

## A novel technique for the individual marking of smaller insects

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### Introduction

Many ecological studies of insects depend on marking. Marks can be simple, i.e., to identify the released specimens in a mark–release–recapture experiment or individual if more detailed data is sought. Simple marks include ink and paint spots (Wineriter & Walker, 1984; Wojcik et al., 2000), dusting with fluorescent powder (Sinsko & Craig, 1979; Reinecke, 1990; Service, 1993), amputating part of a limb (Querci, 1936; Wesoloh, 1985), dyes (Steiner, 1965; Gast & Landin, 1966; Hendricks, 1971), isotope marking (Hagler & Jackson, 2001), and elemental marking (Hamann & Iwanek, 1979; Kipp & Lonergan, 1992).

Several methods have been described for the individual marking of larger insects. Marking insects by placing ink spots in different positions to produce codes has been used in many studies (Gangwere et al., 1964; White, 1970; Harman, 1975; Brenner & Patterson, 1988). Also, beetles have been individually marked by engraving codes onto the elytral intervals either manually with a small needle or with an industrial laser (Best et al., 1981; Unruh & Chauvin, 1993; Griffiths et al., 2001). Many of the methods to individually mark insects can produce a permanent code, however, the techniques are time consuming and expensive, especially the latter technique. Also, both of the above techniques can only be used on larger species, such as mid to large carabids. Smaller insects are more difficult to individually mark. This short communication describes a method for tagging insects, especially smaller species. Tags have been used in a wide variety of studies (Gary, 1971; Hagler & Jackson, 2001). Tags to individually mark honeybees (*Apis mellifera*) are commercially available, but they are too large for smaller insects (Hagler & Jackson, 2001). Small tags have been used to individually label screw-worms (*Cochliomyia hominivorax*) (Rubink, 1988), but the tags were relatively difficult to produce. The tags

described in this method are very simple to produce in large quantities. Tagging has been described as a tedious method of marking that is limited to larger insects (Hagler & Jackson, 2001), however, it is hoped that this method will counter those deficiencies.

### Materials and methods

Marking involved the use of a modified mechanical pencil (Pentel 120 A3 DX) that was to act as a borer. This was made possible by bevelling the edges of the tip of the pencil using a needle file to produce a cutting edge. This device was used to bore out round labels (0.9 mm diameter) from premium quality inkjet paper (Hewlett Packard, Palo Alto, USA) whilst the paper was resting on a clipboard. Codes were printed (Hewlett Packard 950C inkjet) on a sheet of paper and the labels were bored from that sheet. The code was composed of a letter and a number (font size 2) enabling 260 permutations, however this could be increased by using different coloured papers or characters other than the letters in the alphabet. The labels were backed with double-sided tape (Selloptape, Dunsatble, UK), which was found to be very strongly adhesive. The backing of the tape was removed before attempting to stick it to the insect by teasing it from the partially depressed tip of the pencil. The label could be stuck to the mid-portion of the pronotum by depressing the lead in the mechanical pencil. The beetle could be held, carefully, by its legs in the thumb and forefinger. For field studies the label was coated with a small drop of nail varnish using a very fine brush after it had been applied to the insect. The code on the label could be read using a hand lens (Specwell, Japan) with four times magnification.

The technique was used on the captive reared adults of *Cryptocephalus coryli* (Coleoptera: Chrysomelidae) to assess the durability of the tags in the wild. Two releases were conducted at Whisby Nature Reserve, where no wild *C. coryli* have ever been recorded. On 13 May 2001, 69 (21 males and 48 females) tagged beetles were released and 37 (15 males

and 22 females) tagged beetles were released on 4 July 2001. After each release the site was visited as regularly as possible up to 48 days after the release in the first instance and 31 days in the second. Adults were located by visually inspecting trees in and around the release area. The mean eclosion age of the adults used in release 1 was 9.5 days (SD 6.5 days) females and 12 days (SD 7.5 days) males. The mean eclosion age of the adults used in release 2 was 7.4 days (SD 4.2 days) females and 6.8 days (SD 3.6 days) males.

## Results

After release 1, 13 (62%) of the individually marked adult males were recaptured at least once in the subsequent visits to the release site. Thirty-seven (77%) of the females in this first release were recaptured at least once. A greater proportion of males (67%) than females (55%) were recaptured at least once after release 2.

The tags were found to be very durable. Only one male (4%) and one female (2%) of the individuals recaptured were found to have lost their tags. The last recaptured males ( $n = 2$ ) were found 20 days after their release with their tags still in place. Tagged females were recaptured 23 ( $n = 1$ ), 25 ( $n = 1$ ), 29 ( $n = 1$ ), and 48 ( $n = 1$ ) days after release (54 days post eclosion in the latter case) with their tags still easily readable. Four females from release 2 were recaptured 24 days after release with the individually coded tags still in place and readable. The last tagged males ( $n = 2$ ) from this second release were recaptured 12 days after their release.

## Discussion

Recent studies involving the individual marking of insects have not taken advantage of modern printing equipment that enables very small individual tags to be produced without the need of specialist equipment. Marking large Hymenoptera with 'Bee tags' is the only comparable technique, but these tags are produced commercially with specialist equipment.

This study was intended as a pilot to primarily investigate the durability of the individually coded tags in the wild. The tags are very cheap and easy to produce and the technique has the added advantage that it can be conducted in the field, which is not practical with many individual marking techniques (Griffiths et al., 2001). Although non-permanent, the marking technique does not involve the drastic measures that are used to mark insects permanently (Weseloh, 1985; Mikheev & Kreslavskii, 2000), because of this the technique detailed here may not be suitable for long-lived adult insects, such as some carabids. It would be relatively simple to test this technique on longer lived insects to assess the durability of the tags over longer periods and in

leaf litter microhabitats. The technique would lend itself to the study of dispersal, however, as with any individual marking technique the method would be most efficient when the numbers of individuals to be marked was not excessive.

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