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MARKING LEPIDOPTERA AND THEIR OFFSPRING: TRACE ELEMENT LABELLING OF *COLIAS EURYTHEME* (PIERIDAE) WITH RUBIDIUM

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ABSTRACT. Trace elements can be used to label lepidopteran eggs via treated adults, but sources and importance of sample variability are relatively unexplored. Female *Colias eurytheme* (Boisduval) reared on rubidium (Rb)-treated foodplants and their eggs were analyzed for Rb by atomic absorption spectrophotometry. Parent (3 untreated and 4 treated females) and mean egg (25/female) element content were significantly correlated. Compared with untreated adults and eggs, treated samples were reliably marked, although significant egg-to-egg variability in Rb concentration was found within and between sib-groups. Analysis-day differences were not significant. Adult sizes and element exposures may have contributed to between-group variance. Maternal and genetic influences were potential sources of within sib-group variance.

Additional key words: alfalfa butterfly, internal marker, adults, eggs, atomic absorption spectrophotometry.

Many methods for marking and monitoring lepidopteran populations have been proposed, tested, and employed with various degrees of success (Southwood 1978). An internal marking method that shows great potential for use in dispersal studies of phytophagous insects is that of trace element labelling (Berry et al. 1972). These labels can be used in concentrations low enough to permanently mark but not adversely affect the insect or its host. The mark can be acquired without handling the insect because it is obtained as the animal feeds on treated plant tissue (Stimmann et al. 1973) or nectaries (Culin & Alverson 1986). Insects in 7 orders, including 10 species of Lepidoptera in 5 families, have been successfully labelled (Hayes & Hopper 1987:table 1). Dispersal tests employing trace elements as adult markers have been conducted with three lepidopteran species: *Heliothis zea* (Boddie) and *Spodoptera frugiperda* (J. E. Smith) (both Noctuidae) (Graham et al. 1978); and *Pectinophora gossypiella* (Saunders) (Gelechiidae) (Van Steenwyk et al. 1978).

One recently revealed advantage of trace element use over external markers of Lepidoptera is that the mark is passed along to reproductive products, including eggs (Legg & Chiang 1984, Hayes & Hopper 1987) and spermatophores (Graham & Wolfenbarger 1977). Detection of a parental mark in the egg or spermatophore provides, much like a genetic marker, a potential means of assessing gene flow in the field. In measuring dispersal, recovery of eggs may prove superior to recovery of adults because the marked adult transmits multiple signals through the

distribution of marked eggs (Jones et al. 1980). Also, the possibility of using elemental marking of reproductive products in behavioral, physiological, or developmental studies is suggested, but has not been much explored (Engebretson & Mason 1981).

It remains to be seen whether recovery of marked eggs will prove feasible in large-scale field studies. However, the method has been used successfully to monitor small-scale egg dispersal by *Trichoplusia ni* (Hübner) (Geometridae) to adjacent crops (G. Ballmer pers. comm.). Among the many questions that need to be addressed is that of variability among eggs. Individual variability among adults can arise from exposure differences and differences in size or weight, and can occur through time. It has yet to be determined whether variability is passed along to offspring and whether there are intrinsic differences among eggs from the same female.

To investigate these areas and develop methods for more efficient and possibly expanded utilization of trace-element labelling, we analyzed marked adult *Colias eurytheme* (Boisduval) (Pieridae) and their eggs by atomic absorption spectrophotometry (AAS) for trace-element content. In addition to the parent-offspring relation, we examined egg-to-egg variability within sib-groups and among offspring of different parents. Differences between preparation dates were also considered possible sources of variability. Adult samples were prepared from bodies and head capsules and compared with egg samples to examine cost-effective adult sample preparation procedures.

MATERIALS AND METHODS

Insects. *Colias eurytheme*, the alfalfa butterfly, was used because of its tractability in the laboratory and greenhouse (Taylor et al. 1981), and because it is considered a model system for other Lepidoptera (Watt et al. 1974). Like *Heliothis* spp. and a number of other economically important Lepidoptera, *Colias* spp. are highly mobile, polyphagous, and distribute their eggs individually over a potentially large area (Tabashnik 1980). Experimental insects were obtained from a colony originating from eggs collected in November 1986 on commercial vetch, *Vicia villosa* Roth (Leguminosae), on the grounds of the Jamie Whitten Delta States Research Center, Stoneville, Mississippi.

Treatments. Adults were reared in the greenhouse from eggs or neonate larvae on vetch plants treated with rubidium (in chloride form). Host plants were grown from seed in vermiculite, and treated weekly with 1 g RbCl/l water (1000 ppm), initially by foliar application, then by watering the potting soil after insects were placed on the plants. Freshly treated host plants were provided as needed until pupation. Control insects were reared in a similar manner on untreated plants.

Treated and untreated pupae were placed in separate 2-l cardboard cartons with organdy top cloths and moistened paper towel liners. Eclosing adults were marked on the left hindwing with a felt tip pen (/ = treated; // = untreated), and placed in a mating cage. The cage, a 0.6 × 0.6 × 0.3-m wood frame covered with transparent plastic, was provisioned daily with honey-water (1:3) soaked cotton balls, and held at 12°C without light. To stimulate mating, temperatures were elevated to 30°C and light was provided by two banks of fluorescent lights for 2–4 h/day. The cage was checked at 15–30-min intervals for the occurrence of mating. Pairs were removed and held at room temperature (ca. 25°C) until spermatophore transfer was completed. The males were uniquely marked and returned to the cage. For oviposition, females were placed individually on host plants covered by plastic bags and maintained at room temperature (ca. 25°C) and LD 12:12. Ovipositing females were fed daily with honey-water and transferred to fresh host plants as needed.

Twenty-five eggs from each treated female (N = 4) and each untreated female (N = 3) were collected separately by sib-group, and frozen. Eggs were obtained from day 1–2 of oviposition. When a female died, wings were removed and the body frozen.

Sample digestion. Individual eggs were digested, following the method of Hayes and Hopper (1987), by placing an egg directly into the sample cuvette along with 0.025 ml ultrapure nitric acid (HNO_3), heating by microwave for 8–12 min at a low setting, and then diluting with 0.5 ml deionized distilled water (DDI).

The head and body of each female were placed in separate 7-ml scintillation vials with 0.2 ml and 0.4 ml ultrapure nitric acid, respectively. Digestion was allowed to occur at room temperature for 24 h, then samples were microwaved for 8–12 min. Digested material was then diluted with DDI, 4.0 and 5.0 ml, respectively.

AAS analysis. Samples were analyzed using a Perkin-Elmer 3030 with an HGA 400 graphite furnace and AS-40 autosampler. An electrodeless discharge lamp for Rb was used. Wavelength was set at 780 nm. Char and atomization temperatures were 800° and 1900°C (Slavin 1984). Elements were atomized off the wall of pyrolitically-coated graphite tubes.

Data. Initially, 10 eggs from each female (treated and untreated) were individually digested and analyzed by AAS for presence or absence of a detectable Rb signal (=day 1). To increase the sample size and examine between-analysis-day variability, an additional 15 eggs from each female were prepared and analyzed 14 days later (=day 2). To examine within-preparation variability, two separate aliquots of a single preparation from each head and body were decanted and analyzed on

different days. Mean values for head and body samples were used in subsequent analyses since no significant differences were found between dates (Mann-Whitney *U*-test).

Data were analyzed to determine reliability of mark detection for each female and her eggs. Mark thresholds for both eggs and adults (heads and bodies) were determined and compared by two methods: (1) using the high-range value of untreated controls, and (2) using the conservative method of Stimmann (1974), which assumes a normal distribution, three standard deviation units above the mean of untreated control samples. All Rb concentration values are given in units per egg or body part. Variation in egg weight within and between sib-groups was considered negligible for our purposes (mean dry wt = 0.111 mg, SE = 0.0017, N = 10/female). Variation in head and body weight was more extreme (mean dry wt of heads = 0.897 mg, SE = 0.1536; body wt = 16.54 mg, SE = 3.374). Within and between sib-group differences were examined by analysis of variance. Parent-offspring relation was evaluated by correlation of Rb content of a female body or head with the mean Rb content of her eggs. Data analyses were performed using SAS software.

RESULTS AND DISCUSSION

The mean quantity of Rb (in ppm) found in the body, head capsule, and eggs of treated and untreated females is given in Table 1. Samples prepared from the bodies of treated females were found to be 100% reliably marked when compared to thresholds derived from samples prepared from untreated adults. However, only 10–20% of the samples prepared from heads alone produced detectable signals, and head results were not significantly correlated with body results (r = 0.30). It is apparent that detectable quantities of Rb were not evenly distributed throughout the insect's tissues. The time and expense of digesting whole insects makes it advantageous to use the smallest sample that provides consistent results. For *Heliothis* spp. it has been found that a single wing is an adequate substitute for a whole moth (Hayes in press). For butterflies, the wing is not as practical because of large size and the frequent need to retain wings for morph determinations. Thus, *Colias* samples prepared from wingless and headless bodies were used, and they produced reliably detectable signals. If spermatophores are routinely dissected from females, or abdomens are removed for electrophoretic analysis, it would be ideal to be able to rely on a preparation from the thorax alone. However, a feasibility test for use of the thorax has not yet been conducted.

More than 90% of eggs (N = 100; 4 females) were determined to be detectably marked regardless of method used (92% exceeded range of

TABLE 1. Quantity of Rb (mean & range in ppm) in untreated and treated female *Colias eurytheme* (body and head) and their eggs (for 2 analysis days). Mark thresholds, both high range of untreated controls (Mark 1) and Stimmann value calculated from mean of controls (Mark 2), are provided along with mark determination (yes/no) or percentage of marks.

		Rb concentration			
Female no.	N	Mean	Range	Mark 1	Mark 2
Threshold values:					
Body	10			0.0185	0.0309
Head	10			0.0140	0.0213
Eggs (day 1)	30			0.0014	0.0015
(day 2)	45			0.0028	0.0026
			Untreated		
1					
Body		0.0039[a]		no	no
Head		0.0021[a]		no	no
Eggs (day 1)	10	0.0004	0.0000–0.0012	0%	0%
(day 2)	15	0.0011	0.0003–0.0014	0%	0%
2					
Body		0.0182		no	no
Head		0.0046		no	no
Eggs (day 1)	10	0.0006	0.0002–0.0014	0%	0%
(day 2)	15	0.0013	0.0008–0.0022	0%	0%
3					
Body		0.0047		no	no
Head		0.0125		no	no
Eggs (day 1)	10	0.0002	0.0000–0.0006	0%	0%
(day 2)	15	0.0011	0.0004–0.0028	0%	0%
			Treated		
4					
Body		0.0739		yes	yes
Head		0.0208		yes	no
Eggs (day 1)	10	0.0035	0.0011–0.0030	90%	80%
(day 2)	15	0.0038	0.0031–0.0048	100%	100%
5					
Body		0.0999		yes	yes
Head		0.0058		no	no
Eggs (day 1)	10	0.0035	0.0016–0.0160	100%	100%
(day 2)	15	0.0038	0.0030–0.0051	100%	100%
6					
Body	2	0.0378		yes	yes
Head	2	0.0106		no	no
Eggs (day 1)	10	0.0022	0.0011–0.0032	70%	70%
(day 2)	15	0.0045	0.0019–0.0077	73%	73%
7					
Body		0.0995		yes	yes
Head		0.0101		no	no
Eggs (day 1)	10	0.0063	0.0033–0.0169	100%	100%
(day 2)	15	0.0070	0.0049–0.0086	100%	100%

[a] Mean of two aliquots per sample; for further explanation see Materials and Methods.

nonmarks; 91% exceeded Stimmann's value). The proportion of detectably marked eggs per analysis day for each sib-group is given in Table 1. Significant correlation was found on both analysis days between maternal Rb content of the body and mean quantity of Rb in offspring (Table 1; day 1 $r = 0.88$, $P < 0.01$; day 2 $r = 0.84$, $P < 0.01$).

Analysis of variance revealed significant differences between treated and untreated samples ($P < 0.0001$; Table 1). More than 60% of the variance was due to within-sib-group variance, more than 35% to between-group variance, and less than 1% was attributable to analysis-day variance. Low day-to-day analysis difference is reassuring because in field tests large numbers of samples must be processed over several days.

Between-group variance dropped to less than 20% for the untreated sib-groups when treated and untreated groups were analyzed separately. However, within-sib-group variance remained above 60%. Between-group differences could be attributed to differing insect sizes and element exposures. Eggs from a large female or one that has fed consistently on well-treated foliage may show higher levels of Rb than those from a smaller female or one that has fed inconsistently on a treated host plant.

Within-group differences cannot be understood as easily. Each female was mated only once, and use of the first eggs oviposited should lessen age effects. Since Rb is a potassium mimic, results suggest that the female does not supply her eggs with consistently similar quantities of necessary metabolites. Alternatively, inherent differences from egg to egg (genomic differences) may result in the observed Rb content variance. The parent-offspring correlation analysis reveals significant associations which may indicate some degree of inherent relation. Further investigation of these hypotheses could provide valuable insights into development, and might dictate an expanded role for the use of trace elements as an experimental tool.

In the final analysis, specimen-to-specimen, in particular egg-to-egg, variability does not present difficulties for use of this marking technique in field operations. Despite high individual variability, our findings indicate that labelled parents and offspring are readily distinguishable from unlabelled specimens.

Trace element marking has been reported previously with only one other butterfly species, *Pieris rapae* (L.), and then only in the adult stage (Stimmann 1974). The potential to exploit this marking method among all Lepidoptera is great. It seems well justified since mark-release-recapture studies using external markers are commonly used to study pest and nonpest lepidopteran population attributes (Ehrlich & Davidson 1960), but have received considerable criticism (Morton 1984).

Along with problems arising from handling insects, insufficient recapture numbers are a persistent problem. By labelling the egg, the adult signal is amplified, and the concomitant ability to directly assess gene flow is a definite advantage. Increasing the number of unique marks will also improve recapture efficiency per unit area. Along with Rb, other elements such as cesium and strontium are promising adult and possible egg markers.

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ANNOUNCEMENT

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Journal submission categories are being broadened to include front cover illustrations and feature photographs. Submissions in these new categories, like all submissions to the *Journal*, may deal with any aspect of Lepidoptera study. Submissions in both categories must be accompanied by brief captions that include scientific names.

Cover illustration subjects are well depicted by past covers created before submissions were invited. Submissions should be no larger than letter size, with the caption for the inside front cover on a separate sheet. Drawings may be more suitable than photographs because drawings can usually better withstand the coarse reproduction necessitated by present cover stock texture. Submissions will be selected for artistry, novelty, and general appeal. There are no author page charges for cover illustrations.

Feature photographs might show unusual behaviors, unusual habitats, type localities, specimens in nature illustrating identifying marks, or other subjects. Photographic submissions should be mounted on white cardboard no larger than letter size, with a brief caption on a separate sheet. Feature photographs must be suitable for reduction to either half or full *Journal* pages (8.5 cm high by 11 cm wide, or 17 cm high by 11 cm wide, caption included). Submissions will be selected for photographic quality, scientific merit, and general appeal. Regular page charges will apply to accepted feature photographs.

Submissions in both new categories should be sent to the *Journal* editor.

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