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# Spatially aggregated parasitism on pea aphids, *Acyrthosiphon pisum*, caused by random foraging behavior of the parasitoid *Aphidius ervi*

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We investigated the role of the foraging behavior of the parasitoid wasp *Aphidius ervi* in producing nonrandom spatial patterns of parasitism among pea aphids, *Acyrthosiphon pisum*. We measured spatial variability in percent parasitism by determining the number of aphids and percent parasitism in 40 sampling plots (0.65-m² circles) located within a homogeneous alfalfa field. In one replicate of this experiment, mean parasitism of aphids was 18.7%, and percent parasitism showed density-independent aggregation (i.e., greater than random variability in percent parasitism among sampling plots). In the other replicate, mean parasitism was 56.3%, and percent parasitism was not aggregated among plots. We used a combination of field observations of parasitoid foraging and mathematical models to explore these results. In particular, we asked whether the presence or absence of density-independent aggregation at different mean percent parasitism can be explained even if parasitoids forage randomly, without changing their behavior in response to encounters with aphids. Observations show that parasitoids tend to move short distances between nearby alfalfa stems (mean = 10.8 cm), and the turning angle between successively visited stems was uniformly distributed. We incorporated this behavior into both simulation and analytical models of parasitoid foraging. The models show the same pattern as that observed in the field: parasitism is aggregated in a density-independent fashion when mean percent parasitism is low but not when mean percent parasitism is high. Therefore, density-independent aggregation in percent parasitism does not necessarily imply behavioral responses of parasitoids to host encounters and previously parasitized hosts.

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A central theme in research on parasitoid foraging behavior and host-parasitoid population dynamics is spatial heterogeneity in parasitism (Cheke 1974, Hassell and May 1974, Chesson and Murdoch 1986, Casas et al. 1993, Jones et al. 1993, Godfray 1994, Schooler et al. 1996). "Parasitoid aggregation" is used both to refer to the behavior of individual parasitoids in response to variation in host density (Cook and Hubbard 1977, Waage 1979) and to describe any spatial variation in percent parasitism among hosts (Hassell and Pacala 1990). Here, we use aggregation in the latter sense to refer to any spatial variation in percent parasitism. If parasitoids preferentially search in areas of high host density, then percent parasitism may increase with host density to create positive density-dependent aggregation (Stamp 1982, van Alphen and Galis 1983, Lessells 1985, Rosenheim et al. 1989). Conversely, if parasitoids have a saturating functional response, then percent parasitism may drop in areas of high host density to create inverse density-dependent aggregation (Hassell 1982). Variation in percent parasitism can also occur independently of host density if, for example, parasitoids are

better able to find and attack hosts in certain types of resource patches (Andow and Prokrym 1990). This creates density-independent aggregation in percent parasitism.

In this study we investigate spatial patterns in percent parasitism of the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae), by *Aphidius ervi* Haliday (Hymenoptera: Braconidae). We conducted two replicates of a field experiment to quantify the distribution of aphids and percent parasitism in spatially homogeneous alfalfa (lucerne) fields. When mean percent parasitism was low (18.7%), variability in percent parasitism among sampling plots was greater than anticipated if aphids in all plots had the same chance of being parasitized. However, when mean percent parasitism was high (56.3%), parasitism was not statistically different from that expected if aphids in all plots had the same chance of being parasitized. Therefore, there was density-independent aggregation of percent parasitism when mean percent parasitism was low, but not when mean percent parasitism was high.

Density-independent aggregation can be explained as the simple consequence of parasitoids moving in a continuous path through an alfalfa field (for a mathematical development of spatial cluster processes, see Cressie 1991). For example, Fig. 1 gives the foraging paths of two parasitoid individuals moving according to random walks. After searching an alfalfa plant, they fly in a random direction for a fixed distance, and then land on a plant to search for hosts again. The four large circles give hypothetical sampling plots from which hosts are collected and parasitism determined.
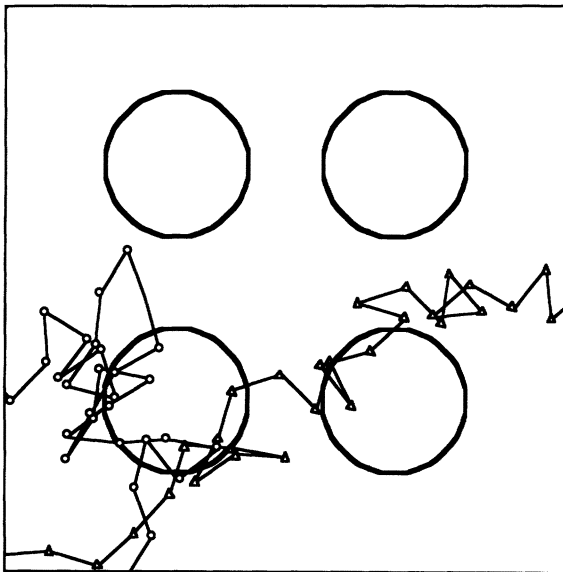


Fig. 1. Hypothetical foraging paths of two parasitoids following a random walk through a spatially uniform habitat. Plants searched by the parasitoids are marked either by small circles or small triangles. The large circles represent sampling plots.

No parasitoid entered two of the sampling plots, while parasitoids searched on 12 and two plants in the lower left and lower right plots, respectively. If percent parasitism were proportional to the number of plants searched by parasitoids per plot, then percent parasitism in this example would be highly variable. Therefore, provided *A. ervi* females fly short distances between plants relative to the size of the sampling plots we used, density-independent aggregation should be expected. This is in fact what we found when mean percent parasitism was low. But what explains the lack of density-independent aggregation when mean percent parasitism was higher?

The goal of our work is to answer this question. We develop a parsimonious explanation for the pattern of percent parasitism expected under the assumption that parasitoids do not modulate their foraging behavior in response either to aphid abundance or to other factors that vary spatially. To quantify the behavior of parasitoids, we observed individual parasitoids foraging naturally in alfalfa fields. We restricted attention to observations in which parasitoids did not encounter hosts, thereby allowing us to characterize foraging without the possibility of behavioral responses to hosts. We then constructed a computer simulation based on the observed foraging patterns of parasitoids and assuming that parasitoids do not alter their foraging patterns in response to encounters with hosts. By comparing the simulated and real data, we can ask whether the presence and absence of density-independent aggregation at low and high mean percent parasitism, respectively, can be explained by random foraging behavior. Finally, we constructed a simple analytical model to investigate how density-independent aggregation changes with the area of sampling plots and the density of hosts within plots.

## Methods

### Study organisms

The pea aphid, *Acyrthosiphon pisum*, is a common pest of peas, beans, and alfalfa in the USA. Population growth rates are potentially huge. Reproduction is parthenogenetic during the summer months, juveniles go through four instars and reach adulthood in about 8 d, and reproductive females can produce 4–6 offspring per day (Hutchinson and Hogg 1984, 1985, Thiboldeaux 1986). Thus, the potential population doubling time is less than 3 d.

*Aphidius ervi* was introduced into North America from France in the 1960s as a biological control agent and has now spread over much North America (van den Bosch et al. 1964, Gonzalez et al. 1978, Mackauer and Kambhampati 1986, Thiboldeaux et al. 1987). In Wisconsin alfalfa fields, its sole host is the pea aphid.

Females parasitize aphids by injecting an egg through the aphid's cuticle. Larval parasitoids kill aphids in roughly 6–8 d and spin a web within the aphid's exoskeleton (mummy) for pupation, which lasts roughly 6 d. Because *A. ervi* larval development takes 6–8 d, mummies are formed only in fourth instar and adult aphids (Thiboldeaux et al. 1987). Furthermore, although females can attack all aphid instars, attacks on fourth instars and adults are rare (Ives et al. 1999), probably because large aphids mount defenses against attack, either dropping off plants or kicking at the attacker (Roitberg and Myers 1978, Weisser 1995).

Numerous studies have investigated the foraging behavior of *A. ervi*. Foraging females are attracted by chemical cues from aphid-damaged plants (Guerrieri et al. 1993, 1997). After encountering hosts on plants, *A. ervi* search plants more intensively (Ives et al. 1999). *A. ervi* can distinguish previously parasitized hosts, and parasitism is reduced on hosts that have been previously parasitized by more than 6 h (Micha et al. 1992). Despite these predictable behaviors shown by foraging *A. ervi*, the number of aphids attacked on plants remains highly variable, owing to differences among females, variability in the distribution of aphids within plants, and other unexplained factors (Ives et al. 1999). Largely due to this variability, the functional response of parasitoids measured at the among-plant scale is roughly type I (Ives et al. 1999).

## Spatial heterogeneity of percent parasitism in the field

To determine the spatial pattern of aphid density and percent parasitism in the field, we sampled a 4-ha alfalfa field at the Arlington Agricultural Research Station in Arlington, WI, USA. The east side of the field was bordered by a gravel road. Forty sampling plots were arranged in eight transects perpendicular to the road spaced 18 m apart. Samples were taken along each transect at 0, 4.5, 9, 13.5, and 18 m from the road. Plots were sampled by placing a 45-cm-radius circular ring (a hula hoop) gently over the alfalfa. Alfalfa immediately outside the ring was flattened to clearly define the plot. A thorough visual scan was conducted to determine the number of mummies within the sample, and the plot was then swept intensively with a net to collect aphids. Although sweep-netting does not capture all aphids, care was taken to sweep all plots in the same way, thereby ensuring that the number of aphids captured is proportional to the true aphid abundance. *A. ervi* adults were collected by performing 20 sweeps in a line moving south (perpendicular) from the transect; the areas swept for *A. ervi* adults were about five times greater than the area of the plots used to sample aphids and mummies.

Table 1. Aphid and parasitoid abundances, and density-independent aggregation of percent parasitism in the field experiments.

| | Expt 1 (17 June) | Expt 2 (31 July) |
| --- | --- | --- |
| Mean number of *A. ervi* per plot | 3.35 | 0.58 |
| Mean number of aphids per plot | 24.3 | 22.9 |
| Mean number of aphids dissected per plot | 12.6 | 11.4 |
| Mean number of mummies per plot | 7.85 | 1.43 |
| Mean% parasitism (mummies excluded) | 56.3 | 18.7 |
| $Z^2$ (mummies excluded) | 1.11 | 2.04 |
| $p$-value for $\chi^2_{39}$ test (mummies excluded) | > 0.2 | < 0.001 |
| Mean% parasitism (mummies included) | 75.0 | 26.8 |
| $Z^2$ (mummies included) | 1.00 | 2.04 |
| $p$-value for $\chi^2_{39}$ test (mummies included) | > 0.2 | < 0.001 |

Aphids were returned to the lab and counted. Fourth instars and adults were dissected to determine percent parasitism. Aphids were considered parasitized if they contained a parasitoid larva that could be detected visibly under a dissecting microscope. Although we did not search dissected aphids for parasitoid eggs, this is unlikely to cause much underestimation of percent parasitism, because fourth instar and adult aphids are rarely attacked by *A. ervi* successfully.

Replicates of this experiment were conducted on 17 June and 31 July, 1998 (Table 1). Mean aphid densities were similar in both experiments, with mean aphid densities of 24.3 and 22.9 per sampling plot on 17 June and 31 July, respectively. In the first experiment, *A. ervi* adult density was relatively high (mean = 3.35 per sample), and average parasitism was 56.3%; in the second experiment, *A. ervi* adult density was relatively low (mean = 0.58 per sample), and average parasitism was 18.7%.

To detect possible density-dependent aggregation of percent parasitism, we regressed percent parasitism (arcsine square-root transformed) against the number of aphids within sampling plots. In the regression we included distance from the edge of the field to account for a possible "edge effect" that could be caused if parasitoids changed their behavior at the field boundary (Fagan et al. 1999). To account for variation in the number of aphids sampled per plot, the data were weighted by the square-root of the number of aphids dissected.

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The regression analysis revealed neither density-dependent aggregation nor an edge effect (see Results, Table 2). Therefore, we could analyze the data for density-independent aggregation using a variant of a $\chi^2$ dispersion test (Southwood 1978, Boulinier et al. 1996). The null hypothesis for this test is that every aphid has the same probability of being parasitized regardless of sampling plot. Specifically, let $n_i$ denote the number of susceptible hosts in sampling plot $i$, and assume all aphids have the same probability $p$ of being parasitized. Then the number of parasitized hosts in sample $i$, $x_i$, will be given by a binomial distribution with parameters $n_i$ and $p$. Thus, the statistic

$$Z_i = \frac{x_i - n_i p}{n_i p[(1-p)]^{1/2}} \tag{1}$$

has expectation 0 and variance 1. A measure of variability in the number of aphids parasitized among sampling plots is

$$Z^2 = \frac{1}{39} \sum_{i=1}^{40} Z_i^2 = \frac{1}{39} \sum_{i=1}^{40} \frac{(x_i - n_i p)^2}{n_i p(1-p)}. \tag{2}$$

This leads to a statistical test of density-independent aggregation, since $39 \times Z^2$ is asymptotically a $\chi^2$ distribution with 39 degrees of freedom (Larsen and Marx 1981). Furthermore, because the mean of a $\chi^2_{39}$ distribution is 39, $Z^2$ has expectation 1, and $Z^2 > 1$ indicates density-independent aggregation.

We determined parasitism by dissecting fourth instar and adult aphids. This method will underestimate the true percent parasitism because we did not include aphids that were parasitized and subsequently killed by the parasitoid before we sampled. To account for this, in addition to analyzing the data for parasitism based solely on dissections, we repeated the analyses including mummies collected in the sampling plots, scoring mummies as parasitized aphids. Including mummies in the analyses implicitly assumes that the survival of mum-

Table 2. Regression of percent parasitism[1] vs aphid density and distance from the edge of the field

| Variable | Coefficient | S.E. | p-value |
| --- | --- | --- | --- |
| 17 June | | | |
| Constant | 0.86 | 0.085 | |
| Aphids | −0.0020 | 0.0029 | 0.50 |
| Distance | 0.0019 | 0.0013 | 0.17 |
| 31 July | | | |
| Constant | 0.24 | 0.11 | |
| Aphids | 0.0073 | 0.0038 | 0.065 |
| Distance | −0.00095 | 0.0018 | 0.61 |

[1] Percent parasitism was arcsine square-root transformed, and data were weighted by the square-root of the number of aphids dissected to account for variation due to variability in sample sizes.

mies in the field is the same as the survival of adult aphids. Although we do not know the relative survivals of mummies and adult aphids, we suspect that mummy survival is higher than adult aphid survival, because the mummies are protected from most predators by a hard shell within which the parasitoid pupa spins a web. Therefore, including mummies in the analyses likely overestimates the true percent parasitism. This provides an upper bound on true percent parasitism, whereas the analyses excluding mummies provides a lower bound.

## A. ervi foraging observations

We conducted field observations of foraging female *A. ervi* to determine their movement characteristics in the absence of encounters with aphids. Field observations were conducted between 15 July and 31 September, 1998, in alfalfa fields at the Arlington Agricultural Research Station and on the main campus of the UW-Madison. Observations were made on parasitoids naturally foraging in the field in late morning and early afternoon. Parasitoids were first located and then followed by at least two observers. One observer kept careful watch of the parasitoid, while another marked its foraging path by placing a numbered piece of tape on each stem that the parasitoid visited. The trial ended when observers were unable to locate the parasitoid. Most observations ended with the parasitoid flying relatively long distances ( > 1 m) that made relocating the parasitoid difficult. At the end of the trial, the parasitoid's foraging path was recorded by measuring the distance and turning angle between successive stems. Measurements were taken from apex to apex of each stem, and turning angles were recorded as the deviation from the direction moved between the preceding pair of stems. No encounters with aphids or other parasitoids were observed in the data we present.

## Simulation model

We constructed a model to simulate data under the assumption that *A. ervi* does not vary its foraging behavior in response to encounters with aphids. The model has two parts. The first simulates the movement of parasitoids in a field, and the second translates the number of stems visited within sampling plots into percent parasitism. To model the movement of parasitoids, we simulated parasitoids flying in a 2.5 × 2.5 m arena containing a 45-cm-radius circular sampling plot in the middle. Parasitoids were 'released' from a random location within 50 cm of the boundary of the arena. An initial direction of movement was selected randomly, and the parasitoid then flew a distance selected randomly from the collection of distances obtained from the field observations. The parasitoid was

assumed to search for aphids on a stem at that location. Then a turning angle and a distance were selected at random from the collections of turning angles and distances observed in the field, and the simulated parasitoid flew to another stem. This was repeated until the parasitoid flew out of the arena.

A problem arises in our field observations of *A. ervi* because we generally lost track of parasitoids when they moved long distances. Therefore, our collection of distances underestimates the true travel distances. To account for this, for each time we lost track of a parasitoid during a long-distance flight, we included a 1-m flight in the collection of observed distances. Because these flights may be longer than 1 m, this procedure still underestimates the average flight distance. We performed simulations (not presented) in which longer flight distances were assigned to parasitoids that we lost during observations. The results of these simulations were quantitatively very similar to those we present here. Because 1 m is far enough to remove a parasitoid from the simulated sampling plot, increasing this distance to greater than 1 m has little impact on the number of times a parasitoid lands within the same sampling plot.

We simulated the field experiments by replicating 40 different arenas to represent the 40 sampling plots. To simulate different parasitoid densities, we created experiments with the number of parasitoids added to each arena varying from 1 to 800. For a given experiment, the same number of parasitoids was allowed to forage in each of the 40 arenas. We recorded the number of stems searched within the sampling plots and the number of parasitoids that visited each plot. From these we calculated the mean and variance in the number of stems searched per plot, and the mean number of stems searched per parasitoid visit to a plot.

The second part of the simulation model translates the number of stems searched per sampling plot into percent parasitism. For the numbers of aphids within the 40 sampling plots, we use the numbers observed in the first field experiment, when the mean was 24.3 aphids/plot; these differ little from the numbers observed in the second field experiment when the mean was 22.9 aphids/plot. To determine percent parasitism, we assume that within sampling plots, aphids are parasitized randomly and independently. Let $v_i$ be the number of stems that parasitoids search within sampling plot $i$ that contains $n_i$ aphids. Further, let $q_i$ be the probability that a given aphid is attacked per stem searched by a parasitoid. To calculate $q_i$, we assume that each plot contains 50 alfalfa stems (roughly the density observed in the field), so there is a 2% (1/50) chance that a particular aphid is on the stem searched by a parasitoid. We assume that an aphid has a 50% (1/2) chance of being parasitized if a parasitoid searches the stem it is on. Therefore, the probability that a particular aphid is parasitized per stem visited by a

parasitoid is $q_i = (1/50)(1/2) = 1\%$. When parasitoids search $v_i$ stems within a sampling plot, the probability of a given aphid being parasitized is $p_i = 1 - (1 - q_i)^{v_i}$. In the simulations, after determining the number of stems searched by simulated parasitoids in each sampling plot, we randomly selected the number of parasitized aphids from a binomial distribution with parameters $n_i$ and $p_i$. We analyzed the resulting simulated data in the same way as the data from the field experiments.

## Analytical model

We developed an analytical model to approximate the density-independent aggregation of percent parasitism created when parasitoids take short flights between stems on which they search for aphids. To derive this approximation, assume that the number of stems visited by each parasitoid visiting a sampling plot is a constant $c$. Although in reality this number will be variable, depending on the path which parasitoids take through a sampling plot, the average number of stems visited will be greater than or equal to 1. Further assume that the number of parasitoids visiting sampling plots is described by a Poisson distribution $Y$ with mean $\lambda$. Then the number of stems searched per plot is $cY$. Letting $q$ denote the probability that a given host is attacked when a parasitoid searches a random stem within a sampling plot, the probability that a host is attacked per plot is given by the random variable

$$P = 1 - (1 - q)^{cY} \tag{3}$$

Using the generating function for $Y$, $G_Y[z] = e^{\lambda(z-1)}$ (Larsen and Marx 1981), the expectation and variance of $P$ can be calculated as

$$E[P] = 1 - e^{\lambda((1-q)^c - 1)} \tag{4}$$

$$V[P] = e^{\lambda((1-q)^{2c} - 1)} - e^{2\lambda((1-q)^c - 1)} \tag{5}$$

To derive a measure of density-independent aggregation comparable to $Z^2$, let $x$ be the number of hosts that are parasitized in a plot containing $n$ hosts. Then

$$E[x] = n E[P] \tag{6}$$

$$E[x^2] = n(n-1) E[P^2] + n E[P]$$
$$= n(n-1) (V[P] + E[P]^2) + n E[P] \tag{7}$$

Define $Z_p$ as

$$Z_p = \frac{x - nE[P]}{[nE[P](1 - E[P])]^{1/2}} \tag{8}$$

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Under the null assumption that the probability $P$ of a host being parasitized is the same in each plot, $Z_p$ has expectation zero and variance 1. The expectation of $Z_p^2$ is given by

$$Z_p^2 = E\left[\frac{(x - nE[P])^2}{nE[P](1 - E[P])}\right]$$

$$= \frac{E[(x - nE[P])^2]}{nE[P](1 - E[P])}$$

$$= \frac{(n - 1)V[P] + E[P] - E[P]^2}{E[P](1 - E[P])} \qquad (9)$$

Thus, $Z_p^2$ gives a measure of the expected variability in the number of hosts parasitized among sampling plots and is equivalent to $Z^2$.

These analyses give two measures of density-independent aggregation: $V[P]$ and $Z_p^2$. $V[P]$, the variance in the probability of parasitoid attack among hosts in different sampling plots, is not directly observable in data, because the observed percent parasitism will depend on the number of hosts sampled in different plots. In contrast, $Z_p^2$ is based on the number of hosts sampled per plot, $n$. Therefore, $Z_p^2$ depends both on the variability in the probability of parasitism among plots and the ability to detect this variability based on the sample size in plots. We will consider both of these measures when using the analytical model to explore the pattern of density-independent aggregation under different assumptions about parasitoid foraging behavior and sampling.

## Results

### Aggregation of percent parasitism in the field

The field experiments were designed to identify density-dependent and density-independent aggregation of percent parasitism among sampling plots. The mean numbers of *A. ervi* obtained in sweep-net samples were 3.35 and 0.58 in experiments 1 and 2, respectively, and the corresponding parasitism based on aphid dissections were 56.3 and 18.7%. Neither experiment revealed evidence of density-dependent aggregation, and percent parasitism was also independent of the distance of the sampling plots from the edge of the field (Table 2). In experiment 1 (17 June, Table 1, Fig. 2A) when average percent parasitism was high, there was no density-independent aggregation of percent parasitism, whether or not mummies are included as parasitized aphids. In contrast, in experiment 2 (31 July, Table 1, Fig. 2B) when average percent parasitism was low, there was strong density-independent aggregation of percent parasitism with and without the inclusion of mummies as
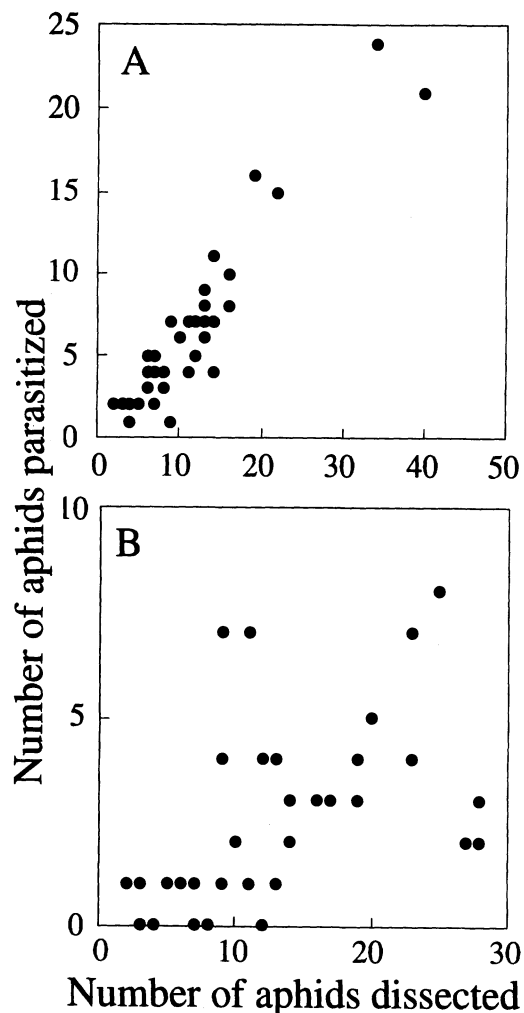


Fig. 2. Number of aphids parasitized vs the number of aphids dissected from the field experiments conducted on (A) 17 June and (B) 31 July, 1998.

parasitized aphids. Finally, the distribution of adult *A. ervi* was not significantly different from a Poisson distribution (experiment 1, $\chi_{39}^2 = 37.34$, $P > 0.5$; experiment 2, $\chi_{39}^2 = 41.35$, $P > 0.5$); note, however, that the sample area for adult parasitoids was roughly five times greater than the area of the plots sampled for aphids, and this larger area could obscure variability at the spatial scale of the sampling plots.

### *A. ervi* foraging observations

Observations of *A. ervi* females in the field yielded 17 foraging bouts consisting of 70 distance and 49 angle measurements (Fig. 3). The mean distance traveled per move between stems was 10.8 cm, although the distribution of distances had a high variance, with many moves covering very short distances and a few moves covering much greater distances. The turning angles
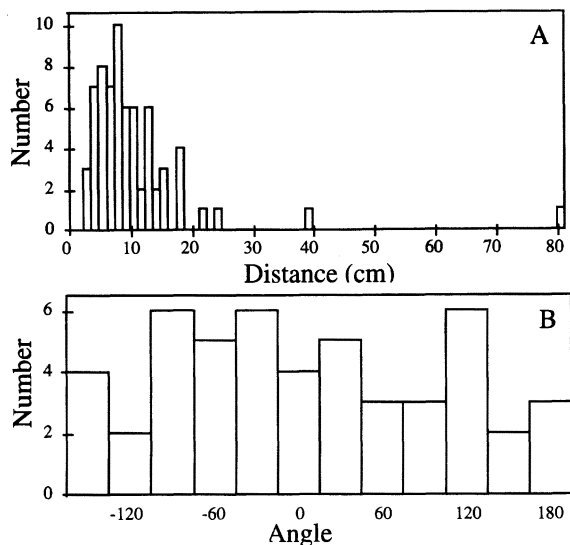
Fig. 3. (A) Distribution of distances moved between stems and (B) distribution of turning angles made between consecutive pairs of stems.

taken by *A. ervi* between successive stems were roughly uniformly distributed over 360°.

## Simulation model

The simulation model was designed to generate data for percent parasitism among sampling plots equivalent to the data collected in the field. The first component of the model simulated the movement of parasitoids in an arena and counted the number of stems visited by
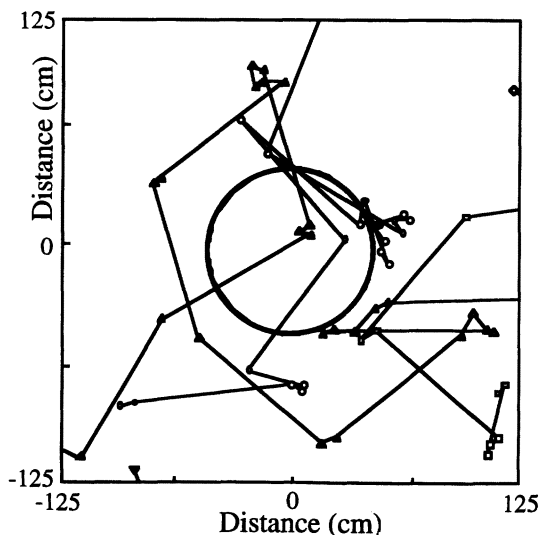


Fig. 4. Foraging paths of parasitoids from the simulation model. Foraging paths are shown as lines with the marks denoting stems searched by the parasitoids. The circle at the center of the arena represents a sampling plot.
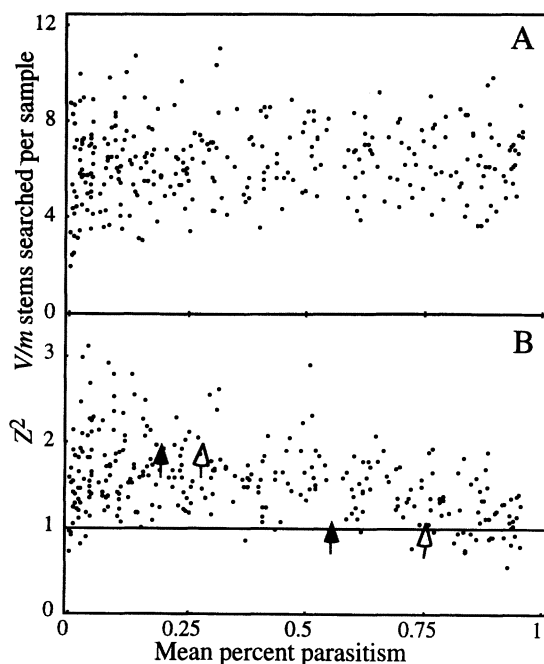


Fig. 5. (A) Variance-to-mean ratio for the number of stems searched by simulated parasitoids in 40 sampling plots and (B) the variance in percent parasitism vs the mean percent parasitism among all samples. Each point represents a separate experiment, and variation among experiments in mean percent parasitism was produced by varying the number of parasitoids in the simulated foraging arena from 1 to 800. Black arrows show values from the field experiments when mummies are excluded from calculations of percent parasitism, and white arrows show values when mummies are included with parasitized aphids. For the simulation, $q = 0.01$.

parasitoids within a sampling plot. Fig. 4 gives a visual realization of the simulation in which five parasitoids were started at random locations in the 50-cm strip around the periphery of the arena. Two of the parasitoids immediately moved in a direction that took them out of the arena, two parasitoids entered the sampling plot, and the remaining parasitoid searched eight stems in the arena, none of which were in the sampling plot. Of the two parasitoids that entered the sampling plot, one searched three and the other searched two stems within the plot. In order to simulate an experiment, 40 repetitions were performed with the same number of parasitoids introduced into the arena.

Because simulated parasitoids moved short distances between stems, parasitoids that visited the sampling plot commonly searched several stems; the average over all simulations was 3.90 stems. The variance in the number of stems searched by parasitoids per plot in the simulated experiments was greater than the variance expected if the searched stems were randomly distributed among the 40 plots. If the number of stems searched per plot were Poisson distributed, the variance-to-mean ratio for the number of stems searched per plot would be 1; however, the average variance-to-

mean ratio was 6.29 (Fig. 5A). This pattern arose because parasitoids searched multiple stems within plots. For example, if the number of parasitoids visiting sampling plots were Poisson distributed and each parasitoid searched exactly four stems per plot, then the mean and variance in the number of stems searched among plots would be four and 16 times the number of parasitoids that visit, respectively. Since a Poisson distribution has a variance-to-mean ratio of 1, the variance-to-mean ratio of the number of stems searched among plots would be four.

To translate the number of stems searched by parasitoids into percent parasitism, we assumed that (1) the number of hosts within sampling plots equaled the observed number of aphids from the first field experiment (mean = 24.3), (2) these hosts were randomly distributed among 50 stems within the plots, and (3) a host had a 50% chance of being parasitized by a parasitoid searching the stem it was on. Because the distribution of the numbers of stems searched by parasitoids per sampling plots was clumped (Fig. 5A), simulated percent parasitism showed density-independent aggregation as measured by $Z^2$ (Fig. 5B). If percent parasitism were not aggregated, $Z^2$ would equal 1. When overall percent parasitism is low, the average value of $Z^2$ in the simulations is about 1.8. With increasing mean percent parasitism, however, $Z^2$ decreases, reaching about 1.1 when parasitism is 90%.

The pattern of aggregation of percent parasitism in the simulation (Fig. 5B) is similar to that found in the field experiments. When mummies are excluded from the count of parasitized aphids, in the first and second field experiments mean parasitism was 56.3 and 18.7%, and $Z^2$ was 1.11 and 2.04, respectively (black arrows in Fig. 5B). At high percent parasitism (experiment 1), the value of $Z^2$ is at the lower range of the simulated values. However, when mummies are included, mean parasitism was 75.0 and 26.8%, and $Z^2$ was 1.00 and 2.04, respectively (white arrows in Fig. 5B; note that at high mean percent parasitism, the $Z^2$ values of 2.04 excluding and including mummies were the same to three significant figures). These estimates fall more centrally in the range of values of $Z^2$ produced by the simulation model.

## Analytical model

In the analytical model, the variance in the probability that hosts are parasitized in different plots, $V[P]$, depends on the number of stems searched by parasitoids when they visit a plot, $c$, the probability that a given host is attacked per stem searched, $q$, and the expected number of parasitoids visiting plots, $\lambda$, which depends on the parasitoid density (Eq. 5). The variability in the observed percent parasitism, $Z_p^2$, depends additionally on the number of hosts sampled per plot, $n$ (Eq. 9). In

Fig. 6 we plot $Z_p^2$ vs the expected percent parasitism, $E[P]$ (Eq. 4), for different values of $c$, $q$, and $n$ by varying values of $\lambda$ to control the density of parasitoids and hence the expected percent parasitism. For a baseline, we selected parameter values that correspond roughly to the experimental and simulated data. The mean number of stems searched by parasitoids per sampling plot in the simulations was 3.90; therefore, for the analytical model we assume that parasitoids search four stems per visit to a plot ($c = 4$). The probability that a host was attacked per stem searched was assumed to be 0.01 ($q = 0.01$) as it was in the simulation model. Finally, the number of hosts sampled per plot was set to 25 ($n = 25$), while the mean numbers of aphids dissected per plot were 24.3 and 22.9 in field experiments 1 and 2, respectively. The baseline assumptions give the central line in each panel of Fig. 6.
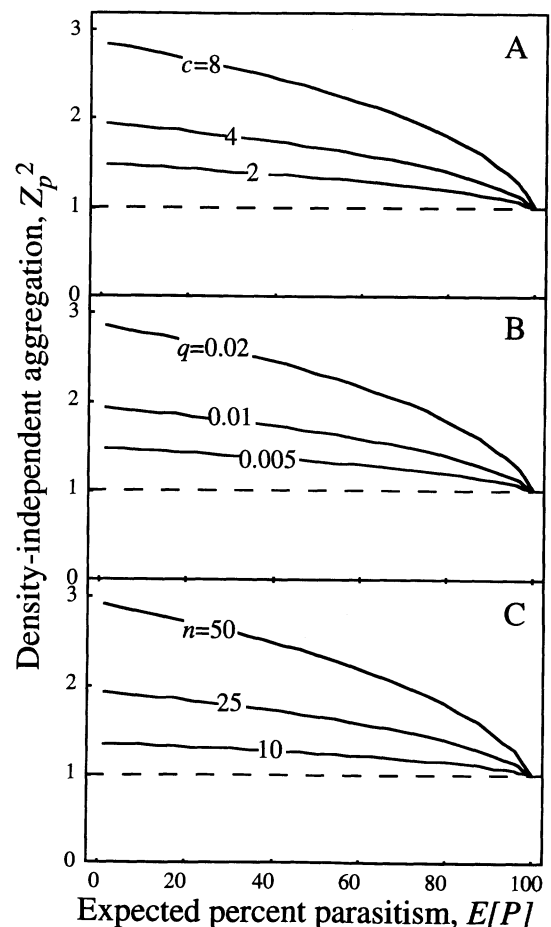


Fig. 6. Density-independent aggregation of percent parasitism measured by $Z_p^2$ vs. the expected probability of parasitism, $E[P]$, for different values of (A) $c$, the number of stems searched by parasitoids visiting a sampling plot, (B) $q$, the probability of a given host being attacked per stem searched within the sampling plot, and (C) $n$, the number of hosts sampled within the plots. The panels were generated from Eqs. (4) and (9) by varying the value of $\lambda$. Unless otherwise labeled, $c = 4$, $q = 0.01$, and $n = 25$.

Increasing the number of plants searched by parasitoids per visit to a sampling plot, $c$, increases $Z_p^2$ (Fig. 6A). This occurs because when a parasitoid searches many stems per plot, attacks on hosts among plots are clustered. Increasing the chance that a host is parasitized per stem searched, $q$, also increases $Z_p^2$. The reason for this result can be explained with the following hypothetical example. Suppose that parasitoids rarely found and parasitized aphids when they searched a stem. Then, even though they might search many stems within a plot, the number of stems they search that lead to parasitism is small. Although the number of stems visited per plot may be clustered ($c > 1$), acts of parasitism will not be as strongly clustered, thereby reducing the variance in percent parasitism among plots. In contrast, if parasitoids attacked hosts on every stem they searched, then the variance in the number of stems searched per plot would translate directly into the variance in percent parasitism. Finally, increasing the number of hosts within plots, $n$, increases $Z_p^2$. This is not because increasing $n$ changes the variance in the probability $P$ that hosts are parasitized, since $V[P]$ is independent of $n$ (Eq. 5). Instead, it is a consequence of increasing the sample size of hosts, which makes detecting density-independent aggregation easier.

The baseline assumptions in the analytical model give a relationship between $Z_p^2$ and $E[P]$ (central lines in Fig. 6) very similar to the relationship between $Z^2$ and mean parasitism produced by the simulation model (Fig. 5B). In particular, $Z_p^2$ decreases from about 1.9 when percent parasitism is low to 1 when parasitism approaches 100%. The decline in $Z_p^2$ can be explained as follows. Density-independent aggregation ($Z_p^2 > 1$) is created because parasitoids search multiple stems per visit to a sampling plot ($c > 1$). However, with increasing numbers of parasitoids visiting plots, and hence increasing mean percent parasitism, the variance in percent parasitism is constrained, because there is an upper limit to the percent parasitism within each plot, namely 100%. The effect of this ceiling can be illustrated by considering the case of very high mean percent parasitism (e.g., 95%). In this case, the upper limit of 100% parasitism will be reached in many plots, thereby restricting the variance. Following from this argument, the variance in percent parasitism must decrease with increasing mean percent parasitism.

For parasitoids that forage by moving continuously through habitat containing hosts, the degree of density-independent aggregation depends on the scale at which parasitism is measured. To illustrate this, consider the consequences of changing the size of sampling plots we used for the pea aphid – *A. ervi* system. Rather than use $Z_p^2$ for this illustration, instead we will use $V[P]$, because we are interested in the underlying pattern of percent parasitism. Using $Z_p^2$ would confound this, because it depends on the sample size of hosts, which in turn likely depends on the size of the sampling plots.
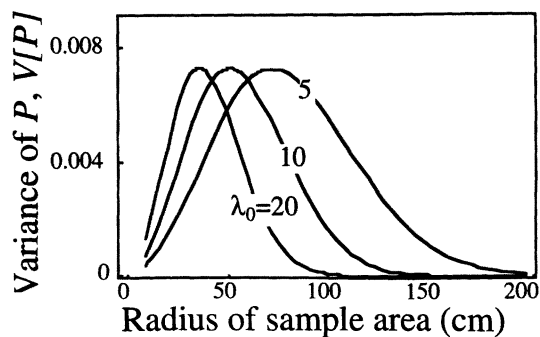


Fig. 7. The variance in the probability of parasitism among samples, $V[P]$, vs the radius of the sampling plots (Eqs 4 and 5). Parameter values were selected so that at the radius of the actual sampling plots (45 cm), $c = 3.90$, $q = 0.01$, and $\lambda = \lambda_0 = 5$, 10, and 20 as labeled.

$V[P]$ depends on the number of stems searched per parasitoid visit to a sampling plot, $c$, the probability that a given host is parasitized per stem searched, $q$, and the expected number of parasitoids visiting the plot, $\lambda$. For a parasitoid showing a uniform distribution of turning angles between successive stops, the area covered during foraging will vary linearly with the number of stems it searches (Kareiva and Shigesada 1983). Therefore, $c$ will be a linear function of the area of the sampling plots. Because larger plots will contain proportionally more stems, the probability that a given host is parasitized per stem searched in a plot, $q$, will be inversely proportional to area. Finally, the mean number of parasitoids visiting the plot, $\lambda$, will be directly proportional to plot area. Assuming $c$ and $\lambda$ are directly proportional to plot area, and $q$ is inversely proportional, makes it possible to calculate $V[P]$ as a function of the size of the sampling plots.

Fig. 7 plots density-independent aggregation measured by $V[P]$ vs the radius of sampling plots for three values of overall parasitoid density, $\lambda_0$. Parameter values were selected so that at the radius of the actual sampling plots (45 cm), values of $c$ and $q$ are those from the simulation model ($c = 3.90$, $q = 0.01$) and the densities of parasitoids are $\lambda = \lambda_0 = 5$, 10, and 20 as labeled. The variance in percent parasitism is always greatest for sampling plots of intermediate size, with the size of the sample plot giving the greatest variance decreasing with increasing parasitoid density $\lambda_0$. This pattern can be explained as follows. At the extreme of very small sampling plots, parasitoids will not search more than one stem per plot, leading to no clustering of stems searched per plot ($c = 1$). Thus, the initial source of density-independent aggregation is removed when sampling plots are small. Conversely, when plots are very large, large numbers of parasitoids visit each plot, and the distribution of percent parasitism is dictated more by the number of parasitoids visiting plots than the number of stems each one searches. Thus, density-inde-

pendent aggregation declines. This also explains why the greatest variance in percent parasitism occurs at smaller spatial scales when parasitoids are more abundant, since the importance of the distribution of parasitoids visiting sampling plots increases with parasitoid density.

## Discussion

In our field experiments, the pattern of percent parasitism by *A. ervi* on pea aphids was not always random. When mean percent parasitism was low, we found density-independent aggregation of percent parasitism among spatial samples, while at higher mean percent parasitism, parasitism showed no density-independent aggregation. These results can be explained without recourse to any assumptions about parasitoids modifying their behavior while searching for aphids. Using both simulation and analytical models, we showed that the patterns of density-independent aggregation of percent parasitism we observed in the field could be the simple consequence of parasitoids searching for aphids among nearby stems as they move through an alfalfa field.

Understanding the factors responsible for the spatial patterns of percent parasitism requires considering two questions: What determines the spatial distribution of the plants searched by parasitoids, and how does the distribution of plants searched by parasitoids translate into spatial patterns of percent parasitism? In answer to the first question, when parasitoids move through a field and search nearby plants for hosts, then those plants searched for hosts will be spatially clustered. This is a simple consequence of the fact that a plant adjacent to a plant that is searched by a parasitoid is itself more likely to be searched. In answer to the second question, how the spatial clustering of plants searched by parasitoids translates into spatial patterns of percent parasitism depends on the overall level of parasitism and the density of hosts. If mean percent parasitism is low, then the pattern of percent parasitism will more closely reflect the pattern of plants searched by parasitoids. This is because hosts will rarely be parasitized by multiple parasitoids, so the spatial clustering of parasitism caused by individual parasitoids will remain intact. Conversely, when mean percent parasitism is high, then the variance in percent parasitism among sampling plots will decline, because all plots will have high percent parasitism; in effect, the signatures of individual parasitoid foraging paths will be swamped by large numbers of other parasitoids.

An important conclusion from these results is that density-independent aggregation of percent parasitism depends not only on the foraging behavior of parasitoids, but also on the density and susceptibility of hosts. It also depends on the spatial scale at which

percent parasitism is measured. Using the analytical model, we showed that the variance in percent parasitism among sampling plots is always greatest at some intermediate spatial scale, with that spatial scale which gives the greatest variance itself depending on parasitoid density (Fig. 7). Thus, when investigating the spatial distribution of percent parasitism in a habitat without clear patches, the degree of density-independent aggregation may vary greatly according to the spatial scale at which parasitism is measured. This is a well-known problem with any quadrat-based spatial sampling techniques (Upton and Fingleton 1985: 26–53): No single scale of sampling can give a complete characterization of the pattern of percent parasitism. Although this problem is particularly obvious for hosts and parasitoids living in a relatively uniform environment, it also occurs for many studies in which it is necessary to decide what constitutes a 'patch' (Addicott et al. 1987, Ayal 1987).

Our study demonstrates that density-independent aggregation of percent parasitism should be expected whenever parasitoids search neighboring areas for hosts. This is an important point for the study of parasitoid-host interactions, because density-independent aggregation may act to stabilize host-parasitoid dynamics (e.g., Chesson and Murdoch 1986, Hassell et al. 1991, Ives 1992, Jones et al. 1993). Density-independent aggregation acts to stabilize host-parasitoid interactions via pseudo-interference (Chesson and Murdoch 1986, Bernstein 1987, Walde and Murdoch 1988). Because density-independent aggregation leads to multiple parasitoids attacking the same hosts, it increases intraspecific competition among parasitoids. To create pseudo-interference among parasitoid individuals, however, density-independent aggregation must be caused by multiple parasitoids aggregating in the same area and attacking the same hosts. The processes generating density-independent aggregation that we demonstrated arise from the foraging patterns of individual parasitoids. Therefore, none of this density-independent aggregation will lead to pseudo-interference and greater stability of host-parasitoid population dynamics (for a more detailed analysis, see Gross and Ives 1999). This can be seen most clearly in the fact that the variance among samples depends on the scale at which samples are taken (Fig. 7) rather than the behavior of parasitoids; thus, the variance in percent parasitism is an artifact of the sampling method that does not give information about host-parasitoid dynamics.

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