

Characterizing Movement of Ground-Dwelling Arthropods with a Novel Mark-Capture Method Using Fluorescent Powder

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Abstract A major knowledge gap exists in understanding dispersal potential of ground-dwelling arthropods, especially in forest ecosystems. Movement of the ground-dwelling arthropod community was quantified using a novel mark-capture technique in which three different colored fluorescent powders in two separate mixtures were applied to the floor of a deciduous forest in concentric bands 3, 8, and 15 m from the center of 30 × 30 m experimental plots. The majority (67.1%) of ground-dwelling arthropods did not cross a colored band when fluorescents were mixed with protein powder in 2014. However, when mixed with sand in 2015, 77.3% of captured arthropods were marked with fluorescent powder, with the majority of individuals crossing one band (41.2%), suggesting limited dispersal by most individuals in the community. Only 2.8% and 15.0% of arthropods crossed all three bands in 2014 and 2015, respectively, which further indicates that individuals have limited dispersal. Responses were taxon-specific, and a high proportion of some arthropods such as millipedes and harvestmen crossed two or three bands. Limited dispersal by most individuals may have important implications for the structure and distribution of ground-dwelling arthropod communities, as well as their responses to natural or

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anthropogenic disturbances. Our results demonstrate the feasibility of this novel technique for self-marking and capturing individuals in the field to investigate dispersal of ground-dwelling arthropods.

Keywords Dispersal · forest floor · insects · invertebrates · sampling

Introduction

Dispersal is a fundamental process influencing population dynamics, patterns of diversity, community assembly, species distributions, and response to disturbances (Clobert et al. 2001). Dispersal potential is a key life history trait that affects a species' ability to move within and between habitat patches for foraging or reproduction (Bowne and Bowers 2004). Terrestrial arthropods may actively disperse by walking and flying, or they may passively disperse through phoresy and ballooning (Southwood 1962). Knowledge of dispersal capacity is necessary to understand how individuals move throughout homogeneous, heterogeneous or fragmented landscapes, and respond to environmental change caused by natural and anthropogenic disturbances (Niemelä 2001; Reigada et al. 2015; Jönsson et al. 2016).

The dispersal potential of ground-dwelling arthropods has been understudied especially in forest ecosystems, and few empirical data are available to describe their movement (Brouwers and Newton 2009). Small ground-dwelling arthropods that inhabit more stable undisturbed environments are considered to be more vulnerable to habitat fragmentation and disturbance if their dispersal potential is limited (Niemelä 2001). Previous dispersal studies are biased towards specific insect taxa such as Carabidae with major knowledge gaps existing for other arthropods (Brouwers and Newton 2009), perhaps due to difficulties quantifying dispersal of taxonomic groups that have cryptic or nocturnal lifestyles. Many ground-dwelling arthropod taxa are flightless, and disperse primarily by walking or running.

A variety of techniques have been used to quantify dispersal of arthropods. Mark-release-recapture methods require mass rearing of individuals in the laboratory or collection of living individuals in high numbers from the field (Hagler and Jackson 2001). Arthropods are then marked with a paint (Wojcik et al. 2000; De Souza et al. 2012), dye (Graham and Mangum 1971; Haagsma and Rust 1993), or dust (Stern and Mueller 1968; Narisu et al. 1999) and released into the environment. Individuals are recaptured at specific time intervals, and/or at distances from the release point and inspected for the marker (Hagler and Jackson 2001). These dispersal methods are most feasible for one or a few species, but pose significant logistical challenges for investigating the movement of members of an entire community.

Mark-capture techniques differ from mark-release-recapture methods in that arthropods self-acquire the marker in the field where it is applied directly to vegetation or other substrates. Thus, individuals only have to be captured once, which increases the sampling efficiency of the study. This is a significant advantage over mark-release-recapture methods, which generally have low probabilities of recapture (Rieske and Raffa 1990; Muir and Kay 1998; Nazni et al. 2005).

Inexpensive markers that can be easily applied to a large area are ideal for mark-capture studies (Hagler and Jackson 2001). Recent development of protein-based markers, including soy protein, bovine casein, chicken egg albumin, and wheat gluten, provides an affordable and easy method to mark arthropods in the field (Jones et al. 2006; Jones et al. 2011; Sivakoff et al. 2012; Sivakoff et al. 2016). Following marker application, individuals are collected and tested for the presence of the protein using enzyme-linked immunosorbent assay (ELISA) (Horton et al. 2009; Hagler and Jones 2010). However, this method also provides challenges for investigating movement of ground-dwelling arthropods. For example, the markers are not visible when using the proteins in liquid form. Following application, invisible proteins could be accidentally transferred to unmarked locations by investigators. When using the markers in powdered form, some ground-dwelling arthropod taxa such as millipedes consume the protein (KI Perry, personal observation).

Fluorescent powders have been used for years in mark-capture studies to investigate the movement of insects (Musgrave 1950; Hogsette 1983; Dodds and Ross 2002; Coviella et al. 2006). Brightly colored powders can be applied directly in the field where they are acquired by arthropods, and detected in the laboratory under ultra-violet light.

In this study, we used fluorescent powder in a novel self-mark and capture technique to characterize the movement of ground-dwelling arthropods in a forest community. The objectives of this study were to 1) assess the feasibility of this technique by determining if ground-dwelling arthropods acquired the fluorescent powder in the field, and whether the powder mixed with protein powder or sand could be detected on a variety of taxa; and 2) characterize the dispersal potential of ground-dwelling arthropod taxa.

Materials and Methods

Study Area

The study was conducted in forests at Powdermill Nature Reserve (PNR) in Rector, Westmoreland County, Pennsylvania, USA. PNR is located in the Laurel Highlands, and was established in 1956 as the field research station of the Carnegie Museum of Natural History. The reserve includes approximately 900 ha of natural habitat of which most is largely contiguous temperate deciduous forest comprised of maple (*Acer* spp.), oak (*Quercus* spp.), beech (*Fagus* spp.), poplar (*Populus* spp.), and hickory (*Carya* spp.) (Murphy et al. 2015). Spicebush (*Lindera benzoin* (L.) Blume), multiflora rose (*Rosa multiflora* Thunb.), and Japanese barberry (*Berberis thunbergii* DC) are abundant understory shrubs along with a diverse community of other herbaceous and woody shrub species, including violet (*Viola* spp.), blackberry (*Rubus allegheniensis* Porter), round lobed hepatica (*Hepatica americana* (DC)), common cinquefoil (*Potentilla* spp.), dewberry (*Rubus hispidus* L.), partridgeberry (*Mitchella repens* L.), bedstraw (*Galium* spp.), sedges (*Cyperaceae* spp.), nettle (*Urtica* spp.), greenbrier (*Smilax* spp.), and several species of ferns (*Polystichum acrostichoides* (Michx.), *Dennstaedtia punctilobula* (Michx.), *Thelypteris noveboracensis* (L.), and *Dryopteris* spp.).

Experimental Design

Pink, blue, and orange fluorescent powders (Rocket Red Pigment A-13 N, Horizon Blue A-19 N, Arch Yellow Pigment A-16 N; DayGlo Color Corp., Cleveland, Ohio) were applied to the forest floor in concentric bands located at 3, 8, and 15 m, respectively, from the center of 30 × 30 m experimental plots ($n = 18$) (Fig. 1). In 2014, each fluorescent powder was mixed with a corresponding dry protein powder (1:8 fluorescent powder to protein powder) to act as a double marker. Protein powders were soy protein (Soy Protein Isolate Powder, Good 'N Natural; Bohemia, New York), bovine casein (Great Value Brand Nonfat Instant Dry Milk; Bentonville, Arkansas), and chicken egg albumin (Honeyville Farms Powdered Egg Whites; Brigham, Utah). The experiment was repeated in 2015, but fluorescent powders were mixed with sand (1:8 fluorescent powder to Quikrete Play Sand; The Quikrete Companies, Atlanta, Georgia) rather than proteins. Sand was incorporated as an adjuvant to improve adhesion of the dust to arthropods (Reinecke 1990).

Flags were placed at 3, 8, and 15 m from the center of each plot in each cardinal direction to guide powder application. Additional flags were occasionally added if the understory vegetation was dense. Bands of powder approximately 0.4 m in diameter were applied to the forest floor using an 18 cm mesh strainer (Fig. 2a). Detectable levels of fluorescent powder were acquired by ground-dwelling arthropods during application or through contact with marked surfaces. In 2014, the experiment was conducted on 14–17 July and repeated on 18–22 August; in 2015, the experiment was conducted on 13–17 July and repeated on 10–14 August.

The fluorescent powder had a fine texture that created dust when handled. Therefore, extreme caution was taken during application to limit cross-contamination of the powders within and between experimental plots. Fluorescent powders were transported in Ziploc bags secured in backpacks. Small amounts of powder were added to the

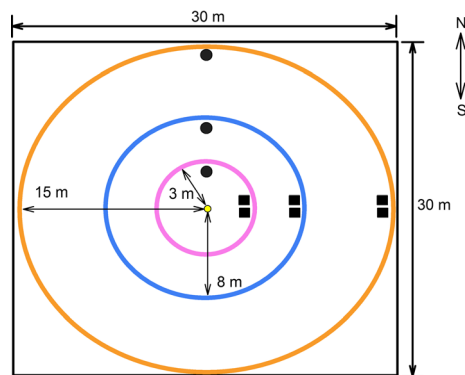


Fig. 1 Design schematic for the mark-capture experiment in forest experimental plots (30 × 30 m) at Powdermill Nature Reserve. Different colored circles represent different fluorescent powders [DayGlo®; pink (Rocket Red Pigment A-13 N), blue (Horizon Blue A-19 N), and orange (Arch Yellow Pigment A-16 N)], applied at 3 m (pink, inner band), 8 m (blue, middle band), and 15 m (orange, outer band) from the center of experimental plots (yellow filled circle). Arthropods were hand collected in 1 × 1 m sampling stations located in each cardinal direction just inside each band of fluorescent powder, although only one cardinal direction is depicted. In 2014, arthropods were collected in the same 1 × 1 m sampling station after 24 and 48 h (represented by black circles). In 2015, arthropods were collected in two adjacent 1 × 1 m sampling stations, one after 24 h (left) and the other after 48 h (right) (represented by black squares)



Fig. 2 Fluorescent powders were applied to the forest floor using 18 cm mesh strainers (**a**; Photo credit: Pamela Curtin). Ground-dwelling arthropods were sampled via five minute hand collections in 1×1 m sampling stations just inside each band of powder in each cardinal direction (**b**). Arthropods were collected meticulously and placed singly in microcentrifuge tubes. Ground-dwelling arthropods were examined microscopically under ultra-violet black light to detect the fluorescent powders; polydesmid millipede with blue fluorescent powder (**c**), ground beetle elytra with pink fluorescent powder (**d**), and polydesmid millipede with orange and pink fluorescent powders (**e**)

strainers using measuring cups, and the strainers were held within 0.3 m to the ground during application. Protective suits and gloves were removed immediately following each powder application and secured in large garbage bags along with the strainers, measuring cups, and bags of powder. All materials used for applying a specific color of powder were isolated from those of other colors.

Arthropod Sampling and Processing

Arthropods were sampled during five minute hand collections at 24 and 48 h following powder application in a 1×1 m quadrat (i.e. sampling station) just inside each band of powder at each cardinal direction adjacent to flags (Fig. 2b), resulting in 12 collections per plot per sampling event. Arthropods collected adjacent to each colored band were pooled across cardinal directions for each experimental plot and for each time interval. In 2014, hand collections occurred at the same sampling stations within each band at 24 and 48 h. In 2015, ground-dwelling arthropods were collected just inside each band of powder, but at different sampling stations; to the left of the flag after 24 h and to the right of the flag after 48 h to avoid any effects of disturbance from the hand collections (Fig. 1). Hand collections involved active searching for arthropods by moving leaf litter, rocks, and small pieces of woody debris, but soil was not displaced. After the five minute time period, all debris was replaced to pre-collection conditions to cover the soil on the forest floor. Arthropods were collected individually using toothpicks and stored

singly in 1.5 ml microcentrifuge tubes. Specimens were kept in a cooler in the field and then transferred to a -80°C freezer until further processing.

Fluorescent powder was detected on arthropods microscopically under ultra-violet black light (Fig. 2c-e). Arthropods occasionally were coated in fluorescent powder, but more often a few flecks of powder were present. Therefore, microscopic examination of arthropods was used to detect the powder. To limit cross-contamination between specimens, arthropods were inspected for powders individually in weighing dishes using a new toothpick to manipulate each specimen. Arthropods were scored positive if fluorescent powder was detected on the body. Depending on the number of powders detected, arthropods were scored as having crossed one, two, or three bands. If powder was identified on debris that was collected with the arthropod, but not on the arthropod itself, it was scored as negative. It was apparent during processing that some of the arthropods had consumed the protein markers. Therefore, protein markers were ignored and only presence and absence of fluorescent powder was scored. Millipedes and insects were identified to family using Shear (1999) and Triplehorn and Johnson (2005), respectively, and other arthropods were identified to order.

Statistical Analyses

Chi-squared analyses were used to determine the probability of arthropods crossing 0, 1, 2, or 3 bands of fluorescent powder at 24 and 48 h following application in 2014 and 2015. The interaction between the number of colored bands crossed (0, 1, 2, or 3 bands) and the collection location (3, 8, or 15 m sampling stations) was analyzed to determine arthropod movement patterns within the experimental plots. Analyses were conducted for total arthropods to identify general trends and for each arthropod taxon with ≥ 30 individuals collected to identify group-specific patterns. Chi-squared analyses were also conducted to compare the two experiments in July and August of each year. Data were checked for statistical assumptions of normality and homogeneity of variance. Each year was analyzed separately. Chi-squared analyses were conducted using SAS software (SAS 2014–2016).

Results

Over the two years of the study, a total of 6581 arthropods comprising 13 arthropod orders and 11 insect families were collected, with 4134 collected in 2014 and 2447 in 2015. Fluorescent powder was detected on at least one individual of every taxon collected. In 2014, the majority of arthropods collected (67.1%) did not cross a band of fluorescent and protein powder ($\chi^2 = 4383.2$, $\text{df} = 3$, $P < 0.001$). Powder was detected on only 32.9% of individuals collected; 25.0% were marked with one color, 5.1% with two colors, and 2.8% with all three colors (Table 1). In 2015, the fluorescent powder mixed with sand was detected on 77.3% of individuals collected with 41.2%, 21.1% and 15% marked with one, two, and three colors, respectively ($\chi^2 = 372.4$, $\text{df} = 3$, $P < 0.001$) (Table 1).

Individuals marked with only one color were collected in higher numbers at stations adjacent to that particular band rather than adjacent to other bands (Fig. 3). Of those arthropods that crossed two colored bands (pink and blue, or blue and orange), similar

Table 1 Ground-dwelling arthropod taxa that crossed 0, 1, 2, or 3 bands of fluorescent powder in 2014 and 2015 in forests at Powdermill Nature Reserve in Rector, Westmoreland County, Pennsylvania, USA

| Arthropod Taxa | | 2014 | | | | 2015 | | | | Total |
|----------------|-------------------|-------------------|---------|--------|---------|---------|---------|--------|---------|---------|
| Class | Order | Family | 0 Bands | 1 Band | 2 Bands | 3 Bands | 0 Bands | 1 Band | 2 Bands | 3 Bands |
| Chilopoda | Geophilomorpha | | 29 | 6 | 1 | 0 | 0 | 0 | 0 | 1 |
| | Lithobiomorpha | | 95 | 23 | 1 | 0 | 35 | 36 | 12 | 5 |
| Diplopoda | Scolopendromorpha | | 33 | 2 | 1 | 0 | 2 | 0 | 1 | 1 |
| | Callipodida | Abacionidae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Chordeumatida | Caseyidae | 69 | 20 | 0 | 0 | 5 | 34 | 25 | 8 |
| | Julida | Julidae | 99 | 24 | 6 | 0 | 29 | 31 | 12 | 2 |
| | | Parajulidae | 494 | 122 | 12 | 1 | 113 | 171 | 93 | 20 |
| | Polydesmida | Paradoxosomatidae | 356 | 378 | 142 | 103 | 9 | 78 | 91 | 188 |
| | | Polydesmidae | 331 | 124 | 16 | 6 | 13 | 41 | 22 | 13 |
| | | Xystodesmidae | 8 | 0 | 0 | 0 | 2 | 4 | 3 | 4 |
| | Polyzoniida | Polyzoniidae | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Spirobolida | Spirobolidae | 54 | 14 | 0 | 0 | 2 | 7 | 11 | 5 |
| Malacostraca | Isopoda | | 42 | 13 | 1 | 0 | 7 | 24 | 8 | 2 |
| Arachnida | Araneeae | | 295 | 104 | 7 | 1 | 76 | 229 | 89 | 37 |
| | Opiliones | | 50 | 41 | 9 | 5 | 1 | 10 | 19 | 37 |
| Collembola | | | 188 | 24 | 2 | 0 | 112 | 113 | 45 | 9 |
| Insecta | Coleoptera | Carabidae | 89 | 40 | 6 | 0 | 12 | 47 | 20 | 13 |
| | | Curculionidae | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| | | Elateridae | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 |
| | | Nitidulidae | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| | | Scarabaeidae | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| | | Silphidae | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |

Table 1 (continued)

| Arthropod Taxa Class | Order | Family | 2014 | | | 2015 | | | 1 Band | 2 Bands | 3 Bands | Total |
|-------------------------|------------|------------------|---------|--------|---------|---------|---------|--------|--------|---------|---------|-------|
| | | | 0 Bands | 1 Band | 2 Bands | 3 Bands | 0 Bands | 1 Band | | | | |
| Hymenoptera | Orthoptera | Staphylinidae | 22 | 9 | 0 | 0 | 1 | 3 | 0 | 0 | 1 | 36 |
| | | Tenebrionidae | 56 | 18 | 1 | 0 | 15 | 33 | 16 | 3 | 3 | 142 |
| | | Formicidae | 433 | 52 | 1 | 1 | 118 | 133 | 33 | 8 | 8 | 779 |
| | | Gryllidae | 6 | 5 | 0 | 0 | 1 | 1 | 3 | 2 | 2 | 18 |
| Total | | Rhaphidophoridae | 16 | 14 | 3 | 1 | 1 | 6 | 9 | 8 | 8 | 58 |
| | | | 2770 | 1035 | 211 | 118 | 555 | 1007 | 516 | 369 | 369 | 6581 |

numbers were collected at the sampling stations adjacent to the 3 and 8 m bands, and at the sampling stations adjacent to the 8 and 15 m bands, respectively, in both years (Fig. 3). More arthropods that crossed all three bands of fluorescent powder were collected at the 3 m sampling station than at the 8 and 15 m sampling stations (Fig. 3). In both years, 4.4% of the total number of arthropods collected had some form of inconsistencies between the color of powders detected on their bodies and the color of the band adjacent to the sampling station (3, 8, or 15 m from plot center) where they were collected. For example, powder from the pink band (inner band), but not the blue band (middle band), was detected on a very low proportion of arthropods collected adjacent to the orange band (outer band) at the 15 m sampling station.

Although these general trends were observed for total ground-dwelling arthropods, movement patterns varied within individual taxa. Two families of millipedes, Paradoxosomatidae (Order Polydesmida) and Parajulidae (Order Julida), were the most abundant arthropod taxa collected, and Paradoxosomatidae also had the highest number of individuals marked with powder (Table 1). The number of bands crossed was a highly significant factor for all arthropod taxa across both years ($P < 0.010$). In 2014, the majority of Parajulidae, Polydesmidae, and Formicidae collected did not cross a band, whereas slightly more Paradoxosomatidae crossed one than zero bands (Fig. 4, top). Most predators (Araneae, Opiliones, Carabidae, and Lithobiomorpha) did not cross a band of fluorescent powder, but more Opiliones were marked than other taxa (Fig. 4, top). In 2015, similar numbers of Formicidae and Collembola crossed zero or one band of fluorescent powder, while more individuals of Parajulidae crossed one band and Paradoxosomatidae crossed three bands (Fig. 4, bottom). A variety of patterns were observed for predators in 2015 (Fig. 4, bottom). Most Araneae and Carabidae crossed only one band of fluorescent powder, but individuals with two or three powders were collected. Powder was detected on all but one individual Opiliones, and most individuals crossed three bands of powder.

Total arthropods ($\chi^2 = 82.5$, $df = 1$, $P < 0.001$) as well as 10 arthropod taxa were collected in greater numbers after 24 h than after 48 h in 2014. A total of 2359

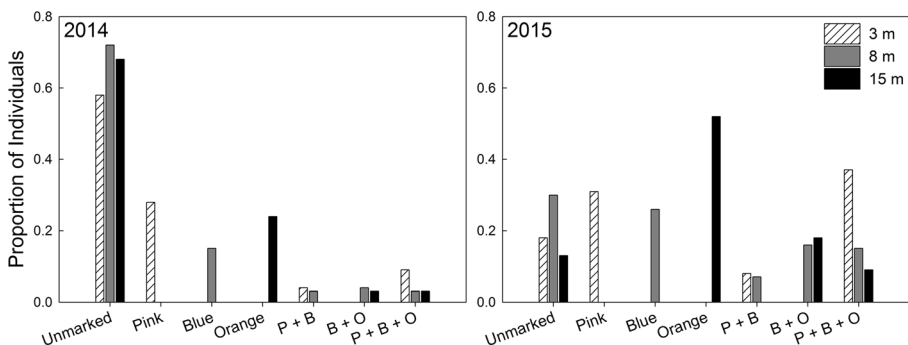


Fig. 3 Proportion of total ground-dwelling arthropods collected at the 3 m (white-striped bars), 8 m (gray bars), and 15 m (black bars) sampling stations that were unmarked (no powder detected), marked with pink powder only (crossed inner band), blue powder only (crossed middle band), orange powder only (crossed outer band), or marked with a combination of colors in 2014 (left) and 2015 (right). Fluorescent powder combinations included individuals that crossed the pink and blue bands (P + B; inner and middle bands), the blue and orange bands (B + O; middle and outer bands), and all three bands (P + B + O; inner, middle and outer bands)

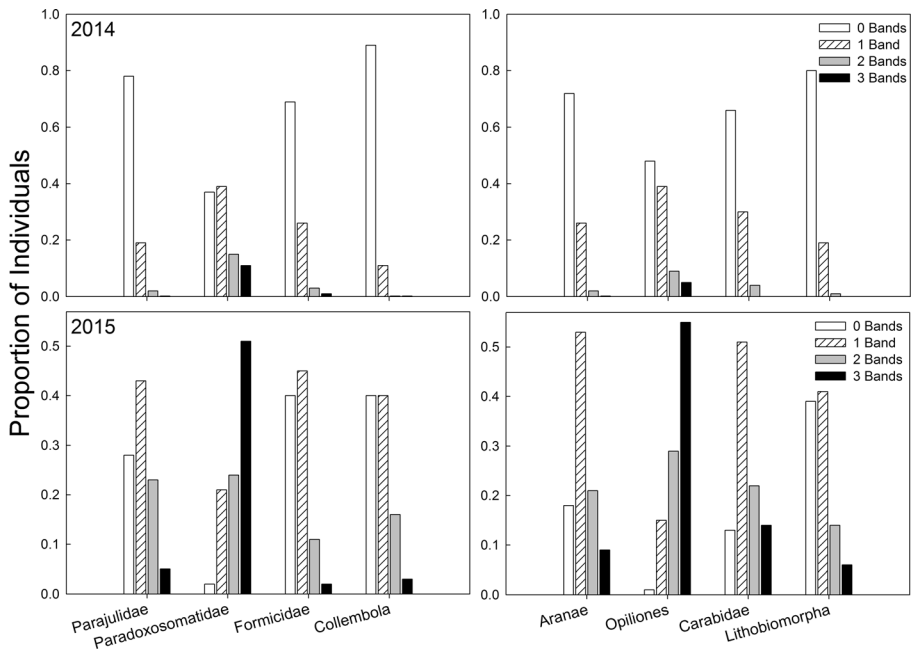


Fig. 4 Proportion of individuals within the most abundant arthropod taxa (*left*) and taxa of common predators (*right*) that were unmarked (crossed 0 bands) or marked (crossed 1, 2, or 3 bands) with fluorescent powder in 2014 (*top*) and 2015 (*bottom*)

individuals were collected 24 h following powder application, while 1775 were collected after 48 h. Geophilomorpha, Scolopendromorpha, Julidae, Parajulidae, Paradoxosomatidae, Polydesmidae, Araneae, Collembola, Carabidae, and Formicidae were all collected in greater numbers 24 h following application ($P = 0.047$ – < 0.001), regardless of whether or not they were marked with powder. This pattern was not observed in 2015, and the total number of arthropods collected after 24 and 48 h was similar ($\chi^2 = 2.42$, $df = 1$, $P = 0.119$). A total of 1262 individuals were collected after 24 h, while 1185 were collected after 48 h. However, collection time was a significant factor for three ground-dwelling arthropod taxa in 2015. Formicidae ($\chi^2 = 5.22$, $df = 1$, $P = 0.022$) and Araneae ($\chi^2 = 6.03$, $df = 1$, $P = 0.014$) were collected in greater numbers 24 h following fluorescent powder application, whereas Paradoxosomatidae were collected in greater numbers after 48 h ($\chi^2 = 5.28$, $df = 1$, $P = 0.021$).

Ground-dwelling arthropods crossed more bands of fluorescent powder after 48 than 24 h in 2014 (Fig. 5a; $\chi^2 = 33.7$, $df = 3$, $P < 0.001$). The same pattern was observed for Geophilomorpha ($\chi^2 = 9.9$, $df = 2$, $P = 0.007$), Paradoxosomatidae ($\chi^2 = 43.3$, $df = 3$, $P < 0.001$), Polydesmidae ($\chi^2 = 9.2$, $df = 3$, $P = 0.025$), and Staphylinidae ($\chi^2 = 4.1$, $df = 1$, $P = 0.041$). Opiliones collected at 48 h were more likely to have crossed one band of fluorescent powder, but individuals that had crossed two or three bands were found more often after 24 h. A different pattern was observed for Carabidae where individuals that had crossed one band were collected more often after 24 h, but individuals that crossed two bands were collected more often after 48 h ($\chi^2 = 5.2$, $df = 2$, $P = 0.072$). Time had no effect on the number of bands crossed by individuals of other taxa in 2014. In 2015, this pattern was not observed for total ground-dwelling

arthropods (Fig. 5b; $\chi^2 = 6.75$, $df = 3$, $P = 0.080$), but more Parajulidae collected after 24 h were unmarked while more individuals had one powder after 48 h ($\chi^2 = 10.2$, $df = 3$, $P = 0.016$).

In 2014, ground-dwelling arthropods crossed more bands of fluorescent powder overall during the second experiment in August than during the first experiment in July ($\chi^2 = 491.3$, $df = 3$, $P < 0.001$). In July, one color was detected on 366 individuals, two colors on 23 individuals, and three colors on one individual. The number of marked individuals increased in August with one color detected on 669 individuals, two on 188 individuals, and three on 117 individuals. In 2015, more arthropods crossed bands during the first experiment in July (1060 individuals) than during August (832 individuals) ($\chi^2 = 296.3$, $df = 3$, $P < 0.001$). However, 282 arthropods collected in August crossed three bands, whereas three colors were detected on only 86 individuals in July.

Discussion

Our results demonstrate that this novel mark-capture technique is feasible and effective for investigating the movement of ground-dwelling arthropods in the field. We were able to investigate the movement of many diverse taxa within the community rather than just one or a few species. Our findings also suggest that the dispersal potential of ground-dwelling arthropods was generally limited, but some mobile taxa such as millipedes and harvestmen moved greater distances.

Dispersal was indicated by the presence of color and the number of different colors found on ground-dwelling arthropods. In 2014 when fluorescent powders were mixed with protein powder, 32.9% of individuals collected were marked, and this increased to 77.3% when powders were mixed with sand in 2015. In both years, at least one individual of each taxon collected was marked with powder, and a much larger percentage of individuals crossed one band (25.0 and 41.2% in 2014 and 2015, respectively) than two (5.1 and 21.1% in 2014 and 2015, respectively) or three bands (2.5 and 15.0% in 2014 and 2015, respectively).

The high proportion of individuals that had not been marked or crossed only one band suggests that the dispersal potential of the ground-dwelling arthropod community

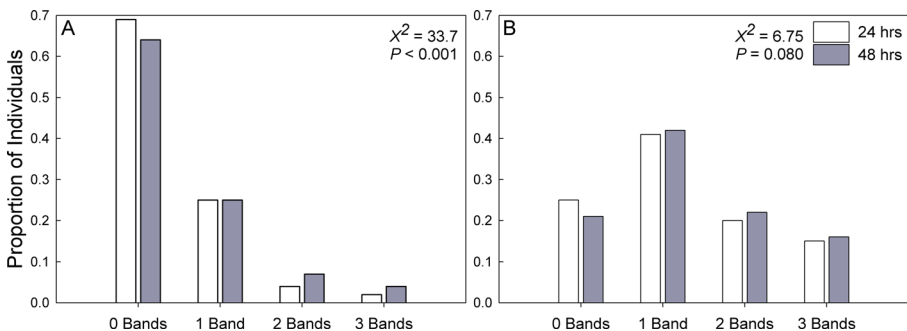


Fig. 5 Proportion of total ground-dwelling arthropods that were unmarked (crossed 0 bands) or marked (crossed 1, 2, or 3 bands) with fluorescent powder when collected at 24 h (white) and 48 h (gray) after application in 2014 (a) and 2015 (b). Statistical results are for chi-squared analyses

was generally limited. Unmarked individuals did not cross a band of fluorescent powder, suggesting the lowest dispersal distance. Arthropods that crossed one band of powder, either the pink, blue, or orange band, could have moved radially <1 m to 5 m, depending on the particular colored band that was crossed. The majority of individuals that crossed one band were collected at sampling stations adjacent to the colored band they were marked with, again suggesting limited dispersal.

The much smaller percentage of arthropods that crossed two colored bands could have moved radially at least 6 m to 10 m, depending on their collection location (3, 8, or 15 m sampling station) and the combination of bands crossed. In both years, arthropods that crossed the pink (inner) and blue (middle) bands were collected in similar numbers at the 3 m (inner) and 8 m (middle) sampling stations, suggesting that equal numbers of individuals were moving in both directions within the experimental plots. Similar patterns were observed for arthropods that crossed the blue (middle) and orange (outer) bands and were collected at the 8 m (middle) and 15 m (outer) sampling stations. However, in 2015, more arthropods crossed the blue and orange bands than the pink and blue bands, suggesting that the distance traveled by most individuals was towards the higher end of the 6 m to 10 m range.

The greatest dispersal potential was represented by the smallest percentage of individuals that crossed all three bands, indicating that they moved radially ≥ 13 m. In both years, arthropods that crossed all three colored bands were collected in greater numbers at the 3 m sampling station (adjacent to the inner band) than at the 8 m and 15 m sampling stations (adjacent to the middle and outer bands, respectively), suggesting arthropods were moving into the experimental plots. If arthropods were moving out of the experimental plots, we would have expected to collect more individuals marked with all three colored powders at the 15 m sampling station (adjacent to the outer band). However, this finding may be an artifact of lower sampling efficiency, which decreased as distance of the band from plot center increased. This is because proportion of area inside the band covered by the sampling station decreased as the area inside the band increased. Depending on the research objectives, it may be important to increase the area or number of sampling stations as the area encompassed by bands of fluorescent powder increases.

Previous dispersal studies are highly biased towards insects compared to other arthropod taxa, and largely focused on ground beetles (Brouwers and Newton 2009). We found that approximately 45% of ground beetles (Carabidae) collected in our two-year study were not marked, 38% were marked with one color, 11% were marked with two colors, and three colors were detected on only 13 individuals (6%), suggesting many ground beetles collected in this study did not move very far over the 48 h study period. These results are consistent with those of other studies that found higher dispersal potential of ground beetle species in more open or disturbed habitats than in forest ecosystems. When measured in the field via other methods, ground beetles moved an average of 5 to 20 m with some individuals dispersing up to 80 m, and species in grassy fields and agricultural landscapes moving the farthest (Mascanzoni and Wallin 1986; Wallin and Ekbohm 1988; Ranjha and Irmiler 2014). A meta-analysis conducted by Brouwers and Newton (2009) revealed a range of 0.6 to 18.4 m day⁻¹ for thirteen ground beetle species in a variety of habitats, with the average within-patch rate of movement for four species of forest ground beetles being 3.0 ± 2.6 m day⁻¹ (range: 0.6 to 8.5 m day⁻¹).

Although our results suggest movement of ground-dwelling arthropods generally was limited, some arthropod taxa expressed greater dispersal potential. A greater percentage of millipedes and harvestmen were marked than were other taxa, indicating the highest dispersal potential, while ants, centipedes, and springtails had the lowest. The millipede families Parajulidae and Paradoxosomatidae were the most abundant taxa collected, and Paradoxosomatidae had the highest proportion of individuals marked (over 70%) with fluorescent powder, which may be explained by their mobile foraging behavior. Millipedes are detritivores and feed primarily on organic debris such as decaying plant material in forest ecosystems (Coleman et al. 2004; David 2009). Senesced leaves and woody debris that cover the forest floor are fairly nutrient-poor, while more nutrient-rich resources such as fruits can be patchily distributed. There is some evidence that millipedes selectively feed on leaf litter high in calcium, and avoid detritus high in phenolic compounds (Neuhauser and Hartenstein 1978).

Surprisingly, arthropod predators that actively hunt for prey had a low proportion of individuals marked with fluorescent powder when mixed with protein powder in 2014. No centipedes or ground beetles collected, and only one individual spider (Araneae), were marked with all three colors. More spiders, ground beetles, and centipedes were marked in 2015 when fluorescent powder was mixed with sand, but usually with just one color.

Most harvestmen (Opiliones) were collected with no or only one color in 2014, suggesting limited dispersal even though they also are considered to be active predators (Adams 1984; Coleman et al. 2004). In 2015, much higher dispersal potential was observed for harvestmen, with all three colors detected on over 50% of individuals, which is more reflective of their active hunting behavior. More harvestmen that had crossed multiple bands were collected after 24 than after 48 h, perhaps because the most mobile individuals crossed the 15 m outer band and exited the plot.

This method also may have missed far-ranging individuals of other taxa if they exited the experimental plot within the 48 h time interval, although the majority of individuals collected were marked with only one color. Long distance dispersal of some arthropod taxa was likely not accounted for in the current design, as the distances moved by individuals originating from outside the experimental plots could not be assessed. Burrowing and nocturnal arthropods, such as many ground beetle species (Thiele 1977; Lövei and Sunderland 1996), may also be under-sampled by this technique. The spatial scale of this self-mark and capture method should be calibrated to the dispersal potential of the taxa under study.

Ants (Formicidae) were collected in relatively high numbers, which is expected owing to their general abundance and active foraging behavior of workers. As with harvestmen, fluorescent powder was detected on very few individuals in 2014. In 2015, however, a greater percentage of ants were marked, most with a single color, suggesting many were foraging within close proximity to their nests. Nests of *Aphaenogaster picea* (Wheeler) and *Lasius* spp. were occasionally found within the leaf litter or under rocks during hand collections. In 2015, two colors were detected on 33 ants, and only eight individuals had three, suggesting a smaller proportion foraged for resources at greater distances.

The much greater percentage of total arthropods collected that were marked in 2015 (77%) than in 2014 (33%) may be explained by the different marking procedures and sampling protocols used in each year. In 2014, protein powder was used as a secondary marker in combination with the fluorescent dust. Some arthropods were observed

consuming the powder, and the presence of the protein may have stimulated grooming in others. Alternatively, the protein powder may have been a poor adjuvant compared to the sand that replaced it in 2015. The protein could have adhered to the fluorescent powder, making it more sticky and difficult for arthropods to acquire as they crossed the bands.

Collection methods also varied between years, which may have contributed to the greater percentage of marked arthropods collected in 2015. In 2014, many more arthropods were collected after 24 than 48 h following application of the fluorescent powder, perhaps because arthropods were collected at the same sampling station at each time interval. Although great care was taken to restore the litter layer after collecting, arthropods may have been slow to recolonize areas disturbed by sampling. In 2015, the sampling protocol was changed such that arthropods were collected at adjacent sampling stations at the two time intervals, rather than in the same location. Following this change in 2015, the number of arthropods collected during the 48 h interval increased to numbers similar to the 24 h interval. This suggests that when using this marking technique, repeated collections should be made in different locations.

In 2014, a greater percentage of arthropods were marked with multiple colors when collected after 48 h than after 24 h following application of powder. However, this pattern was not observed in 2015, making it difficult to conclude whether more individuals traveled farther in 2014, overall movement was slower in 2014, or whether the difference between years was affected by the variation in methodology. Perhaps the time allotted for hand collections at the sampling stations in each cardinal direction could be increased and the number of sampling periods decreased, depending on the study objectives.

The number of marked arthropods also varied between the July and August sampling periods during both years. Rain occurred during the experiment in July 2014 and August 2015, which may explain why there were fewer individuals marked with powder during these sampling periods. Because of protection from the forest canopy, the powder can withstand light showers, but heavy rain can erase the bands. Therefore, extending arthropod sampling past 48 h following fluorescent powder application may increase the chances of losing the marker in the field.

In summary, we found that most ground-dwelling forest arthropods had limited dispersal potential, which may have important implications for the structure and distribution of ground-dwelling arthropod communities, such as limit their ability to recolonize habitats following natural and anthropogenic disturbances. We also found that this mark-capture technique using fluorescent powder is a feasible and efficient method for investigating the dispersal of ground-dwelling arthropods in natural habitats without having to rear, release, and recapture individuals. It allows for the convenient study of diverse arthropod taxa and community-level comparisons of dispersal ability. Another advantage is that it can be easily tailored to specific objectives by changing the spatial arrangement of the bands of fluorescent powder. For example, decreasing the distance between bands would allow for finer scale study of small ground-dwelling arthropods such as Collembola. Increasing the distance between bands may more accurately characterize movement of larger, active ground-dwelling arthropods such as harvestmen and millipedes.

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