often used, and what has been done (works of Stimmann and of Van Steenwyk, this supplement) followed by a paper on the instrumentation and analytical methodologies (*i.e.*, the how-to-do-it work of Akey and Burns, this supplement). A series of papers follow that describe original research and the field application of labeling. The concluding paper deals with the complicated aspects of experimental design and data analysis (work of Hopper, this supplement).

The investigations in this field have been wide-ranging and reflect the diversity of the insects and the numerous ecological problems that can be studied with elemental markers. We believe that interest in this subject is keen and we are hopeful that this report will be useful and will stimulate additional research endeavors in this field.

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