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Recapture studies of the harvester ant, *Pogonomyrmex owyheei* Cole, using a fluorescent marking technique

SANFORD D. PORTER and CLIVE D. JORGENSEN Department of Zoology, Brigham Young University, Provo, Utah

ABSTRACT. 1. Mark–recapture sampling must be stratified because populations of foragers and defenders are partitioned by trunk trails and order of emergence respectively.

2. Foragers and defenders form overlapping subsets of the total colony population, each of which is correlated with total colony population size.

3. Foragers and defenders have an average life expectancy of approximately 2 weeks.

4. The fluorescent marking procedure did not significantly affect harvester ant mortality and only temporarily affected their behaviour.

5. Combinations and mixtures of fluorescent ink allow at least eight groups in a colony to be distinctly marked for periods exceeding 4 months.

Introduction

Use of the Lincoln index to estimate ant colony populations showed promise when first proposed by Chew (1959), since previous methods of determining colony size usually required laborious excavations (Pickles, 1940; Brian, 1950; Lavigne, 1969). However, researchers soon found that the usefulness of these estimates in ant population studies was limited by two important technical problems: the lack of a rapid durable marking technique for large samples and the difficulty in obtaining mark–recapture samples representative of the entire colony population.

Development of suitable marking techniques for large-scale mark–recapture studies has been particularly difficult. Paint, 'techpens' and wire rings have been used with some success, but their use is time-consuming since each ant must be marked individually (Ayre, 1962; Whitford & Ettershank, 1975; Kruk-de Bruin *et al.*, 1977). Use of radioactive phosphorus (^{32}P) allows large groups of ants to be mass marked, but only one type of mark is available and marked ants must be retained 3–4 days before release to avoid mark transfer through trophallaxis (Stradling, 1970). Our use of fluorescent inks avoided these problems by allowing the rapid application of durable multicoloured marks to large numbers of ants.

The second problem encountered by most researchers was the population structure of ant colonies which usually made it difficult to obtain a representative sample of the entire colony. Consequently, most researchers had to settle for estimates of the foraging subpopulation, in the hope that these estimates correlated with the total colony size (Golley & Gentry, 1964; Erickson, 1972; Whitford & Ettershank, 1975; Kruk-de Bruin *et al.*, 1977).

The purposes of our study were first to test the use of fluorescent ink in mark–recapture studies and second to determine if Lincoln index estimates of foraging and defending workers were correlated with the

Correspondence: Mr Sanford D. Porter, Department of Biological Science, Florida State University, Tallahassee, Florida 32306, U.S.A.

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colony size. Hopefully, this work will facilitate future studies of ant populations and social structure in *Pogonomyrmex* spp. and other genera.

Materials and Methods

We studied colonies of *Pogonomyrmex owyheei* Cole during the summers of 1977–79 in the Raft River Valley of southern Idaho, U.S.A. Study sites were located in big sagegreasewood (*Artemisia tridentata, Sarcobatus vermiculatus*) plant communities (Jorgensen, 1979).

Mark–recapture procedures

We used the unbiased Lincoln mark–recapture index (Lincoln, 1930) as modified by Bailey (1951) and Leslie (1952) to estimate worker populations:

$$N = \frac{\left(\begin{matrix}\text{No. in mark}\\ \text{sample}\end{matrix}\right)\left(\begin{matrix}\text{No. in recapture}\\ \text{sample}\end{matrix} + 1\right)}{(\text{No. marked recaptures} + 1)}$$

Marking and recapture samples were restricted to foraging or defensive ants (foragers and defenders, respectively). Foragers were caught as they travelled approximately 1 m from the mound on foraging trunk trails (Hölldobler, 1976). Defenders were caught as they emerged from the mound entrance after a general defence response was intiated by blowing into the entrance hole through an aspirator partially filled with the defenders being captured.

Marking ants with fluorescent ink was quick and simple: ants were sufficiently etherized so as to immobilize them for about 30 s, then spread evenly onto tissue and quickly marked with a fine mist of nearly invisible fluorescent ink sprayed from an atomizer. As many as 500 ants could be marked at one time. Marked ants were allowed 2–4 h to recover from the ether and then released onto their respective mounds. Recapture samples were taken about 24 h later, etherized so as to immobilize the ants for 5–10 min, and then placed under long wave UV light in a 'dark viewing box' where the marked individuals fluoresced brightly. Marked ants were easily hand-sorted by colours under the UV light.

We first used fluorescent ink removed from felt-tip markers* in our field tests of the mark–recapture procedure. However, because this ink is no longer manufactured we switched to fluorescent printing ink† (5 g in 50 ml of ethyl ether) for general field use. Four colours of printing ink are available, allowing for at least eight groups in a colony to be marked concurrently by different mixtures and/or combinations of these colours.

Mark effectiveness

A suitable mark for recapture studies must be durable and neither injure the ant nor affect its behaviour. We tested durability of the fluorescent ink marks and their effect on harvester ant behaviour using thirty-three groups of twenty ants each. Each group was placed in a separate laboratory colony chamber and provided with ample food and water (Spangler, 1973). Ten of these groups were etherized for 1 min and sprayed either twice or four times with one of the following concentrations of fluorescent printing ink: 1, 2, 3, 4.5 or 8 g of ink in 50 ml of ethyl ether. Five groups were also etherized for 1 min and sprayed with fluorescent marker pen ink. To determine the effects of etherization, eleven groups were exposed to ether for periods ranging from 45 s to 4 min. Finally, three groups were exposed to ether for 2 min every fourth day, and four groups were retained as controls. Mortality and abnormal behaviour within each group were checked and recorded periodically for 31 days.

Social structure

We stratified our mark–recapture samples for studies of harvester ant social structure according to several potential subdivisions in the surface-active population. These subdivisions were sampled and marked with different colours to determine by dispersal of marked individuals if the subdivisions were

* Fluorescent Magic Marker® Studio Liners, Magic Marker Corp., Glendale, New York 11227, U.S.A.

† Day-Glo® Fluorescent 'Super' Bases, colours: invisible blue, rocket red, saturn yellow and arc yellow, Gans Ink Co. of Utah, 1919 West 2300 South, Salt Lake City, Utah 84119, U.S.A.

non-overlapping, partially overlapping or completely overlapping.

First we marked and recaptured foragers and defenders at fifteen mounds to determine relative sizes and relationships of both groups. Twelve of these mounds were excavated later using a backhoe as described by Lavigne (1969). All excavated workers and allates were collected, counted and checked for marks. Total excavated populations were then correlated with the forager and defender mark–recapture estimates. Second, foragers from each of four foraging trunk trails at one mound were marked and then recaptured 1, 3 and 5 days later to determine if they drift between trails. Third, we subdivided a defender sample at each of three mounds into five equal groups of fifty ants and marked them according to the order they emerged in defence of the mound. On the following day five samples of fifty ants at each colony were recaptured to determine if the marked defenders released the previous day were recaptured randomly or sequentially in the order in which they had previously emerged from the mound.

Results

Mark effectiveness

Etherization and marking procedures did not significantly affect the mortality or behaviour of the marked ants during any period of the experiment or at any treatment level. A two-way analysis of variance showed no significant differences among the treatment means ($P > 0.20$), nor was there significant interaction between time and treatment. Also we found no significant linear correlation betweeen ant mortality ($r = -0.517$, $P > 0.10$, 9 d.f.) and ether doses ranging from 45 s to 4 min, or between ant mortality ($r = -0.422$, $P > 0.20$, 8 d.f.) and printing ink concentrations ranging from 1 to 8 g of ink per 50 ml of ether. Ants marked with 1–2 g concentrations of ink retained their marks for several days, while those marked with 5–8 g retained distinct marks even after 4 months. Finally high ink applications (four sprays) failed to affect mortality but did increase mark durability.

In field tests of the printing ink, the ink elicited temporary rejection behaviour,

TABLE 1. Comparison of forager, defender and composite population estimates ± standard deviation, for fifteen mounds

Mound	Forager estimate	Defender estimate	Composite estimate	Forager estimate as a percentage of defender estimate
1	493 ± 79	650 ± 50	669 ± 30	75.8
2	110 ± 10	178 ± 23	182 ± 7	61.8
3	357 ± 40	473 ± 48	572 ± 31	75.5
4	277 ± 20	374 ± 42	477 ± 23	74.1
5	255 ± 28	374 ± 33	430 ± 18	68.2
6	77 ± 12	55 ± 8	108 ± 9	140.0
7	189 ± 15	233 ± 19	329 ± 12	81.1
8	269 ± 35	503 ± 32	579 ± 25	53.5
9	135 ± 16	227 ± 25	231 ± 11	59.5
10	300 ± 50	473 ± 36	497 ± 23	63.4
11	76 ± 11	187 ± 11	205 ± 7	40.6
12	180 ± 19	192 ± 24	263 ± 15	94.2
13	173 ± 52	337 ± 36	365 ± 31	51.3
14	255 ± 50	353 ± 57	304 ± 20	72.2
15	155 ± 34	231 ± 27	297 ± 24	67.1
Means				
Estimate	220 ± 31*	323 ± 31*	367 ± 19*	71.9
Sample size	82.2	140.1	222.3	—
Per cent recapture	41.6	41.9	41.8	—

* Mean of standard deviations.

resulting in marked individuals being carried 0.1–2.0 m from the mound and released unharmed. This reaction lasted less than 2–3 h and injury to the marked individuals which usually returned quickly to the mound was never observed. Such rejection behaviour, however, was rarely observed when using fluorescent marker pen ink. Interior and callow ants marked in late July 1978 with the marker pen ink were recaptured foraging in late June 1979 still clearly marked after 11 months.

Social structure

Foragers and defenders form two overlapping populations of unequal size. The estimated forager population averaged 72% of the defender population (Table 1). Foragers were always more likely to be recaptured foraging, although many were recaptured defending. Conversely, defenders were always more likely to be recaptured defending, but many were also recaptured foraging. Composite estimates, calculated after summing forager and defender mark–recapture data, were only slightly larger than defender estimates because of the partial overlap between these populations (Table 1).

The forager population was further subdivided into groups according to the trunk trails they were using. Only 3–6% of the foragers shifted trails each day (Table 2); however, this shift is cumulative so that after 5 days 23% of the marked foragers had shifted trails. These observations agree with similar results reported by Hölldobler (1976) for *Pogonomyrmex barbatus* (Smith) and *Pogonomyrmex rugosus* Emery.

TABLE 2. Drift of marked foragers between trunk trails

Day	Recapture sample size	Marked recaptures			
		Total	No. shifted	% shifted	% shifted/day
1	168	93	3	3.2	3.2
3	214	67	11	16.4	5.5
5	177	30	7	23.4	4.7

The defenders were stratified according to order of emergence from the mound. Defensive ants marked emerging in a particular sequence were most likely to be recaptured emerging in that same sequence (Table 3). Because the defenders were stratified by order of emergence, increasing the size of the mark–recapture samples also increased the estimated size of the defender population (Fig. 1).

Excavations

The percentage of marked ants remaining in a colony from the initial marking was a function of the time elapsed between marking and excavation (Fig. 2). Very few ants remained from samples marked more than 2 weeks prior to excavation. Of the marked ants found while excavating, 89% were removed in the defender samples taken just prior to excavation. The remaining 11% were removed from the mound or just beneath it, even though the majority of the colony was still

TABLE 3. The stratification of successively marked defenders in successive recapture samples; data represent average percentages of three colonies

Marking samples	Per cent of recapture sample				
	1	2	3	4	5
First	29.6*	23.0	14.3	13.2	11.7
Second	21.7	30.4*	18.8	6.6	5.5
Third	11.8	21.6*	21.4	15.2	11.0
Fourth	15.8	16.2	18.2	24.5*	18.6
Fifth	8.6	6.1	14.3	13.9	25.5*
Unmarked	12.5	2.7	13.0	26.5	27.6*
Total	100.0	100.0	100.0	100.0	100.0

* Highest recapture percentage for each mark sample.

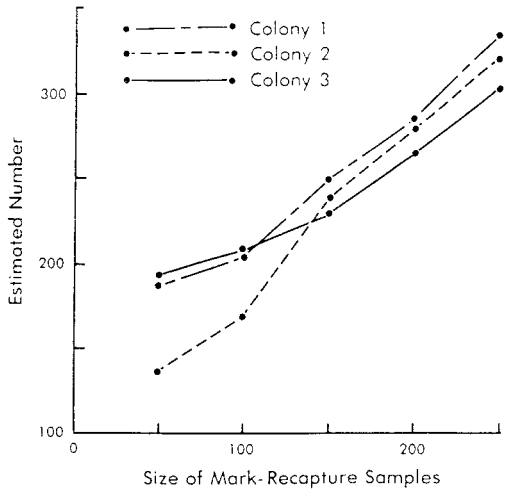

FIG. 1. Effect of increasing the size of mark–recapture samples on the estimated numbers of defenders: •–.–•, Colony 1; •– – –•, Colony 2; •——•, Colony 3.

well below this level. Finally, the estimated forager and defender populations were correlated ($P < 0.001$) with the total population of adults in the excavated mounds (Fig. 3).

Discussion

Because of its application efficiency, durability and colour variety, the fluorescent ink marking procedure has potential for use in future studies of ant colony populations and social structure. Different mixtures and colour combinations of fluorescent printing ink allow large numbers of ants in at least eight groups in a colony to be quickly and distinctly marked for periods exceeding 3 months. Fluorescent printing ink marks had no significant effect on mortality and only a temporary effect on the behaviour of *P.owyheei*, but possible effects on other species need to be tested. Although Weir (1957) reported that heavy use of ether increased mortality in *Myrmica rubra* Linn., we found no evidence that any level of etherization used in this study affected *P.owyheei* mortality or behaviour, even when repeated every fourth day for a month.

Harvester ant colonies were not homogeneous but rather highly structured populations. As in previous investigations, we found that surface-active marked ants rarely ventured below mound level (Golley & Gentry, 1964; Erickson, 1972). We also observed that foragers and defenders form two overlapping but distinctive populations with the foragers averaging 72% of the estimated defender population. Furthermore, foragers were subdivided by the trunk trails on which they foraged and defenders were stratified by their order of emergence in defence behaviour.

As a consequence of these divisions, sampling for the Lincoln index and other

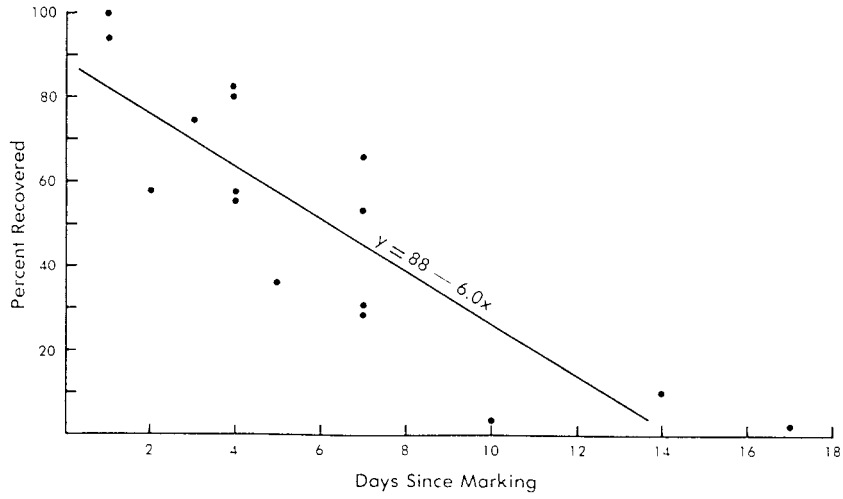

FIG. 2. Per cent of marked ants recovered by excavation, as a function of the days since marking and releasing the sample ($r = 0.85$, $P < 0.001$).

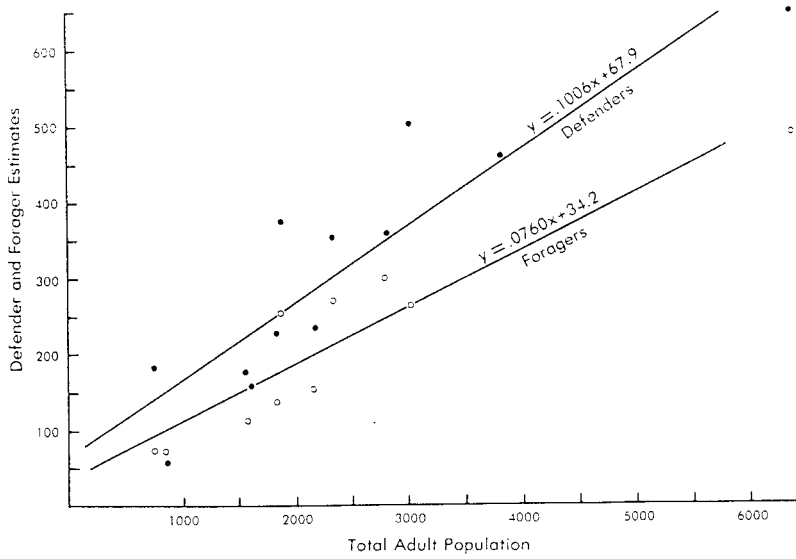

FIG. 3. Correlations between defender (solid circles, $r = 0.93$, $P < 0.001$) and forager (open circles, $r = 0.90$, $P < 0.001$) population estimates, and the total adult population determined by excavation.

mark–recapture studies should be stratified according to the colony's social structure. Foraging workers should be sampled separately from defenders. Also, foragers should be sampled so that each trunk trail is separately marked or proportionately represented. Defenders should be collected in comparably sized marking and recapture samples with the sampling effort adjusted to their attack response to avoid unnecessarily inflating or deflating the defender population estimates (Fig. 1). While potentially more subjective, estimating defenders was more efficient than estimating foragers because a sufficient defender sample could be obtained in less than 25% of the time necessary to sample foragers.

The strong correlation between the percentage of marked ants recovered and the time since marking suggests that the average life expectancy of foragers may be as short as 2 weeks (Fig. 2). As a result, recapture sampling should be done within 1 day to minimize the effects of high forager mortality. These studies indicate that mortality, and not continued dispersal, explains the increasing population estimates reported by Golley & Gentry (1964) and Erickson (1972) in their continuous tagging experiments. High mortality probably also contributes to the 'mark loss' reported by Chew (1959) and Kruk-de Bruin *et al.* (1977).

Finally we established that there is a significant correlation between the number of foragers and defenders and the total adult population of a colony (Fig. 3). Kruk-de Bruin *et al.* (1977) found a similar correlation between foragers and total colony populations of fourteen laboratory and three field colonies of *Formica polyctena* Forst. Even though total harvester ant colony population cannot be directly estimated with the mark–recapture method, forager and defender estimates appear to be good indices of total colony population.

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References

Ayre, G.L. (1962) Problems in using the Lincoln index for estimating the size of ant colonies

(Hymenoptera: Formicidae). *Journal of the New York Entomological Society*, 70, 159–166.

Bailey, N.T.J. (1951) On estimating the size of the mobile populations from recapture data. *Biometrika*, 38, 293–306.

Brian, M.V. (1950) The stable winter population structure in species of *Myrmica*. *Journal of Animal Ecology*, 19, 119–123.

Chew, R.M. (1959) Estimation of ant colony size by the Lincoln index method. *Journal of the New York Entomological Society*, 67, 157–161.

Erickson, J.M. (1972) Mark–recapture techniques for population estimates of *Pogonomyrmex* ant colonies: An evaluation of the ^{32}P technique. *Annals of the Entomological Society of America*, 65, 57–61.

Golley, F.B. & Gentry, J.B. (1964) Bioenergetics of the Southern Harvester Ant, *Pogonomyrmex badius*. *Ecology*, 45, 217–225.

Hölldobler, B. (1976) Recruitment behaviour, home range orientation and territoriality in harvester ants, *Pogonomyrmex*. *Behavioral Ecology and Sociobiology*, 1, 3–44.

Jorgensen, C.D. (1979) Raft River Environmental Report. Report to EG & G Idaho, Inc. and Department of Energy.

Kruk-de Bruin, M., Röst, L.C.M. & Draisma, F.G.A.M. (1977) Estimates of the number of foraging ants with the Lincoln-index method in relation to the colony size of *Formica polyctena*. *Journal of Animal Ecology*, 46, 457–470.

Lavigne, R.J. (1969) Bionomics and nest structure of *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *Annals of the Entomological Society of America*, 62, 1166–1175.

Leslie, P.H. (1952) The estimation of population parameters from data obtained by means of the capture–recapture method. II. The estimation of total numbers. *Biometrika*, 39, 363–388.

Lincoln, F.C. (1930) Calculating water fowl abundance on the basis of banding returns. *U.S. Department of Agriculture Circular*, 118, 1–4.

Pickles, W. (1940) Fluctuations in the populations, weights and biomasses of ants at Thornhill, Yorkshire, from 1935–1939. *Transactions of the Royal Entomological Society of London*, 90, 467–485.

Spangler, H.G. (1973) Ant nests for observation and study. *Annals of the Entomological Society of America*, 66, 691–692.

Stradling, D.J. (1970) The estimation of worker ant populations by the mark–release–recapture method: An improved marking technique. *Journal of Animal Ecology*, 39, 575–591.

Weir, J.S. (1957) Effect of anaesthetics on workers of the ant *Myrmica*. *Journal of Experimental Biology*, 34, 464–468.

Whitford, W.G. & Ettershank, G. (1975) Factors affecting foraging activity in Chihuahuan desert harvester ants. *Environmental Entomology*, 4, 689–696.

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Current Contact Information (Oct. 2004)
Sanford D. Porter

Mailing Address:
USDA-ARS, CMAVE
P.O. Box 14565
Gainesville, FL 32604 USA

Street Address:
USDA-ARS, CMAVE
1600 S.W. 23rd Drive
Gainesville, FL 32608 USA

Office: 352 374-5914
Secretary: 374-5903
FAX: 374-5818
E-mail: sdp@nersp.nerdc.ufl.edu (preferred)
E-mail: sdporter@gainesville.usda.ufl.edu (alternate)
Official Web Site: http://www.ars.usda.gov/pandp/people

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