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MARKING TECHNIQUES FOR RECOGNIZING INDIVIDUAL INSECTS

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The current revolution in ecological theory is based on the preeminence of individual reproductive success. Testing such theory requires that individuals be recognized and monitored as they play out their reproductive lives. Insects have the diversity, abundance, and accessibility that make them especially attractive as research subjects, but getting to know individuals is often difficult. Their small size, short lives, and great mobility complicate the task of the field observer. It is not surprising that lizards, ground squirrels, and hyenas are better known as individuals under field conditions than are their insect counterparts.

Southwood (1966, 1978) extensively reviewed the literature of insect marking methods, including those suited to identifying individuals. We will build on his contribution by listing and discussing techniques that are broadly applicable and by outlining the principles important in developing a marking method or in deciding which existing method to adopt.

Marking systems that permit marking 50 or more insects for individual recognition fall into three principal categories: (1) *Mutilation*—changing the insect itself, (2) *Labeling*—attaching a label to the insect, (3) *Direct marking*—using the insect as a blank label.

MUTILATION

The grosser forms of mutilation—such as amputation of all or parts of appendages or wings—should be avoided because they are likely to disrupt or alter behavior. Gangwere et al. (1964) found pronotal notching to be satisfactory for marking saltatorial Orthoptera and cockroaches and pointed out that the notches, unlike conventional marks, remained readable through one or more molts.

The hard, thick, often smooth cuticle of beetles provides a suitable substrate for scratching or burning coded spots or numbers. For example, Murdoch (1963) used a piece of safety razor blade to mark the elytra of carabids, and J. C. Schuster (pers. comm., 1974) used an insect pin to engrave numbers into passalid pronota.

The chief advantage to mutilation techniques is permanence; the chief disadvantage is damage to the insect or infection.

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LABELING

In this procedure a prepared label, often printed, is attached to the insect. In some cases the writing may be small enough and the label large enough to allow the insect to carry instructions for the finder and a return address. In an entomological equivalent of bird banding, Urquhart and Urquhart (e.g. 1978, 1979) labeled Monarch butterflies to learn their migration routes. The alar tags they used were 9x13 mm self-adhesive labels folded over the de-scaled costal margin of the forewing (Fig. 1A). The tags remained in place as the Monarchs traveled 1000's of kilometers on their way to overwintering sites. Few insects can carry a label as heavy as those used by the Urquharts. Roer (1957, 1969) devised a much lighter butterfly label, composed of two 6 mm discs glued together through a hole in the forewing (Fig. 1B). The upper disc was aluminum foil that glinted in the sun making it easier to spot marked individuals.

If a label has only a number on it, it can be very small and light-weight. German apiculturists developed plastic "sign-platelets" (*Zeichenplättehen*) to be glued to the pronota of queen bees making them easy to see and identify[1]. The 2 mm-diameter platelets, numbered 00-99, come in 5 colors and weigh only 1.3 mg each. They are suited to labeling a variety of insects (Fig. 1C) and are legible nearly a meter away. Gary (1971) added a disc of shim steel to each numbered plastic disc (total weight 11 mg) and retrieved the labels from returning honeybees with powerful magnets mounted at the hive entrance.

DIRECT MARKING

This is by far the largest and most varied set of techniques for marking for individual recognition. The variety is only partly a result of different

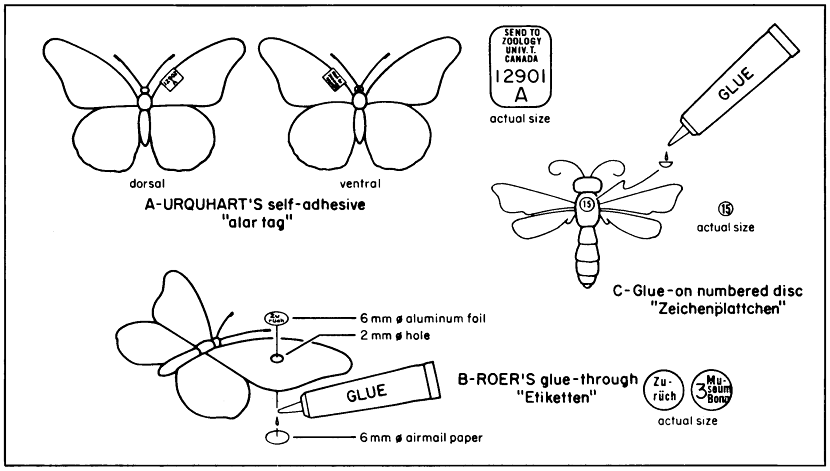

Fig. 1. Labeling techniques. Weights of labels are (A) 11-14 mg (including self-adhesive), (B) 1.2 mg (excluding glue), (C) 1.3 mg (excluding glue).

constraints imposed by different insect sizes, surfaces, habits, and habitats, and by different requirements of the researcher. Much of the variety results from scientists settling on the first technique they try that works fairly well for them. They are, after all, not concerned with developing optimal marking techniques but with learning something about "their" insects. If buying a can of paint at the variety store and applying it as coded dots with the head of an insect pin works, then no more time need be wasted. Southwood (1978) reviews a large number of *ad hoc* solutions to direct marking problems. We will develop below a framework for evaluating and improving direct marking methods.

Marking Materials

The variety of substances available to the researcher as potential marking materials continually increases. Generally, one or more of the first few materials a researcher tries will work, so the problem is not in finding a workable material but in failing to find an equally available material that is superior.

A perfect marking material would combine these properties (and all prospective marking materials should be evaluated in these respects):

Durable. The material must resist wear and abrasion for the duration of the study.

Adhesive. The material must not flake or chip from the insect. Some materials that are durable are prone to chip—for example, butyrate dope sold for painting model cars. If the entire mark[2] flakes off, the insect reverts to unmarked status; if part of a mark flakes off, the insect may be made the identical twin of another marked insect (Fig. 4C).

Non-toxic. Neither the material nor its solvent, in the amounts applied, should kill or permanently alter the behavior of the insect[3].

Easy to apply. Ease of application is a function of the applicator (see below) as well as the material and becomes more important as the number of insects to be marked and the number of persons doing the marking increases.

Quick-drying. Insects may need to be held after marking until the material can no longer smear or act as an adhesive for organic debris. Therefore, slow-drying materials are undesirable.

Light-weight. Some insects are so small (e.g. mosquitoes) that the weight of the mark may prove an important criterion.

Available in several easy-to-distinguish colors[4]. In many instances more than one color must be used in order to produce enough unique marks.

Invisible except to researcher. Although desirable so that predators will be no more or less likely to take marked insects than unmarked ones, this criterion is seldom met. Using pigments that can be seen only in the dark under UV illumination is generally impractical for studies of diurnal insects. For cave insects, or for nocturnal insects that stay in the dark during the day, pigment invisibility is of no consequence. Only for nocturnal insects that remain exposed during daylight is the use of UV-fluorescent materials worth considering[5].

Materials that have been successfully used to mark insects for individual recognition are *paints* (e.g. artist's oils, enamels, tempera, and those in Fig. 2—Tech-Pen "ink," acrylics, Liquid Paper), *lacquers* (e.g. nailpolish, bu-

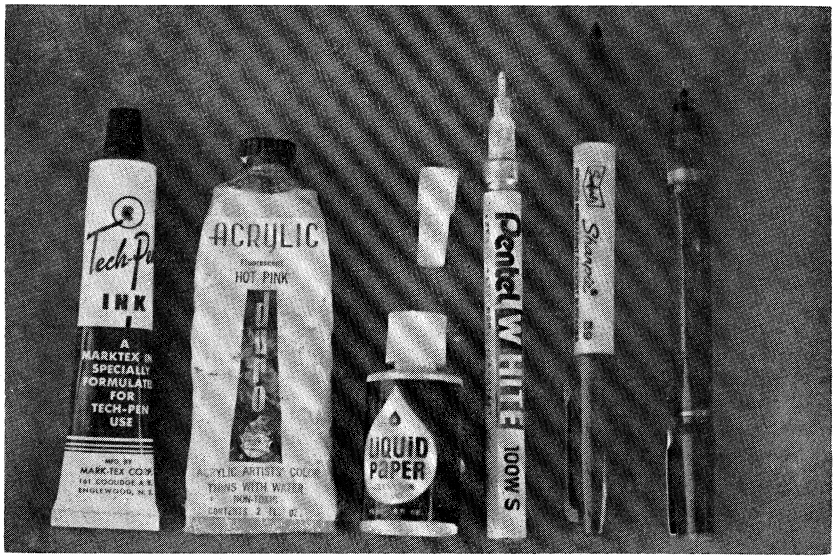

Fig. 2. Some especially useful marking materials (left to right) Tech-Pen "ink," developed for marking laboratory glassware, is a thick paint that comes in 11 colors[6]; acrylic paint with day-light fluorescent pigments[7] (acrylics can be thinned with water but are waterproof when dry); Liquid Paper© correction fluid comes in 9 colors[8]; Pentel pens deliver a fine line of opaque-white, oil-base ink that drys quickly[9]; Sharpie© marking pens have "fine" or "extra fine" points that deliver quick-drying, water-resistant inks (8 colors available)[10]; technical pens[11] filled with india ink produce uniform lines or dots that can be applied to transparent wings or blots of Liquid Paper (Fig. 3H).

tyrate dope, nitrocellulose lacquers), *inks* (e.g. stamp pad ink, india ink, permanent inks in marking pens as in Fig. 2), *copper wire*[13]. Most of these materials have been tried by 50 or more graduate students on 10 or more kinds of insects. Although all will serve at least to some extent on some insects, only a few mark a variety of insects well. We took the four materials with the highest student ratings and tested them on three difficult-to-mark species. Tech-Pen ink and Liquid Paper won the competition (Table 1). We are presently testing a much wider variety of materials in a similar fashion (Wineriter and Walker, in preparation).

Application Methods

Techniques for applying a mark may be as important as the choice of marking material.

Holding the insect firmly yet without injury during marking is often difficult. When fingers fail, devices employing netting or suction[14] can be used (Fig. 3A-D). Anesthetizing the insect should be avoided if at all possible because of potential effects on the physiology or behavior of the insect. Chilling the insect—for example, by placing the insect in a vial in an ice-filled vacuum jug—is apparently safer than using carbon dioxide[3] or ether.

TABLE 1. DURABILITY (IN WEEKS) OF FOUR HIGHLY RATED MARKING MATERIALS APPLIED AS A SPOT ON ONE QUADRANT OF THE PRONOTUM OF 20 INDIVIDUALS OF EACH OF THREE SPECIES SELECTED FOR DIFFICULTY OF MARKING: RED FLOUR BEETLE, *Tribolium castaneum* (SMALL SIZE), PASSALID BETTLE, *Odontotaenius disjunctus* (HARD, SMOOTH SURFACE), AND AMERICAN COCKROACH, *Periplaneta americana* (GREASY SURFACE).

	T. castaneum		*O. disjunctus*		*P. americana*	
Marking material*	first**	median†	first	median	first	median
Tech-Pen ink (orange)	3	6	7	13	5	8
Liquid Paper (white)	(could not apply)		3	4	6	12
Pentel pen	1	1	(would not adhere)		2	3
Hyplar acrylic‡ (lemon yellow)	1	1	1	1	1	1

*Application methods were as follows: Liquid Paper, in-bottle brush or bristle of brush; Tech-Pen ink and acrylic, bristle attached to swab stick (for *T. castaneum*) and splintered end of swab stick (for others); Pentel pen, point or portion of the point splayed to one side.

**Average no. of weeks before loss of *first* mark (4 replications of 5 individuals each).

†No. of weeks before loss of *median* mark by those individuals still living. For example, a value of 6 means that not until the sixth week did more than half of the surviving individuals, in the four replications combined, lose their mark. In no species were fewer than 15 individuals alive when the median mark was lost.

‡M. Grumbacker, Inc., New York, N.Y. 10001.

An important criterion in the choice of an applicator for a marking material is often the minimum size of spot it can make consistently. Therefore we have arranged the following list of examples from fine to coarse: single bristle, minutin pin, headless insect pin, technical pen (Fig. 2, 3H), shaft of pin (Fig. 3E), head of fine pin, grass stem, pine needle, syringe (Fig. 3F), paper clip (Fig. 3G), swab stick, marking pen (Fig. 2), rubber stamp (Fig. 3I).

With many marking materials, proper viscosity is important but difficult to maintain. If the material is too thin, it spreads out-of-control on the insect, interfering with sensory areas and increasing exposure to often-toxic solvents; if the material is too viscous, it adheres poorly to the insect and makes a high-profile spot or line, increasing the likelihood that the material will flake off. A paint pot, such as the one designed by W. D. Hamilton (Fig. 3E),

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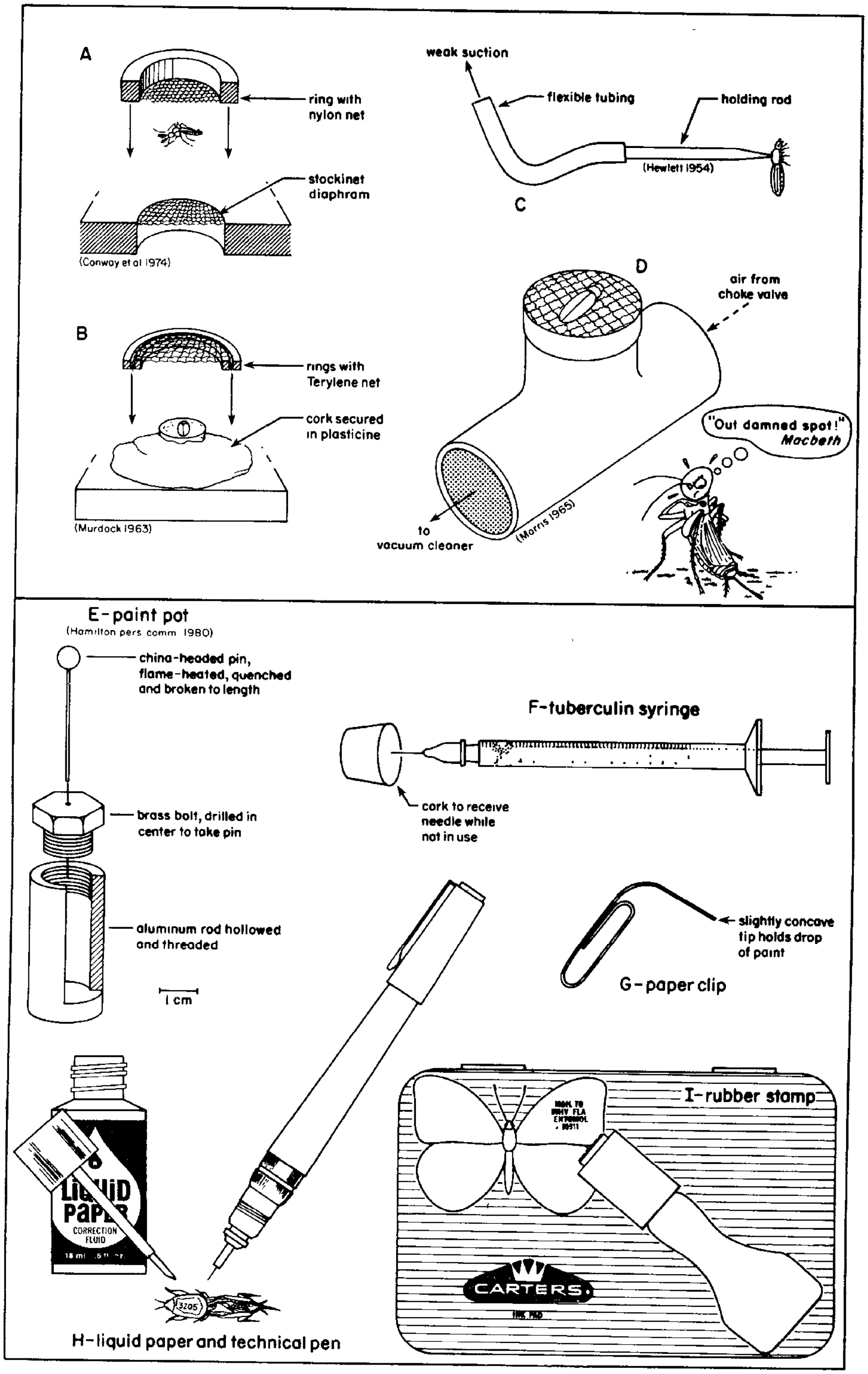
Fig. 3. A-D Holding techniques. A-B. Devices using netting (mark is applied through the mesh of the net). C-D. Devices using suction. E-G. Marking techniques[18]. E. Paint pot keeps paint from drying out and assures a standard amount of paint with each withdrawal of the pin (W. D. Hamilton, pers. comm., 1980). F. A small quantity of Tech-Pen ink[6] can be squeezed directly into the barrel of disposable plastic syringe with needle removed, the plunger reinserted forcing the paint to the needle-end of the syringe, and the needle replaced. So long as the ink is not allowed to dry and clog the needle, uniform droplets can be produced by gentle pressure on the plunger. Inserting the needle into a cork retards the drying[12]. G. Paper clip. H. Technical pen[11] used to write on spot of Liquid Paper[8]. I. Rubber stamp used to mark wing of cloudless sulphur butterfly with return address.


A
ring with nylon net
stockinet diaphram
(Conway et al 1974)
B
rings with Terylene net
cork secured in plasticine
(Murdock 1963)
weak suction
flexible tubing
holding rod
(Hewlett 1954)
C
D
air from choke valve
to vacuum cleaner
(Morris 1965)
"Out damned spot!" Macbeth
E-paint pot
(Hamilton pers comm 1980)
china-headed pin, flame-heated, quenched and broken to length
brass bolt, drilled in center to take pin
aluminum rod hollowed and threaded
1 cm
F-tuberculin syringe
cork to receive needle while not in use
slightly concave tip holds drop of paint
G-paper clip
I-rubber stamp
CARTERS
LIQUID PAPER
CORRECTION FLUID
3205
H-liquid paper and technical pen

or a syringe (Fig. 3F) can be used to maintain optimal viscosity during a marking period. (Hamilton's device also solves another problem—that of getting a uniform amount of material on the applicator.)

Coding Systems

In addition to selecting a marking material and a way to apply it, the researcher desiring to uniquely mark a large number of insects must decide upon a code.

These are four important standards a code should meet:

Enough unique marks[2]. A system adequate for identifying 1000 individuals must be more complex than one that permits identifying only 100 individuals. The coding system selected should be as simple as the study permits yet easily expandable (say by adding another color) if the study need be increased in scope.

Marks easy to apply. Application is facilitated by keeping the marks simple and by using only one color per individual. Two dots are generally easier to make than are one triangle or one star (for an example of overly complex marks, see White 1970). When more than one color is used in marking an individual, the process is seriously complicated by having to switch applicators.

Easy to read and verbalize. If the mark can be seen all at once and quickly translated into a few words (or a figure[15]), note-taking and record keeping are simplified (Fig. 4D).

Failsafe from misidentification. The most serious problem that can arise in a study requiring recognition of individuals is that one marked individual is mistaken for another. The most likely way for this to occur is through partial loss of a mark (Fig. 4C). If every mark has the same number of components, partial loss can generally be detected and misidentification avoided. For example, *364* ("three sixty-four") cannot become "thirty-six" through loss of the *4* if "thirty-six" is coded *036*. With systems using positioned dots, the same number of dots should be used for each individual (Fig. 4A,C) (Michener et al. 1955). A disadvantage of using complex symbols as components of marks, in addition to the difficulty of application, is that partial loss may transform the symbol: a star becomes a circle if it loses its points, and an *8* becomes a *3* if it loses its left side.

For large insects, coding systems present few problems. An arabic number (of constant number of digits) or even a return address (Fig. 3I) can be inscribed on a wing, elytron, or pronotum. If a less conspicuous mark is desired, enough easily identifiable positions are available to make positioned dots easily translated into arabic numbers. For example, the system in Fig. 4A permits marking 100 individuals per color by positioning one dot on each forewing. However, 20 identifiable positions are required. For insects having fewer positions for symbols, some other coding system must be used[17]. The use of symbols more complicated than dots or varying the numbers of symbols per mark violates important principles explained above. The two remaining ways of expanding a code of positioned spots are (1) using more colors and (2) allowing more than one color per mark[17] (Table 2). The former is limited by the number of colors that remain easily identifiable under field conditions[5]; the latter makes marking a more complicated process. Both are subject to error from the fact that pigments are apt to differ in

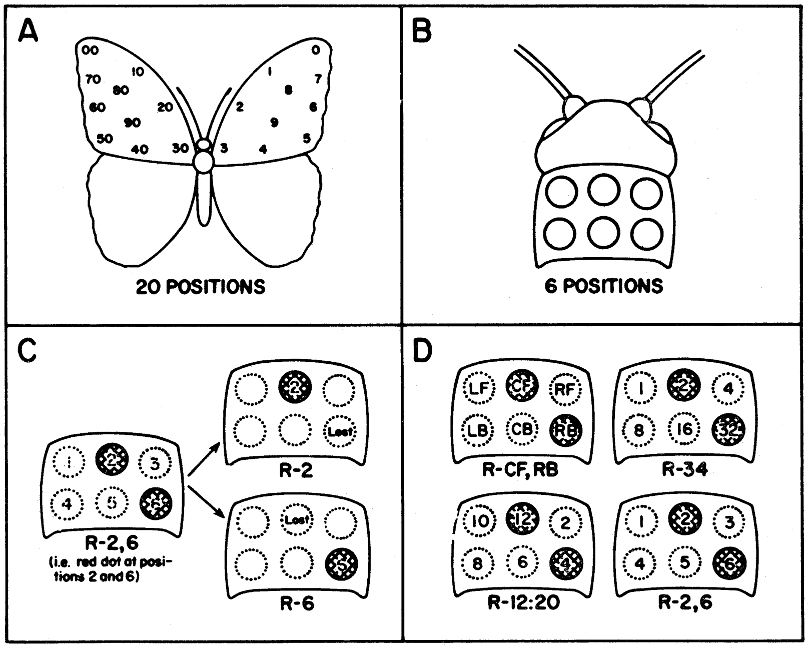


Fig. 4. Positioned dots. A-B. Effect of number of positions. A. Using two dots (one on each wing) 100 individuals can be marked distinctively for each color used. B. Using two dots per individual only 15 can be marked for each color used. C. Need for a constant number of dots per mark. Without this safeguard, loss of a dot can cause the researcher to misidentify the individual. (Individual "R-two-six" can become indistinguishable from "R-two" or "R-six.") D. Different systems for naming marked individuals. "R-CF,RB" (short for "red dot at center front and at right back") is unnecessarily cumbersome; "R-thirty-four" requires adding the values of the positions (e.g. Sheppard et al. 1969) and is of no benefit if a constant number of dots per individual is used; "R-twelve-twenty" depends on imagining the pronotum as the face of a clock with the first dot starting clockwise at "12" being the hour hand and the second dot being the minute hand (adopted from Jackson 1933); "R-two-six" is the easiest, most direct way to name the individual bearing the pronotum pictured[16].

their properties—especially in their likelihood of fading or flaking. For example, consider the effects on the results of a study when a color used early in a study adheres well, while another, used late, does not.

EPILOGUE

Hasty adoption of a less-than-optimal marking technique may seriously limit what can be learned from a study or even compromise its results. We have tried to identify some serious pitfalls and to give tips on how to avoid them.

TABLE 2. INCREASING THE CAPACITY OF A POSITIONED-DOT CODE BY (A) USING MORE THAN ONE COLOR AND (B) USING 1 OR 2 COLORS PER INDIVIDUAL[17]. *Premises*: 2 DOTS/INDIVIDUAL, 6 POSITIONS.

No. of colors used*	No. of unique marks 1 color/indiv. (A)	1 or 2 colors/indiv. (B)
1	15	—
2	30	60
4	60	240
8	120	960

*In many instances no more than 4 or 5 colors remain easily distinguishable under field conditions.

ACKNOWLEDGEMENTS

Persons who have generously shared their ideas relative to marking insects are too numerous for us to list here; furthermore, the ideas they shared were generally offered not as original but as useful methods they had learned in conversation or research with their colleagues, students, or professors[18]. Our greatest debt is to 240 graduate students who took Insect Ecology at University of Florida 1966-1980. Their enthusiasm and verve for marking arthropods of nearly every description seemed too valuable to seclude in the pages of a bulging loose-leaf compendium labeled "Insect-Ecology Marking Manual."

For special help with various aspects of the manuscript we thank J. E. Lloyd, R. C. Littell, Jane Brockmann, Frank Robinson, and Susan Jungreis.

Finally, we must thank T. R. E. Southwood, who published his extensive survey of marking methods (1966) and then updated it (1978). We, as others, have benefited greatly.

APPENDIX

[1] Zeichenplättehen are not available in the United States but can be obtained in Germany from apicultural suppliers—e.g. Hamman Bienenzuchtgeräte, Postfach 225, 6633 Hassloch. Smith (1972) described a technique developed by N. E. Gary for making numbered discs manually. Fresneau and Charpin (1977) described how to make similar labels photographically.

[2] Throughout this article we use *mark* to denote all marking material used to make one insect identifiable: a mark consists of one or more dots, figures, or characters.

[3] The possibility of subtle, chronic effects of chemicals is amply demonstrated by effects of CO_2 anesthetization (Edwards and Patton 1965).

[4] Colors that are easy to distinguish in large amounts in good light may be difficult or impossible to tell apart on the insect under field conditions. Some daylight fluorescent colors[5] become indistinguishable when fluorescing under UV.

[5] Fluorescence is the emission of light of some hue (i.e. a particular wavelength) upon absorption of radiation of shorter wavelengths. Ultraviolet (UV) radiation will cause numerous substances to fluoresce (e.g. calcium fluoride, zinc silicate) and these can be applied in amounts that are difficult to see in daylight but easy to detect under UV.

Daylight fluorescent materials (Day-Glo© Color Corp., 4732 St. Clair Ave., Cleveland, Ohio 44103) appear unusually bright in daylight by reflecting some wavelengths of visible light and transforming some of the absorbed wavelengths (via fluorescence) into emitted light of the same hue as that being reflected. These pigments, widely used in group-marking, can be added to various paint bases to produce colors that are conspicuous in daylight and brilliant under UV. *When dilute,* they are inconspicuous in daylight and bright under UV.

[6] Mark-Tex Corp., 161 Coolidge Ave., Englewood, N.J. 07631.

[7] The brand illustrated has been discontinued. Day-light fluorescent pigments[5] can be mixed with acrylic base to produce extra-bright colors.

[8] Liquid Paper Corp., 9130 Markville Drive, Dallas, Texas 75243.

[9] Pentel of America, 1100 Arthur Ave., Elk Grove Village, Ill. 60007.

[10] Sanford Corp., 2740 W. Washington Blvd., Bellwood, Ill. 60104.

[11] Pen shown is a size 00 Rapidograph©, Koh-I-Noor Rapidograph Inc., 100 North St., Bloomsbury, N.J. 08804.

[12] Another technique for keeping a marking syringe operable is to squeeze a drop onto the point after each marking. The drop dries but can be brushed off to open the needle prior to marking the next individual (J. E. Lloyd, pers. comm., 1980).

[13] Dave Synder (pers. comm., technique developed in Insect Ecology lab, spring 1975) and Mirenda and Vinson (1979) independently discovered that if small pieces of fine copper wire from a lamp cord are tied around various portions of an ant's anatomy, the ant is identifiable and neither it nor its nestmates can remove the mark—as they generally do for more conventional marking materials.

[14] Hand-held battery operated vacuum cleaners are available in increasing variety, making practical the use of suction devices in the field.

[15] A distinction must be made between oral and written note taking. It is easy to write *768*, but if one is talking into a tape recorder, the same mark becomes 'seven hundred sixty-eight" (or the shorter and more ambiguous "seven, six, eight").

[16] Yet another way to read the six positions illustrated is to picture them as three columns of two to be read from the cricket's left to right. If the anterior positions are *1*'s and the posterior positions are *2*'s, the marked cricket becomes "R-O,1,2." A cricket marked red in positions CF and CB would be read "R-O,12,2" (or "R-O,3,2") (J. E. Lloyd, pers. comm., 1980).

[17] The formula for calculating the number of unique marks (N) for a coding system using a fixed number of positions (n) and a fixed number of dots (of one color) per mark (k) is

$$N_{(n),k} = \frac{n!}{k!(n-k)!}$$

For example with 6 positions and 2 dots per mark, $N_{(6),2} = 15$; with 6 positions and 3 dots per mark, $N_{(6),3} = 20$.

If the number of dots per mark is allowed to vary, the number of unique marks is the sum of the N's for each number of dots permitted. For example, with 6 positions and 1, 2, or 3 dots (of one color) per mark,

$$N_{(6),1,2,3} = N_{(6),1} + N_{(6),2} + N_{(6),3} = 6 + 15 + 20 = 41.$$

If more than one color is used, but only one color is applied to each individual, the total number of unique marks for the coding system becomes

$$CN_{(n),k}$$

where C is the number of colors available. For examples, see Column (A) of Table 2.

If more than one color is used and one or more is applied to each individual, the total number of unique marks for the coding system becomes

$$C^kN_{(n),k}$$

For examples, see column (B) of Table 2.

[18]We realize that we cannot properly acknowledge the originators of most of the marking methods we describe. We not only are unable to back-track an idea from our informer to its source but we are also aware that good marking techniques may have more than one original source—they may have been independently invented two or more times (for example, copper bands for ants[13]). Nonetheless we wish to acknowledge *our* sources for these ideas: paper clips as applicators (Fig. 3G) (F. A. Lawson, pers. comm., 1980), Tech-Pen ink as a marking agent and tuberculin syringe as an applicator (Fig. 2 and 3F) (W. A. Banks, pers. comm., 1972) (see also Freeman 1964), pen-and-Liquid-Paper technique (Fig. 3H) (Alan Bolton, pers. comm., 1977, who learned the technique from Donald Windsor), rubber stamping addresses on butterfly wings (Fig. 3I) (Richard Mankin and Basilios Mazomenos, pers. comm., 1977) (see also Neilsen 1961). Those who know earlier sources for these ideas, especially if published, should send us the information if they wish that more proper credit be given in the future.

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