Technique Notes

Potential use of marker pen ink as a marking method for drosophilids.

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Introduction

Mark-release-recapture methods have been widely employed to estimate absolute parameters like dispersal and density in mobile animal populations (Southwood, 1978). These parameters are primordial to elucidate the population structure, that has been used from conservation purposes to evolutionary researches and has long been recognized as a key factor for understanding genetic variation in nature (Wright, 1938) and for estimating the size of panmictic units (Begon, 1976). Mark-release-recapture methods are also the basis for the classic theories of population gene flow (Dobzhansky and Wright, 1943), based on the dispersal of *Drosophila pseudoobscura* Frolova, 1929, and for a unifying mathematical relationship between density and distance of dispersing insects (Taylor, 1978, 1980).

A prerequisite for the use of mark-release-recapture methods is a technique for marking animals, which has important implications for the reliability of the results. Desirable attributes for marking methods are the retention of marks for a sufficient period of time and that the marker does not adversely affect the biology of the organism altering, for example, the mortality rate of the species. Besides, the material needs to be inexpensive, nontoxic to the environment, clearly identifiable (on recapture), and easily applied (Southwood, 1978).

Many materials and methods have been proposed to mark insect since the seminal studies of Geiger *et al.* (1919) and Dudley and Searles (1923), in which paints, dyes, and stains were used to investigate population dynamics. Such materials, as well as micronized dusts, are the most successfully, commonly used and inexpensive materials for marking insects (Southwood, 1978; Wineriter and Walker, 1984). Tags are also promising marking methods for arthropods because they are inexpensive and can identify organisms on an individual basis; however, they are generally too large and heavy for small specimens like drosophilids, and their application is tedious and time-consuming (Hagler and Jackson, 2001). Mutilation, on the other hand, can be applied only for large or heavily sclerotized insects (as beetles) or insects with large wings (as butterflies and dragonflies) and, therefore, can not be applied for drosophilids. Recently, methods like Elemental Marking, Protein Marking, Genetically Engineered Marking and Biochemical Genetic Markers have been used, but they are usually expensive, time-consuming, and require an arduous workout for researchers and the death of the specimens after recapture. In addition, in some cases, specimens must be reared in the laboratory and markers may promote non-visible but detrimental mutations or physiological changes affecting fitness (Hagler and Jackson, 2001).

For wild drosophilids micronized fluorescent dusts have been by far the most successfully and commonly used marking method (Crumpacker, 1974; Begon *et al*. 1975; Begon 1976; Hagler and Jackson, 2001). In this paper, we test for drosophilids the effectiveness of a marking method commonly used for lepidopterans – which consists in marking the body of the insect with marker pen ink – and discuss the advantages and disadvantages of this method compared to micronized dusts.

Material and Methods

We investigate the effect of marking specimens with marker pen ink on persistence of marks and longevity – since some paints and inks are toxic to insects (Southwood 1978) – of South American strains of *Drosophila simulans* Sturtevant, 1919, *D. malerkotliana* Parshad and Paika, 1964, and *Zaprionus indianus* Gupta, 1970. Virgin flies, anesthetized with ethylic ether, were painted in the thorax with a marker pen. The number of spots (one to seven), their position (among ten possibilities) and the assignment of a specimen to the marked or non-marked categories were randomly determined.

After awakening, ten marked flies of the same sex and species were reared with ten non-marked flies – handled in the same way (control) – in vials with cornmeal medium (Marques *et al*., 1966), in a controlled chamber (25 ± 1^{o}C, 60% r.h.). Four vials for males and four for females were utilized for each species. Every two days, food (*Saccharomyces cerevisae*) and water were added to the medium. Every four days, the flies were transferred to a new vial with culture medium to maintain the quality of resource and to avoid toxic effects of metabolites. The vials were inspected daily to detect dead flies, which were removed of the vial. After removal, information on the sex, marking category (marked or non-marked), arrangement of dots for marked specimens (for assessment of the persistence of dots) and longevity were compiled.

A one-way ANOVA was performed using Statistica 5.1 software (StatSoft, Inc, 1998) to detect differences of longevity between marked and non-marked flies ($p \leq 0.05$) for each sex of each species. Data transformation was required for males of *D. malerkotliana* (√x), *D. simulans* (√x), and *Z. indianus* (sin (x)) to fit the ANOVA assumptions. Normality and homoscedasticity were assessed by Lilliefors and Bartllet tests, respectively, using the Genes 2007.0.0 software (Cruz, 2006). During the transference procedures, some flies escaped and were not considered for the analysis.

Results and Discussion

Longevity did not differ between marked (mean ± sd = 22.2 ± 12.3 days, N = 32) and non-marked (20.5 ± 12.9 days, N = 38) *D. simulans* females ($F = 0.325$, $p = 0.57$, df = 1), but it differed between marked (12.7 ± 7.6 days, N = 35) and non-marked (17.9 ± 11.1 days, N = 34) *D. simulans* males ($F = 4.841$, $p = 0.03$, df = 1). For *D. malerkotliana*, longevity differed neither between marked (21.7 ± 11.8 days, N = 30) and non-marked (24.8 ± 14.2 days, N = 31) females ($F = 0.854$, $p = 0.36$, df = 1) nor between marked (23.8 ± 9.2 days, N = 31) and non-marked (26.5 ± 10.0 days, N = 34) males ($F = 1.147$, $p = 0.29$, df = 1). In the same way, longevity differed neither between marked (27.0 ± 7.4 days, N = 40) and non-marked (32.9 ± 11.1 days, N = 32) *Z. indianus* females ($F = 3.668$, $p = 0.06$, df = 1) nor between marked (25.9 ± 18.9 days, N = 36) and non-marked (18.7 ± 1.8 days, N = 38) *Z. indianus* males ($F = 3.137$, $p = 0.08$, df = 1).

Our data suggest that marker pen ink is a reliable method for marking drosophilids, as also suggested by Morton (1982), who marked the upperside forewing and the underside hindwing of the marbled white butterfly *Melanargia galathea* (L.) (Satyridae). Although our results suggest almost

no relevant effect of such marker on longevity, a high variability in the data set as observed in our study increases the probability of error Type II, *i.e.*, a false negative and, consequently, caution is required in its use. Since longevity is naturally highly variable, we encourage further analysis.

The use of dust is frequently preferred for marking insects due to its inexpensiveness, environmental safeness, and the easy and rapid application (by shaking a container in which both dust and insects are put together) and detection (Hagler and Jackson, 2001). Several laboratory studies suggest that micronized dusts have a negligible effect on the viability (Crumpacker, 1974) and mortality (Crumpacker, 1974; Moth and Barker, 1975) of drosophilids and other dipterans, such as *Liriomyza trifolli* (Jones and Parrela, 1986). It has been also observed for drosophilids, in field work, that multiple marking have no significant effect compared to single marking (Rosewell and Shorrocks, 1987) and that different colors and compositions of dust do not affect the recapture rate (Begon, 1976).

Inks are also an inexpensive, clearly identifiable (on recapture), and rather easily applicable for marking insects. Furthermore, such material can not be transferred from marked to unmarked insects, which can occur for dust particles in the field or in traps and sweep nets used for sampling (Miller, 1993), affecting the results. Besides, ink is applied on specific structures while dust particles do not, producing adverse behavioral effects (as on dispersion) (Chang, 1946) through dust impregnation on sense organs (Cook and Hain, 1992).

Ink marks persist for a longer period of time than dusts. Several authors report that inks are very durable, mainly the non-water-soluble inks (Wineriter and Walker, 1984). For drosophilids we observed 100% of retention of marks after ten days and almost 85% at the end of the experiment (65 days). Laboratory experiments point out from 11 to 28 days of persistence for dusts (Crumpacker, 1974; Moth and Barker, 1975) with l00% retention after three days and 80% retention after 10 days (Shorrocks and Nigro, 1981). Such shorter period of persistence of dusts can be inappropriate for long-term studies.

Maybe the major advantage of inks compared to dust is the possibility to mark individual (but also groups) insects. Simple numbering systems (Southwood, 1978) or elaborate coding systems (Opp and Prokopy, 1987) can be used to differentiate them. For the studied flies, we considered viable the use of at least ten dots, which in a dot binary-coding system using just one color allows the recognition of 1023 (2^{10}-1) individuals. Distinct colors of dusts can be used in the same order of using distinct dots, but the differentiation of several colors in the same individual is more arduous (personal observations). For the marking procedure, we spent around three seconds per dot, what we consider viable. However, depending on the number of dots per fly and the number of flies to be marked, the process can be very time-consuming, in contrast to the use of micronized dusts.

Finally, it is important to emphasize that prior to such experiment, we tried to mark the specimens on the wings but the ink was removed by cleaning activities of the flies up to one week after marking, sometimes damaging the wings. Another consideration is that for species with thorax partially or totally dark, this method was not effective due to the hard detection of dots, as tested for *D. mercatorum* Patterson and Wheeler, 1942. For this species, we also set marks with a correction white water-soluble fluid but marks were easily missed after death due to the medium moisture. Thus, this ink was not necessarily ineffective for marking drosophilids, but our method to detect the dots only on dead flies did not allow us to make a reliable estimation of longevity.

Marker pen ink is inexpensive, clearly identifiable on recapture, and, has no relevant effect on the longevity of three species of Drosophilidae (*D. simulans*, *D. malerkotliana*, and *Z. indianus*). Since this material allows marking a higher number of individuals, does not impregnate sense organs throughout the body and is more persistent on the insect, we suggest its use for individual-based studies, long-term studies, and those in which sense organs are determinants for trustworthy results.

For other studies, the use of dust is preferable, since it is less time-consuming and a more conventional marking technique for insects (Hagler and Jackson, 2001).

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Effect of Fly Nap® on ovipositing and fertility in *Basc* mutant and wild type *Drosophila melanogaster*.

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Abstract

The objective of this study is to determine whether wild and *Basc* mutant genotypes of *Drosophila melanogaster* treated with the anesthetic Fly Nap® displayed significant differences in their egg deposition and subsequent egg fertility when compared to etherized wild type and *Basc* mutants. Some mutant genotypes are more sensitive to environmental insult than are the wild type (Nguyen *et al.*, 1979). We were particularly curious to note any significant effects of treatment with Fly Nap® on the *Basc* mutant as compared to the wild genotype. Flies were kept in egg-laying chambers at 25ºC, and ovipositing was quantified for each treatment group by counting the number of eggs deposited on agar at post-treatment intervals of 16, 24, 40, 48, 64, and 72 hours. Ovipositing of *Basc* mutants exposed to Fly Nap® was significantly ($p < 0.05$) lower than etherized *Basc* during the first 24 hours post-treatment. After 40 hours the most significant ($p < 0.01$) difference appeared between *Basc* mutants and wild type flies both treated with Fly Nap®. Wild type flies treated with Fly Nap®, while initially displaying lower egg deposition than etherized wild flies, recovered to control levels after 64 hours. The ovipositing of *Basc* mutants treated with Fly Nap® remained significantly ($p < 0.01$) lower than that of similarly anesthetized wild types from 40 hours post-