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A New Marking Method Using a Sewing Needle: Effects on Survival Rate of Twenty-Eight-Spotted Lady-Beetles, *Henosepilachna vigintioctopunctata* FABRICIUS (Coleoptera : Coccinellidae)

Kohji HIRANO

Laboratory of Applied Entomology and Nematology, School of Agriculture, Nagoya University, Nagoya 464, Japan

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Adults of the twenty-eight-spotted lady-beetle were marked by piercing their elytra with a sewing needle. Sixteen of the twenty-eight natural spots seen on the elytra were used as piercing points. Marking-and-recapture experiments made in the field suggested that there was no significant difference between the mortality rates of needle-marked and unmarked adults. Marking with a needle seemed to have no harmful effect on the number of recaptures. No difference between survival rates of needle-marked and lacquer-marked individuals was seen in a field-cage experiment.

INTRODUCTION

The habitats of any animal species are heterogeneous and discontinuous in time and space. They usually consist of many 'patches', relatively isolated units of habitats including food and other resources. Studies on the population dynamics of insects should, therefore, be carried out with due consideration of the patchy nature of habitats. The marking-and-recapture technique is important for assessing the movement of individuals within and between patches.

A population of the twenty-eight-spotted lady-beetle, *Henosepilachna vigintioctopunctata* F., a pest of solanaceous plants which repeats two generations a year, was studied from 1976 to 1978 in a study area consisting of many patches of host plants. An individual marking system was applied to gather ecological information on adults. Marking adults with spots of nitrocellulose lacquers, which has been the most extensively used methed in entomology, was not appropriate for this beetle, because (1) it is time-consuming when we want to discriminate a large number of individuals with combinations of different colours and (2) some marks may flake off when the time interval from marking to recapture is long. To avoid this, adults were marked by piercing with a sewing needle. The present paper reports the results of analyses on the effect of two marking methods on survival rate of adults.

MATERIALS AND METHODS

Study area. The study was carried out in an area, a suburb of Nagoya surround-

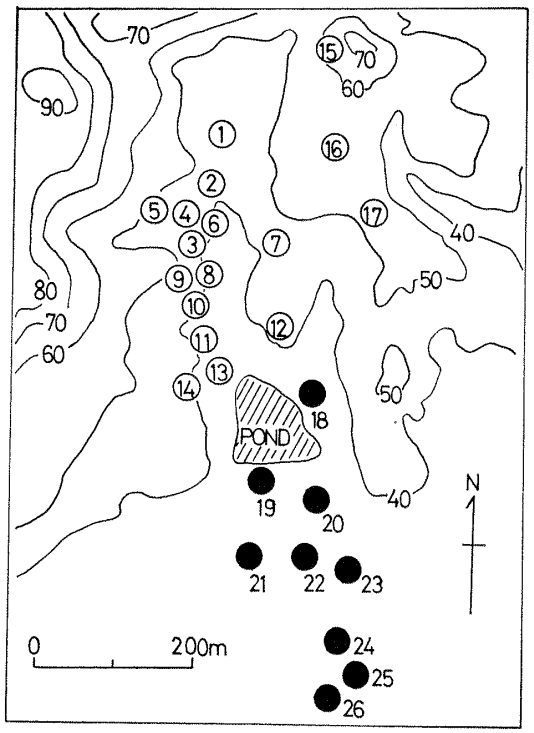

Fig. 1. Map of the study area. Circles indicate patches of food plants. The study was focused on open circles. For other explanation see text.

ed by wooded hills except for the south (Fig. 1). Many groups of one or more small cultivated fields of host plants were present in the area and these were designated as 'patches'. Aside from these patches, there was no field of host plants within a distance of 1 km from the area. South of the pond (patches No. 19–No. 26), the density of adults was low. The investigation was carried out in an area including patches No. 1 to No. 18 in 1976 and patches No. 1 to No. 17 in 1977. In this area (about 150,000 m^2) there were 4,112 potato plants, 346 egg-plants and 337 tomato plants in 1976, and 3,136 potato plants, 352 egg-plants and 234 tomato plants in 1977. All unmarked adults were marked individually at the time of first capture, and the dates and places of captures and recaptures were recorded.

Piercing method. The adults have twenty-eight black spots on the elytra. Marking was made using 16 of these spots, as shown in Fig. 2–a. Coding was made with a 1–2–4–7 marking system (Southwood, 1978) which allowed us to describe all the numerals from 1 to 9 with one or two spots. Marking was made by piercing the centre of these spots with a sewing needle (0.4 mm in diameter). By this system we can identify up to 9,999 individuals. On catching adults by hand, the elytra were opened wide with the fingers while piercing to avoid wounding the hind wings. Beetles were not anaesthetized, but there was no sign of any nervous action after their release.

In a routine census, since teneral adults (two or three days after emergence) were difficult to mark with this method, they were first marked with lacquers and piercing

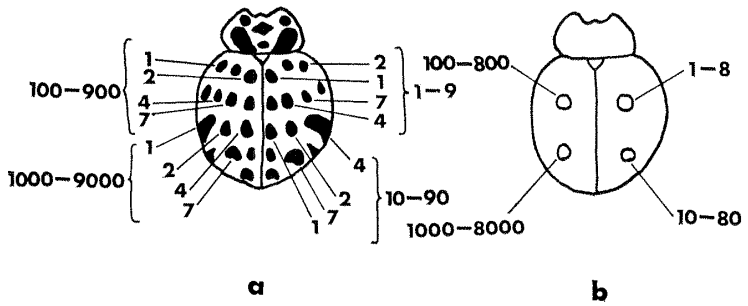

Fig. 2. Method of marking twenty-eight-spotted lady-beetles.
a: Marking points with a needle. Piercing was made at centre of natural spots.
b: Marking points with lacquer paint (see text for further explanation).

was done when they were recaptured after maturation.

Paint method. Four places on the elytra were marked with spots of lacquer paint of nine different colours (Fig. 2–b), which indicated numerals from zero to eight. 'Nine', 'ninety', etc. were discarded in coding. Thus, by this method we could identify up to 8^4 (4096) individuals. As the marking system included a zero-class, we could know the rate of loss of marks when we found an individual with less than four spots.

Field census. To test whether the pipercing method has any affect on the survival rate of marked beetles and whether the number of times of capture affects the recapture rate of marked beetles, the marking-and-recapture data obtained for overwintered adults in 1976 and 1977 were analyzed. All the adults discovered during the experimental periods were marked with a sewing needle.

Field cage experiment. To test whether adults marked with a sewing needle and with lacquer paint are equally subject to mortality factors, an experiment was made in a field cage (3.6×3.6 m in area, 2.7 m in height) covered with 18-mesh wire netting. Twenty-two eggplants were planted before the beetles were released. Nineteen (ten females and nine males) first generation adults collected from the fields were released in the cage on 24th June 1977. One hundred forty-seven second generation adults, which were progeny of the released adults, were marked with a sewing needle or with lacquer paint regardless of the hardness of the elytra on 8th August and released in the cage (in this case, adults marked with paint were not marked again with a needle). In both cases, the sex ratio was about 0.5. Recaptures were made till 10th September.

RESULTS

Mortality rate of unmarked beetles and beetles marked with a sewing needle

LESLIE (1952) described a method to test the absence of dilution in a population to which a marking-and-recapture method had been applied. A prerequisite for this method is that marked insects suffer the same mortality as unmarked ones. Therefore this method can be applied to test whether a marking method has any effect on the survival rate, under an assumption of no dilution (IWAO et al., 1963).

Assuming that no dilution took place and that there was no differential mortality between the marked and unmarked individuals, the expected proportions of the number of individuals marked at a time x and recaptured at a time t ($t=x+1, x+2, \ldots, T$) to the total number of individuals caught at time t should be constant for any t. Therefore c_{xt}, the number of individuals marked at x and recaptured at t may be replaced by

$$\hat{c}_{xt}=R_t \times \sum_{t'=x+1}^{T} c_{xt'} / \sum_{t'=x+1}^{T} R_{t'},$$

where R_t is the total number of individuals caught at t. The expected number of unmarked individuals caught at t is obtained by subtracting $\sum_{x=1}^{t=1} \hat{c}_{xt}$ from R_t.

Overwintered twenty-eight-spotted lady-bettles immigrate into potato fields from overwintering sites in the spring. After settling on potato plants they do not move so actively. Marking-and-recapture data showed that the rate of movement among patches was very low (270 and 109 recaptures were recorded for this stage in 1976

and 1977, respectively, but cases where recaptures were made in patches other than patches where the beetles were released were only 4 in 1977). Based on the number of adults found in the fields, it was estimated that immigration from overwintering sites was mostly finished by the second week of May. Therefore we used marking-and-recapture data after this period to examine the assumption of no differential mortality (Tables 1 and 2).

The expected numbers of marked and unmarked individuals on each sampling day were calculated using the above equation as shown in Table 3. The χ^2-test showed that differences between expected and observed numbers were not significant in either year. Thus the mortality rate of individuals marked with a sewing needle may be taken as the same as that of unmarked ones.

Effects of times of capture

In the marking-and-recapture technique, it is probable that the rate of recapture of a marked animal depends on the number of times it has previously been captured and/or that sluggish individuals are more vulnerable to capture than active ones. LESLIE et al. (1953) presented a method to test these possibilities. If r_t animals are released at time t, of which a_i have been captured i times ($i=1, 2, 3, \ldots$; $\sum_i a_i = r_t$), and c_{t+1} of these are recaptured at $t+1$, we can expect, under assumptions of (1) random sampling, (2) no differential mortality, and (3) no differential recapture rate, that $a_i c_{t+1}/r_t$ out of a_i would be recaptured at $t+1$. The results of marking-

Table 1. RESULTS OF THE MARKING-AND-RECAPTURE EXPERIMENT IN THE STUDY AREA (1976)

		Dates of sampling						
		18 May	22 May	27 May	1 June	7 June	15 June	21 June
Total catch		129	115	117	122	39	50	40
No. of unmarked insects captured		129	78	59	55	7	12	2
No. of marked insects recaptured	18 May	—	37	34	33	11	15	10
	22 May	—	—	24	15	9	11	5
	27 May	—	—	—	19	7	8	10
	1 June	—	—	—	—	5	4	9
	7 June	—	—	—	—	—	0	3
	15 June	—	—	—	—	—	—	1

Table 2. RESULTS OF THE MARKING-AND-RECAPTURE EXPERIMENT IN THE STUDY AREA (1977)

		Dates of sampling				
		9 May	18 May	31 May	6 June	14 June
Total catch		153	123	69	89	15
No. of unmarked insects captured		153	99	47	35	6
No. of marked insects repaptured	9 May	—	24	10	22	2
	18 May	—	—	12	23	4
	31 May	—	—	—	9	1
	6 June	—	—	—	—	2

Table 3. COMPARISON OF OBSERVED AND EXPECTED NUMBERS OF UNMARKED INDIVIDUALS AND NEEDLE-MARKED ONES IN THE STUDY AREA IN 1976 (TOP) AND 1977 (BOTTOM)

Sampling dates	No. marked		No. unmarked	
	Observed	Expected	Observed	Expected
22 May	37	33.35	78	81.65
27 May	58	54.29	59	62.71
1 June	67	77.96	55	44.04
7 June	32	30.38	7	8.62
15 June	38	40.60	12	9.40
21 June	38	33.48	2	6.52

$\chi^2=10.324$ $(0.10>P>0.05)$

Sampling dates	No. marked		No. unmarked	
	Observed	Expected	Observed	Expected
18 May	24	24.11	99	98.89
31 May	22	29.05	47	39.95
6 June	54	46.01	35	42.99
14 June	9	9.75	6	5.25

$\chi^2=5.993$ $(0.25>p>0.10)$

Table 4. DISTRIBUTION OF RECAPTURES WHEN INDIVIDUALS MARKED WITH A NEEDLE WERE CLASSIFIED ACCORDING TO THE NUMBER OF TIMES THEY HAD BEEN CAPTURED (1976)

No. of times captured	Released on 22 May	Caught on 27 May	Released on 27 May	Caught on 1 June	Released on 1 June	Caught on 7 June	Released on 7 June	Caught on 15 June	Released on 15 June	Caught on 21 June
1	78	24(25.8)	59	19(17.1)	55	5(5.4)	7	0(0.7)	12	1(1.7)
2	37	14(12.2)	45	13(13.1)	48	6(4.7)	19	2(1.9)	19	2(2.7)
3	—	—	13	2(3.8)	17	1(1.7)	10	2(1.0)	14	1(2.0)
4	—	—	—	—	2	0(0.2)	3	0(0.3)	4	3(0.6)
5	—	—	—	—	—	—	—	—	1	0(0.1)

Expected numbers of recaptures are given in parentheses.

and-recapture study shown in Table 1 were arranged as Table 4. Numbers of recaptures expected by the model were given in parentheses. There was no clear difference between the observed and expected numbers. Thus there seems to be no tendency for animals which had been captured many times to be caught more easily (or rarely) in the next survey.

Comparison of survival rates between adults marked with piercing and paint methods

Suppose that the adults marked with a needle and lacquer paint suffer the same mortality and that they are recaptured at the same rate, we can obtain Emp_{xt}, the expected number of adults recaptured at time t ($t=x+1, x+2, \ldots\ldots, T$) out of the adults originally marked with a needle at a particular time x;

$$Emp_{xt}=\frac{mp_x(mp_{xt}+ml_{xt})}{mp_x+ml_x}$$

Where

mp_x: the number of adults marked with a needle at x,

mp_{xt}: the number of adults recaptured at t out of mp_x,

ml_x: the number of adults marked with lacquer paint at x,

ml_{xt}: the number of adults recaptured at t out of ml_x.

The expected number of recaptures for adults marked with lacquer paint at time t is obtained by subtracting Emp_{xt} from $[mp_{xt}+ml_{xt}]$. The expected numbers of recaptured adults marked with a needle or lacquer paint on each sampling day in a field cage were thus calculated (Table 5). It was shown that there was no consistent difference in the rate of survival between adults marked with a needle or with lacquer paint.

Table 5. Observed and Expected Numbers of Recaptured Adults Marked with a Needle and Lacquer Paint in the Field Cage during 8th August to 10th September in 1977

		Marked	
		with a needle	with lacquer paint
No. released on 8th August		80	67
21 August	No. recaptured	15 (10.9)	5 (9.1)
	No. not recaptured[a]	65 (69.1)	62 (57.9)
		$\chi^3=3.923$	$(0.050>p>0.025)$
31 August	No. recaptured	8 (10.9)	12 (9.1)
	No. not recaptured[a]	72 (69.1)	55 (57.9)
		$\chi^2=1.963$	$(0.250>p>0.100)$
10 September	No. recaptured	12 (10.9)	8 (9.1)
	No. not recaptured[a]	68 (69.1)	59 (57.9)
		$\chi^2=0.282$	$(0.750>p>0.500)$

Expected numbers of insects recaptured and not recaptured were given in parentheses.

[a] For method of calculation of the expected number of insects not recaptured, see text.

In the field cage experiment, some of the adults marked with lacquer paint lost one mark, but the date of the first release could be recongized. The rate of loss of all 4 points calculated by Cormack's (1968) method was negligible, and there was no need to correct the number of adults recaptured.

DISCUSSION

Although mutilation for marking animals, which has been widely used for vertebrates, was sometimes used with insects (Querci, 1936; Skuhravý, 1957; Grüm, 1959; Schjøtz-Christensen, 1961; Murdoch, 1963; Gangwere, et al., 1964), the only experiment to determine whether this method affects the longevity or behavior of marked insects was made by Murdoch (1963). He found that there was no significant difference between the survival rates of marked and unmarked marsh carabid beetles, *Agonum fuliginosum*, in field cages.

As mentioned by BUGHER and TAYLOR (1946) and GANGWERE, et al. (1964), the ideal marking procedure should meet several conditions: (1) ease of application, so that large numbers of individuals may be marked without excessive labour requirements, and, preferably, without elaborate equipment and procedures; (2) minimal manipulation of the organism to avoid injuring or otherwise altering its behavior and role within the ecosystem; (3) ease of recognition of the marked organisims; (4) certainty of persistence of the marking throughout the life stages of the marked organism.

It was suggested that the method for marking twenty-eight-spotted lady-beetles by piercing with a needle had no harmful effect on their longevity and recapture rate in the field, so that criterion (2) may be satisfied. As to criterion (1), the method of marking with a needle is more appropriate than marking with lacquer paint, because it does not take as much time as lacquer paint, where the need to change colours increases the handling time when we wish to discriminate a large number of insects. I could mark about 150 adults per hour with a needle. As to criterion (3), marks could be recognized easily with the naked eye, and as to criterion (4), the holes are permanent. The only defect with this method is the difficulty of marking teneral adults. It was considered, therefore, that the piercing method was suitable for individual marking of twenty-eight-spotted lady-beetles.

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