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Proximate cues for predator searching: a quantitative analysis of hunger and encounter rate in the ladybird beetle, *Coccinella septempunctata*

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Many mobile predators use different foraging behaviours depending on whether prey are clumped or uniformly distributed in space. It is believed that predators can increase foraging efficiency on patchily distributed prey if they alternate intensive searching (slow speeds and frequent, acute turns) with extensive searching (high speeds and infrequent, shallow turns). We used Fourier transforms, fractal dimension analysis and traditional single-value measures of movement to determine which proximate cue caused these changes in behaviour: frequency of encounter or degree of satiation. Over a range of encounter rates and levels of hunger, we found that larval ladybird beetles showed intensive search behaviour for varying levels of encounter frequency, but did not alter their behaviour when hunger level was manipulated. We provide a conceptual model that explains why encounter frequency is more likely than hunger level to determine search behaviour. We discuss the relative merits of Fourier transforms in relation to fractal dimension and single-value measures.

A basic tenet of modern behavioural ecology is that organisms behave in such a way to maximize their fitness. In particular, when fitness is most affected by energy intake, foraging behaviour should maximize fitness (Stephens & Krebs 1986). Tests of this basic theory occur on two levels. First, one may derive behavioural patterns to be expected assuming an organism forages optimally followed by a test that the pattern exists (e.g. prey size selection by bluegills, *Lepomis macrochirus*: Werner & Hall 1974). A second approach is to determine the fitness associated with particular behavioural mechanisms (rules of thumb, Stephens & Krebs 1986), followed by a determination of the rule of thumb used by the organism. For example, Green (1984) proved for a particular foraging situation that an optimal predator should leave a patch if too few prey were located after a given set of foraging attempts. That is, a predator should leave a patch when the encounter frequency (encounters per attempts) is less than a threshold. Waage (1979) reported evidence supporting this in parastic wasps.

It is possible, however, to extend this second approach and study the physiological mechanisms underlying Green's assessment rule of thumb. Many physiological variables change during a foraging bout and these may be correlated. For example, because a predator might use memory to count the number of encounters and the number of attempts Green's rule of thumb can be implemented. On the other hand, when foraging does not result in a capture, the predator expends metabolic energy. The use of energy results in the predator becoming progressively hungry, so that the mechanism of environmental assessment may not be counting but determining hunger level. Studying the mechanisms underlying rules of thumb is important, since some rules of thumb (e.g. counting) may be more or less unlikely depending on the cognitive prowess of the predator. The research reported here attempts to untangle two such confounded mechanisms: encounter rate and satiation level.

In response to clumped distributions of resources, many animals of restricted sensory perception switch between extensive and intensive searching behaviour to efficiently search their environment. Extensive searching behaviour

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is characterized by fast speeds and relatively straight paths, allowing an animal to move quickly from one area to another. In contrast, intensive searching behaviour is characterized by slower speeds and more tortuous paths, and has the effect of keeping an individual in a localized area. This strategy is often referred to as area-restricted search and it is beneficial if an animal searches intensively only in areas of high prey density and extensively only in areas of low prey density (Cook & Hubbard 1977; Bond 1980; Hodek & Honěk 1996; Walsh 1996). Examples of this behaviour include a variety of organisms, such as parasitoids (Laing 1937; Waage 1978; Strand & Vinson 1982), bees (Hartling & Plowright 1979; Kipp 1984), herbivores (Miller & Strickler 1984), birds (Smith 1971), flies (Nelson 1977; White et al. 1984; Bell et al. 1985), neuropterans (Fleschner 1950) and beetles (Fleschner 1950; Banks 1957; Dixon 1959; Carter & Dixon 1982, 1984; Mols 1993; McIntyre & Wiens 1999).

As a group, larval ladybird beetles provide a good model for studying the behaviour of switching between searching strategies. They spend a majority of their time searching for aphids (Banks 1957; Dixon 1959; Hodek & Honěk 1996), which are commonly found distributed in dense colonies. In the absence of cues, a ladybird searches its environment extensively and upon encountering an aphid, begins searching intensively (Banks 1957; Chandler 1969; Nakamuta 1982, 1985; Ferran & Dixon 1993). If no further prey are encountered, the larva gradually reverts to extensive searching.

For area-restricted search to be effective, larvae must search extensively outside aphid colonies and intensively within. Since larval ladybirds are largely unable to detect their prey by sight, sound, or smell, their ability to perceive prey is predominantly based upon physical contact using their mandibles or forelegs (Fleschner 1950; Banks 1957; Dixon 1959; Nakamuta 1985; Ferran et al. 1997). The interesting question becomes, how do ladybirds determine when they are inside or outside a colony and, consequently, when to search intensively and when to search extensively?

Two possible cues in the choice between searching behaviours are the predator's hunger level and the density of prey in the local area. Experiments examining the influence of hunger level on a ladybird's choice of behaviour found that the duration of intensive search following an encounter increases as a beetle's satiation level decreases (Carter & Dixon 1982; Ferran & Dixon 1993; Ferran et al. 1997). A study by McIntyre & Wiens (1999) showed predator satiation to influence the search behaviour of *Eleodes extricata* Say (darkling beetles). This association is often explained by the hypothesis that hungrier beetles have a stronger motivation to find prey, and thus, spend more time thoroughly searching an encounter site.

A second cue potentially influencing search behaviour is prey density. Hemptinne et al. (1996) found that adult female *Adalia bipunctata* (two-spot ladybird beetle) spent more time in intensive search at high prey density than when prey were scarce. Similarly, Dixon (1959) found that ten-spot ladybird beetle, *Adalia decempunctata*, larvae turn more frequently at high prey densities.

In addition to predator hunger and prey density, other factors influence the length of the intensive search period following an encounter. Nakamuta (1985) found that the type of encounter (contact, biting, or consumption) affects the duration of the ensuing intensive search. Although prey encounters elicit the switch to intensive search, and although other factors appear to be involved, many researchers (Carter & Dixon 1982; Ferran & Dixon 1993; Mols 1993; Ferran et al. 1997) conclude that satiation is the factor most responsible for guiding a larva's ultimate searching behaviour.

To clarify the roles of predator satiation and prey density, we recorded ladybird searching behaviour under different foraging regimens while imposing a range of prey encounter rates during the experimental observation period. To address this question statistically, we used and compared the efficacy of three different approaches to quantitifying insect movement. We compared single-value measures (e.g. turning frequency), fractal dimensions and Fourier transforms. While commonly used, single-value measures have a number of disadvantages that suggest the need to examine the more numerically sophisticated latter two methods. Among these disadvantages is the property that single-value measures represent a mean from a sample over a time interval of movement. This arbitrarily chosen interval has the potential to introduce bias and reduce statistical power. Too short or too long, an interval may fail to reveal the true behaviour of the organism's response to an environmental stimulus. The latter two methods, while more difficult to compute, use a long temporal sequence of moves and numerically characterize different components of this sequence. As described below, properties of these components may be associated with different qualities of movement behaviour.

METHODS

Experimental Design

One of the primary food sources of the ladybird beetle, *Coccinella septempunctata* L., is the pea aphid *Acyrthosiphon pisum* (Harris) (Hodek & Honěk 1996). To test the effect of prey encounter rate on searching behaviour of the second-instar larvae of *C. septempunctata*, we performed experiments at six different encounter rates: an encounter every 10, 30, 60, 90, 135 and 180 s. The effect of different satiation levels was tested using ladybirds deprived of food for different lengths of time. At each encounter rate, we imposed six deprivation times on the beetles: 0, 3, 6, 12, 18 and 24 h. These deprivation times comprise a range of times, the maximum of which is the time to evacuate about 80% of stomach volume (Carter & Dixon 1982). We used a full factorial design, and replicated each of the 36 treatments four times, using a different individual each time, for a total of 144 individuals. During each 3-min experiment, we recorded on videotape the movement of one ladybird larva.

Adult ladybirds collected from local fields were maintained in the laboratory on a diet of larval and adult pea aphids. Each day, we collected eggs laid by adult females and incubated them at 20 ± 1 °C in a 9-cm petri dish with a small vial of water stopped with cotton. The eggs hatched after 4–8 days, at which time the first-instar larvae were transferred to a clean petri dish and fed a diet of pea aphids ad libitum. After 4–6 days, when the ladybirds moulted to the second instar, we transferred them to a new dish where they were supplied with a satiating number of aphids. After several hours of feeding, the food deprivation periods began when we transferred individuals to dishes without food at designated times before the experiments.

To control the rate of prey encounters, we presented aphids to the beetle until the ladybird contacted the aphid with its forelegs or mandibles. The aphid was then promptly removed before feeding began. For our experiments we consider this contact without consumption to be our standard for an encounter. Nakamuta (1985) defines various encounter types and explores the strength of beetle reactions to each type. He found that physical contact between a ladybird and an aphid was enough to elicit a behavioural response.

Controlled encounters between a ladybird and an aphid were accomplished using a live, adult aphid glued by the dorsal surface of its abdomen to a fine metal wire using Elmer's brand school glue. The aphid was presented at the beginning of each experiment, and then at specific intervals throughout the experiment (for example, at $t = 0$, $t = 30$, $t = 60$ s). Offering and removing aphids in this way made it possible to manipulate the encounter rate but maintain a constant satiation level. It also allowed the beetle to search between encounters throughout the entire 3 min rather than spend time feeding. Dixon (1959) suggested that encountering and not consuming an aphid is a natural and common scenario. He found that second-instar ladybirds successfully capture only about 50% of the first-instar aphids they encounter. The frequency drops to about 20% for second-instar aphids, then near zero for third- and fourth-instar and adult aphids.

These experimental procedures require some assumptions about larval ladybird behaviour and biology. We assumed that larvae were engaged in searching behaviour during the entire 3 min of an experiment. This is supported by Dixon's (1959) estimate that second instars spend about 95% of their time searching for food. We also assumed that 3 min is a short enough time that a larva's satiation level would not change during the experiment. Mols (1988) found that adult *Pterosticuhus coerulescens* (Carabidae) satiation levels change on the timescale of hours and days. We also assumed that ladybirds would behave the same in response to an encounter with an aphid glued to a wire as they would to an aphid encountered under more natural circumstances. In preliminary anecdotal observations, individual ladybird behaviour did not appear to be influenced by the presence of dried glue. Larvae readily consumed a live aphid with a drop of dried glue on its back as well as an aphid glued to a wire rod. Others have also found that glued aphids are acceptable to ladybirds (Banks 1957; Ferran et al. 1997).

The experimental arena was a flat white surface on which a black 60-cm diameter circle was drawn. The surface was covered with clear plastic that allowed the arena to be cleaned with an alcohol-wetted cloth between experiments to remove any chemicals potentially left by the beetles or aphids. Anecdotal observations showed no effect of the alcohol swabbing on larvae behaviour. We recorded search paths using a video camera mounted on a tripod 1.3 m directly above the arena. The arena and tripod were positioned on a large table and lit by fluorescent lights and skylights approximately 2.3 m above.

All experiments were conducted between 1100 and 1800 hours from 19 August to 22 September 1999. Each treatment was replicated four times. To minimize pseudoreplication, all treatments of a single replicate were performed before any experiments of the next replicate; within a replicate, however, the order of the 36 treatments was randomly determined.

Behavioural Analysis

After a ladybird's path was recorded, the video record was digitized as a series of X–Y coordinates using locally written software. Displaying the video image on a computer monitor, the pixel location of the ladybird was recorded every 0.5 s. The shrunken image of the 60-cm arena on a monitor was approximately 430 pixels wide, or about 1.4 mm of arena distance per column of pixels. Encounters with aphids were also recorded. Before any further analysis was performed, each recorded path was smoothed using the Fourier transform analysis described below. After smoothing, we calculated the search speed and turning rates. Using Mathematica (Martin 1996), we calculated these measures for every recorded move and used mean speed, mean degrees turned per distance, and mean degrees turned per time as appropriate in statistical analyses. The Fourier slope and fractal dimension (see below) were also calculated for each search path. (The Mathematica code is available from the first author).

Prior to all analyses, digitized paths were smoothed by removing the high-frequency noise components (see below). For each of the five path measures, we evaluated proximate cues using two-way ANOVA with food deprivation time and encounter frequency as factors.

Quantitative Analysis

In addition to evaluating the proximate cues used by searching animals, we also tested the efficacy of three approaches to quantifying movement paths. To distinguish between intensive and extensive search behaviour, the tortuosity of search paths is often measured. Predators following a more tortuous path search their local environment more intensively. Search speed and turning rate are metrics that can be calculated at each step in a pathway and then collapsed into a single value, such as the mean, the 90th percentile, or the minimum to quantify a search path as intensive or extensive. Of the many traditional, single-value methods to quantitatively characterize search behaviour (Bell 1991), we chose three for comparison with the two nontraditional techniques: speed, degrees turned per distance and degrees turned per time. These are particularly well suited to capture the qualities of behaviour that distinguish rapid, relatively linear searching from slow, tortuous searching (Banks 1957; Carter & Dixon 1982; White et al. 1984; Turchin et al. 1991).

The angle turned per distance was computed by dividing the angle described by the first, second and third points by the distance from the first to the second to the third point. Repeating this for the second, third and fourth points, the degrees turned per distance for the second angle was calculated. Once all path steps had been used, the average was calculated. The average degrees turned per time was found similarly. For consistency, we calculated the speed of the ladybird as the sum of path lengths from the first to the second to the third point divided by the elapsed time.

Single-value measures represent a path as a single number without regard for the effect that the type of movement (e.g. sharp or shallow turn) has on the overall path. Analyses based on the distribution of the moves over the path, therefore, have the potential to be more powerful. In the study and analysis of animal pathways, the Fourier transform adds two important advantages. First, it can be used to remove motion erroneously introduced into a digitally recorded pathway. This smoothing process can be used in conjunction with many other path analysis techniques, thus improving their ability to accurately describe animal behaviour. Second, the Fourier slope analysis is a novel and powerful addition to the list of methods for quantifying path tortuosity.

A complete discussion of the Fourier transform is beyond the scope of this paper, but to understand the transform's applicability to behavioural analysis, we must understand its basic principles and procedures (see Rameriz 1985 for an introductory account; Briggs & Henson 1995, for an advanced treatment). The Fourier transform is a method of decomposing a complex wave in time or space into an infinite number of component sine and cosine functions by calculating the amplitude and frequency of each function. The amplitude of each component measures the importance of its corresponding frequency to the overall signal. An amplitude of zero implies that the frequency is missing. When mathematically summed, these components exactly reproduce the complex wave. Since in practice it is not possible to use an infinite number of components, truncated Fourier transforms of real signals introduce a measurable amount of error whose magnitude is determined by the number of sine–cosine components included in the analysis. In addition to decomposing a signal to these components, the transformation can be mathematically reversed to recover the original signal.

The Fourier transform of an animal's path may be calculated by plotting separately the X and Y coordinates of a path over time. Figure 1a, b shows the X coordinates for two different paths. By applying the Fourier transform to these sequences of positions, the two physical coordinates can be treated as complex waves described by functions of various amplitudes and frequencies (Fig. 1c, d). Once the path is transformed to this frequency domain form, it can be manipulated and analysed.

Smoothing a noisy signal is the first useful application of Fourier transforms. Whenever a continuous path is recorded using a discrete grid, inevitably the true path passes between recordable grid locations. Use of the discrete grid locations introduces errors. As automatic and digital recording techniques have developed, this problem has become more common. Even though a ladybird follows a straight path, the computer will record it as moving back and forth between pixel locations as the animal moves between the grid locations. Instead of recording coordinates describing a linear path, the computer records a zigzag path, which artificially inflates the complexity of the ladybird's behaviour. The Fourier transform can remove this erroneous motion by manipulating the frequency domain description of the path (DuChateau & Zachmann 1986; Hastings & Sugihara 1993; Kreyszig 1993). The digitization error in a pathway due to movement back and forth between pixels appears as small, rapid changes in the amplitude of X or Y coordinate waves. These rapid changes appear as high-frequency components in the Fourier transform (Fig. 1c, d) and can be removed by setting their corresponding amplitudes to zero. When the time domain path is reconstructed from the altered frequency domain, the new path does not have the high-frequency noise (Fig. 2). Since X and Y coordinates are smoothed independently, this procedure does not remove cusps, sharp turns, or loops actually found in a path.

The object of the smoothing application of the Fourier transform differs from that of other techniques used to aggregate fine-scale tortuosity into coarser directional change. For example, Wiens et al. (1993) suggested renormalization as a method to decrease the complexity of a path while maintaining essential pathway attributes, such as the root mean squared displacement. The object of the Fourier smoothing process is to remove motion that is introduced by digitization and, therefore, not representative of an individual's true behaviour. However, the ability to analyse and manipulate the frequency domain of a pathway makes the Fourier transform a new tool for aggregating fine-scale behaviour into coarser behaviour, which permits close control of the degree of aggregation.

In our study, the removed frequencies corresponded to a wavelength of 1.4 mm or less (Fig. 2). This is the distance corresponding to the highest resolution of our video images. In other words, any component waves describing motion on a scale smaller than the actual distance that could be resolved by the monitor's pixels was removed. To test the sensitivity to different degrees of smoothing, we removed different percentages of high-frequency components. Because the range of high-frequency removal that we examined did not affect the statistical outcomes, we used the smoothed path for all statistical analyses.

The second application of the Fourier transform is to characterize the degree of tortuosity of a path by the amplitudes and frequencies of its component functions. Plotting each component sine and cosine function as a point with its frequency on one axis and its amplitude on the other gives a graphical representation of the relative contribution of each frequency to the complex wave. Extensive searching means that the animal travels longer distances with fewer turns; this produces paths with large,

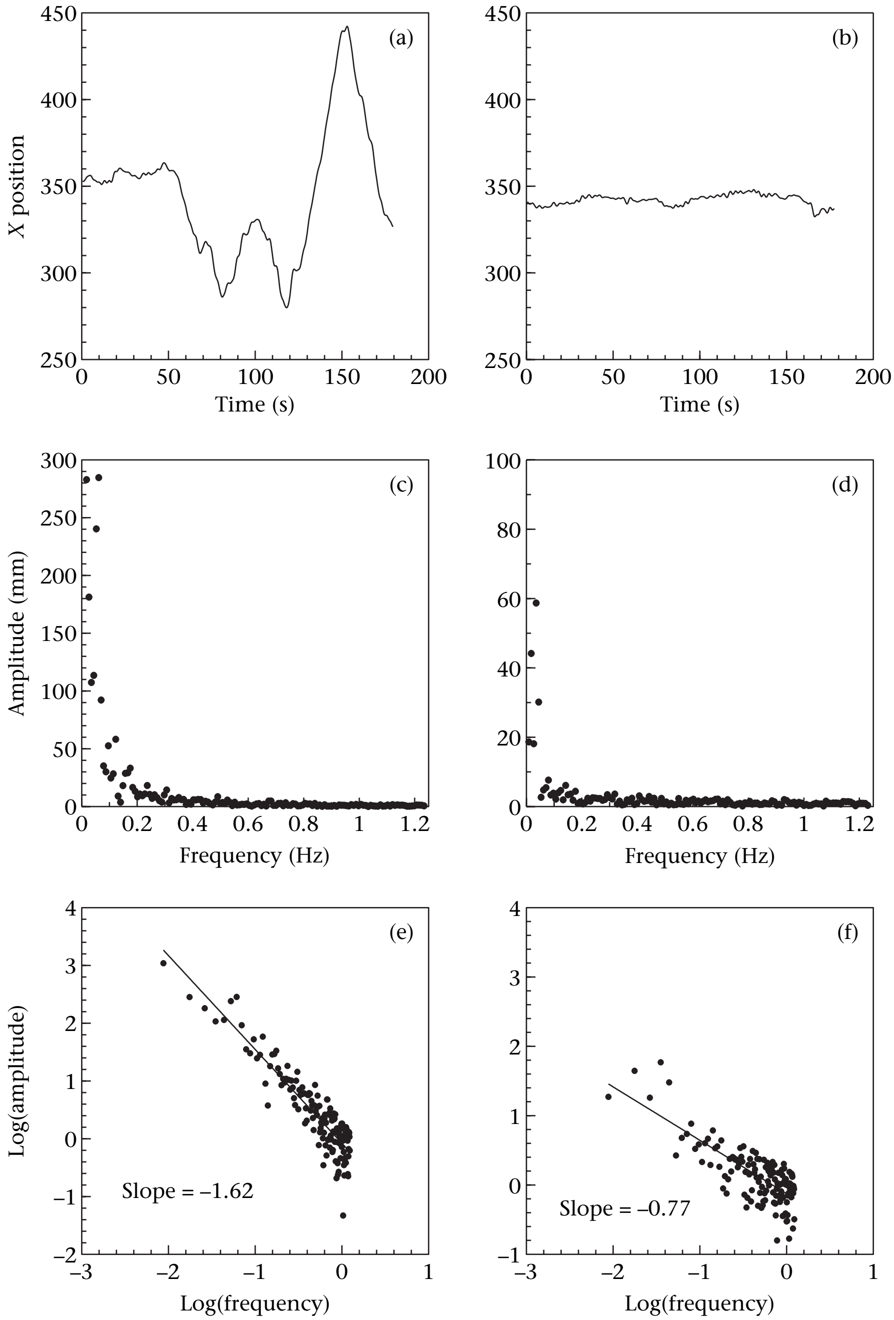

Figure 1. Example analysis of two ladybird searching behaviours using the Fourier transform. On the left is an example of extensive searching; on the right is intensive searching. Plots (a) and (b) are the *X* positions over time. Intensive searching (b) shows little movement from the starting position *X*. Plots (c) and (d) show the Fourier transform of the time domain data to the frequency domain. The amplitude of each frequency of the sine–cosine component is plotted. Note scale differences. Large excursions in plot (a) correspond to high-amplitude waves with low frequency in plot (c). Plots (e) and (f) show the regression line in the log–log graph of the frequency domain. Extensive searching (e) has a steep slope (relatively large amplitudes at low frequencies). Intensive searching has a shallow slope (relatively low amplitude at all frequencies).

slow changes (Fig. 1a) so that low-frequency sine–cosine components have large amplitudes (Fig. 1c). Intensive search paths moving shorter distances become paths with only small changes (frequent turns, Fig. 1b). These paths have low-frequency components of small amplitude (Fig. 1d). A log–log plot of frequencies against amplitudes produces a linear relationship (Fig. 1e, f). The slope of this relationship is a measure of the tortuosity of a search path. Extensive searching produces a steep slope whereas intensive, more tortuous paths produce more shallow slopes (compare Fig. 1a, e and Fig. 1b, f). Completely random movement has a slope of 0.

The Fourier analysis can also be used as an index of the strength of directionality in correlated random walks; as the correlation between successive moves strengthens, the Fourier slope grows steeper. By defining a critical

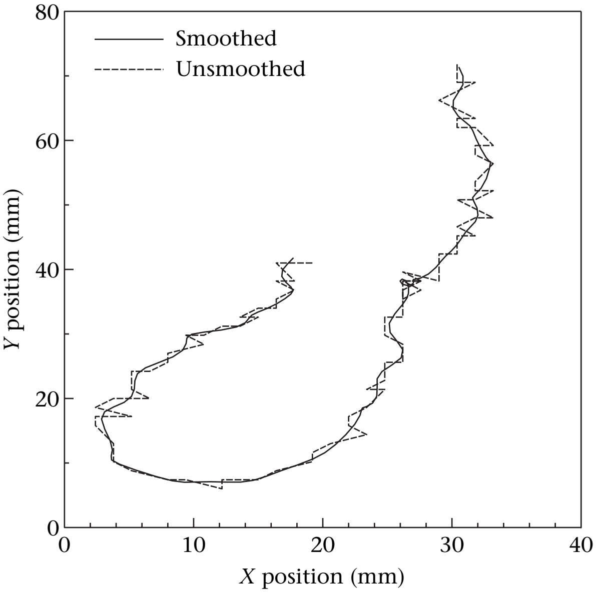

Figure 2. A recorded ladybird search path. The dashed line represents the path as recorded by a computer before smoothing with the Fourier transform. The solid line shows the same path after smoothing.

correlation value, departures of a path from a correlated random walk can be detected. Future assessment can clarify the effectiveness in detecting such departures.

Fractal dimension analysis is a second method that examines the distribution of moves that has been used to quantify the tortuosity of a path (Turchin et al. 1991; Hastings & Sugihara 1993; Wiens et al. 1993; Turchin 1998) and has been used to compare movements of different species (Crist et al. 1992; Wiens et al. 1993; With 1994) and different orders (Wiens et al. 1995) of insects. We used the fractal dimension of search paths to compare the behaviour of individuals of the same species in response to different environmental conditions. Two methods for calculating the fractal dimension, d, are based upon the mean squared displacement from a point of origin or the mean time to exit a circle centred at a point of origin (Johnson et al. 1992). We choose a third, common, method for calculating the fractal dimension, the dividers method (Dicke & Burrough 1988; With 1994). A thorough comparison of the advantages and disadvantages of different methods of calculating the fractal dimension would be useful for assessing the appropriateness of using one calculation over another. Since our concern here is the comparison of fractal dimension between treatments and not the actual value of dimension, the importance of the problem is reduced. In the dividers method, the length of a path is repeatedly measured using rulers of decreasing size. This produces a series of path lengths that increase with decreasing ruler length. The fractal dimension is estimated by the slope of the regression of the log transform of path length against the log transform of ruler length (Hastings & Sugihara 1993).

A common problem with this method is that over a sufficiently large range of divider lengths, the relationship becomes nonlinear at small lengths (Turchin 1998). We experienced this problem in our data and, as a result, used only the subjectively determined linear region to estimate d for each search path. In general, the relationships became nonlinear when the fractal ruler became smaller than 1.4 mm, the actual distance between locations shown on adjacent pixels. This nonlinearity can be explained by the fact that when the fractal ruler becomes smaller than the smallest recordable move, no additional pathway tortuosity is captured. At that point, a plot of tortuosity versus ruler length ceases to follow the linear relationship, and ceases to provide information. While not a serious problem in our analyses, Nams (1996) describes methods for investigating this problem.

RESULTS

The means and standard deviations of the five metrics of ladybird paths are reported in Table 1 and grouped according to encounter rate and satiation level. Each encounter interval represents four replicate experiments at six satiation levels and each satiation level represents four replicate experiments at six encounter intervals, so that in all cases $N = 24$. For each path measure we performed a two-way ANOVA using deprivation time and encounter frequency as factors (Table 2).

High prey density, quantified as a frequent encounter rate, significantly increased the tortuosity of a ladybird's search path. The encounter rate had a significant effect on the natural log-transformed degrees turned per distance, whereas satiation level showed no effect. Considering the degrees turned per time, the encounter rate showed a highly significant effect. Again, satiation level had no effect. Neither factor showed an effect on the searching speed of ladybird larvae. Given that the speed did not vary significantly across treatments, one would anticipate that different methods of measuring turning rate would give similar conclusions. The Fourier slopes steepened significantly (indicating more extensive searching) in response to slower encounter rates, while satiation level had a weak effect. A two-way ANOVA of double natural log-transformed fractal dimensions showed that the encounter rate significantly affected path tortuosity. Satiation level showed no effect.

There were no significant interactions between encounter rate and satiation level in any of the measures (Table 2). To better isolate the treatment effects and account for subtle daylight conditions, we also performed two-way ANOVAs using the hour in which an experiment was performed and the replicate number as blocking factors. In these analyses, the statistical conclusions were unchanged.

DISCUSSION

Proximate Cues

This study evaluated the effect of different proximate cues on the search behaviour of larval ladybird beetles. Regardless of the method of path analysis used, we consistently observed changes in larval behaviour due to encounter rate, but not satiation. We did not adjust

Table 1. Effects of encounter interval and satiation level on movement metrics

	Speed (mm/s)		Degrees/mm		Degrees/s		Fourier slope		Fractal dimension	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Encounter interval (s)										
10	3.5	1.4	26.2	15.6	32.4	6.7	−1.226	0.159	1.132	0.085
30	4.7	2.7	20.6	21.1	25.7	9.4	−1.338	0.232	1.109	0.153
60	4.7	1.8	17.2	18.1	22.3	5.8	−1.438	0.154	1.047	0.036
90	4.4	2.0	15.3	15.9	20.6	7.2	−1.439	0.169	1.050	0.061
135	3.8	2.1	24.7	20.1	24.8	8.1	−1.401	0.171	1.075	0.077
180	4.3	2.0	16.1	17.0	19.4	7.7	−1.482	0.193	1.049	0.054
Satiation level (h)										
0	4.0	1.4	14.6	10.4	22.8	6.1	−1.365	0.160	1.056	0.036
3	4.5	2.3	18.1	17.0	23.0	8.7	−1.432	0.173	1.073	0.077
6	4.2	1.9	18.2	14.9	23.3	8.5	−1.436	0.150	1.064	0.059
12	4.5	2.1	20.5	19.0	23.3	7.5	1.431	0.207	1.057	0.058
18	4.2	2.3	22.8	19.5	26.0	9.6	−1.344	0.224	1.082	0.071
24	3.9	2.4	26.0	25.1	26.7	10.4	−1.316	0.239	1.129	0.168

Sample size is 24 in all cases.

significance levels in Table 2 due to lack of agreement among statisticians that this is advisable (Hurlbert 2003; Moran 2003; but see Rice 1989). Although the five analyses reported in Table 2 are all based on the same data set, all but one of the significant cases ($\alpha = 0.05$) showed extremely strong evidence that encounter rate is biologically meaningful. Four of five probability levels for an effect of satiation were very much larger than 0.05. The only case in which encounter rate was not significant was the measure of speed, which showed no changes in behaviour in response to either encounter rate or satiation. None of the interactions were significant.

These results contradict other studies that found satiation to be the dominant factor guiding behaviour (Carter & Dixon 1982; Nakamuta 1982; Ferran & Dixon 1993; Ferran et al. 1997). Furthermore, some have concluded that prey density plays no role at all (Carter & Dixon 1982). There are several explanations for this discrepancy.

Table 2. Two-way ANOVA results for the effects of encounter rate and satiation level on movement behaviour

Response variable	Factor	df	Mean square	*F*	*P*
Degrees/mm	Encounter	5	2.3185	2.79	0.0207
	Satiation	5	0.5176	0.62	0.6824
	Interaction	25	0.7331	0.88	0.6277
Degrees/s	Encounter	5	522.9197	9.07	<0.0001
	Satiation	5	69.0635	1.20	0.3150
	Interaction	25	53.8015	0.93	0.5602
Speed (mm/s)	Encounter	5	6.1825	1.42	0.2214
	Satiation	5	1.4639	0.34	0.8894
	Interaction	25	3.9928	0.92	0.5783
Fourier slope	Encounter	5	0.2061	6.26	<0.0001
	Satiation	5	0.0654	1.99	0.0863
	Interaction	25	0.0263	0.80	0.7347
Fractal dimension	Encounter	5	5.1853	7.37	<0.0001
	Satiation	5	0.6016	0.85	0.5142
	Interaction	25	0.6238	0.89	0.6227

First, it may be that satiation does have a role in ladybird behaviour but our experimental design, with only four replicates, was insufficient to detect its effect. Nevertheless, the fact that our design was sufficient to detect the effect of encounter frequency suggests that the cue is at least as important as satiation level.

Second, the cues governing ladybird search behaviour may vary with species or life stage. While we used second-instar *C. septempunctata*, Banks (1957) used first-instar *C. septempunctata* and *Propylea quatuordecempunctata*, Carter & Dixon (1982) used fourth-instar *C. septempunctata*, Nakamuta (1982, 1985, 1987) used adult *C. septempunctata*, and Hemptinne et al. (1996) studied adult *A. bipunctata*. This explanation is unlikely, however, since all of the above studies used organisms of similar mobility and evolutionary history. Comparative studies of different species and life stages will most likely resolve this issue.

Third, our choice of food deprivation times, 0–24 h, may have been inappropriate. This explanation is unlikely to be true. Carter & Dixon (1982) used deprivation times of 5 and 25 h and detected a difference in searching behaviour of the fourth-instar of *C. septempunctata*. Nakamuta (1985, 1987) and Hodek & Honěk (1996) also reported the use of a 24-h deprivation period. Also, when studying satiation, it may be that food deprivation time is a poor surrogate. However, in a detailed study of adult *P. coerulescens*, Mols (1988) found a close link between deprivation time and the amount of food in the gut. Extending this relationship to larval *C. septempunctata* seems reasonable considering the finding that the amount of faeces produced by food-deprived, fourth-instar *C. septempunctata* drops to about 80% of the maximum in 25 h (Carter & Dixon 1982).

A fourth explanation for the differences between previous experiments and the current experiment is the way in which prey encounter rates were chosen and administered. To study the effect of different prey encounter rates on ladybird search behaviour, one representative design

(Carter & Dixon 1982) imposed encounter intervals of 7 and 21 min for nearly 2.5 h prior to an observation period. During the observation period no encounters were experienced and no statistical differences in behaviour were seen.

Such designs may not detect the true influence of prey encounter rates because the choice of encounter intervals is inappropriate. The encounter intervals used in the present paper were much shorter, ranging from 10 to 180 s. Differences in search behaviour were most evident in response to the highest encounter rate, every 10 s. This suggests that, to a searching ladybird, if prey encounters do not occur frequently enough, such as every 10 s (indicating to the ladybird that it is in an aphid colony), it does not matter how infrequently the encounters occur. Any lower frequency, such as every 3, 7 or 21 min, is interpreted as being located outside a colony and leads to overall extensive search behaviour.

Finally, in spite of these possible explanations for the discrepancies, there is a more fundamental reason for the disparity between the present research and previous work. This reason hinges on the nature of the experiments that report changes in behaviour of food-deprived animals. In many designs, researchers fed an aphid to a ladybird and recorded the duration of the ensuing intensive search behaviour. They found that hungry larvae searched intensively significantly longer than satiated larvae (e.g. 18 versus 10 s; Carter & Dixon 1982). Such experimental designs focus on the immediate, short-term response. The present experiment, monitoring a 3-min period, evaluates ladybirds' integrated searching decisions following many interactions with potential prey.

Even if the short-term behaviour of a hungry and a satiated ladybird differs, the long-term behaviour will be controlled by the encounter rate, not hunger level. The following conceptual model illustrates this effect. Figure 3 shows the behaviour of two hypothetical animals, one hungry and one satiated, at three different prey densities. Assume that satiation does influence search behaviour so that hungry animals search intensively longer than satiated animals. When prey are rare (Fig. 3a), both animals will begin to search intensively when a prey is encountered. However, if no subsequent aphids are encountered, according to Carter & Dixon (1982), the satiated beetle will revert to extensive searching in about 10 s. The hungry beetle will return to extensive searching in about 18 s, only 8 s longer than a satiated animal (dashed line in Fig. 3a). Even though their immediate reactions to an encounter are different, over long observational periods, when encounter rates are low (suggesting that they are outside an aphid colony), both animals will shift to extensive searching (low turning rates; Fig. 3a).

If these same two ladybirds found themselves within an aphid colony (high encounter rates), their overall behaviour would again be the same in spite of their differing immediate behaviours (Fig. 3b). After an initial encounter, they would both search intensively. But in contrast to low prey density, in a dense colony, it is likely that a second, and then a third encounter would occur before either abandoned intensive search. As a result, over long times, both search behaviours will be dominated by high turning rates, or intensive searching. Again, the short-term responses do not guide overall behaviour (intensive searching). In both scenarios, the prey density, not predator hunger, guides long-term ladybird behaviour.

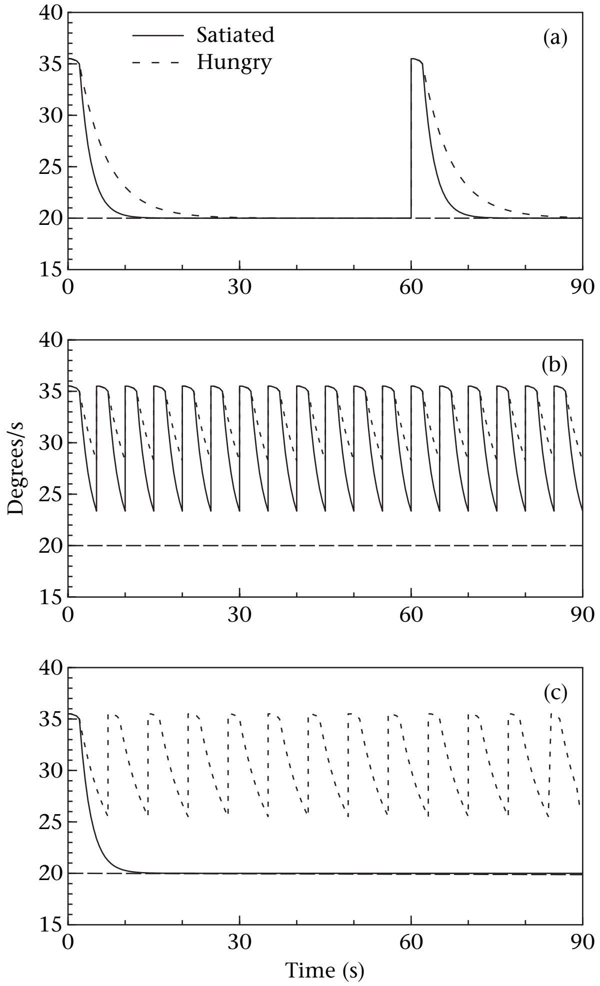

Figure 3. Hypothesized ladybird behaviour in areas of (a) low, (b) high and (c) medium aphid density. Dotted and solid lines represent behaviour of hungry and satiated ladybirds, respectively. More intensive searching is characterized by a more tortuous path, higher degrees turned per second. Peaks indicate times when aphids were encountered. The horizontal dashed line represents the turning frequency associated with extensive search. See text for explanation.

At intermediate encounter rates, however, satiation level can influence long-term behaviour (Fig. 3c). The density may be such that after an initial encounter by a satiated beetle, a second encounter would occur only after the beetle has resumed extensive searching and has left the area. At the same aphid density, however, a hungry beetle will encounter the second aphid before abandoning intensive searching and will therefore remain in the area. Repeated encounters by the hungry beetle will continually reinforce intensive searching (Fig. 3c). Thus, in this case, hunger level does distinguish behaviour.

The frequency with which we observe animals being controlled by hunger or by prey density (Fig. 3a versus Fig. 3c) will depend on how often animals forage at 'intermediate' prey densities. Clearly, there will be an interaction between the time to resume extensive searching and prey density. To make this argument quantitative, suppose aphid encounter rate is the product of aphid density (prey/area) and search rate (area/time). The inverse of encounter rate is mean time between prey encounters. Based on Fig. 3, we expect that hunger levels will control searching behaviour if the time between prey encounters is greater than the time for a satiated beetle to resume extensive searching but less than the time for a hungry beetle to resume extensive searching. It follows that for hunger to control behaviour, the encounter rate must be greater than the inverse of the time for a hungry beetle to resume extensive searching and less than the inverse of the resumption time for a satiated beetle. Or, encounter rate must be greater than 0.055 (1/18 s) and less than 0.10 (1/10 s). To translate encounter rate to aphid density requires that we know the search rate, but suppose it is 5 mm^2/s. Then, an 'intermediate' range of aphid density over which hunger controls searching behaviour will be between about one and two aphids/cm^2. Densities outside this very narrow range will show either extensive searching (<one aphid/cm^2) or intensive searching (>two aphids/cm^2). Aphid colony densities are typically much greater than two/cm^2, and outside an aphid colony, densities are much lower than one/cm^2. So, although short-term behaviour of hungry and satiated aphids is different, we believe that encounter rate dominates control of larval ladybird beetle behaviour in natural systems.

Analysis Techniques

In addition to the biological question, we also applied a numerical technique, Fourier transform, that is not commonly used in behavioural studies of movement. In our study, both the new techniques (Fourier transform and fractal dimensions) and two single-value, traditional measures showed statistical differences when ladybird larvae were presented with different prey encounter rates (Table 2).

Nevertheless, both of these new techniques have some additional advantages. First, as digital recording of movement becomes more common, the ability of the Fourier transform to remove erroneous movement introduced by the recording process is a valuable tool. We have shown that using the Fourier transform for high-frequency filtering of movement data is a practical tool that can be applied to any study using other movement analysis techniques.

Another advantage shared by the newer methods is that they are based upon the entire distribution of behaviours, not simply the mean behaviour. Traditional measures almost always rely on the average as the single value to concisely quantify a range of behaviours. Regardless of whether the mean, maximum or minimum behaviour is used, some information is lost, especially when the distribution of behaviours is non-normal. The way in which the Fourier slope and fractal dimension analyses measure behaviour eliminates the problem of having to ignore part of a behavioural distribution.

We and others have also found that both the Fourier and fractal techniques are more robust over a range of measurement scales (fractal analysis: Wiens et al. 1995) than common techniques. The inappropriate choice of a metric is less likely to result in incorrect conclusions. Indeed, fractal dimension analysis may even suggest an appropriate measurement scale.

The new methods are not without disadvantages, however. The mathematical bases of the techniques are not trivial and the relationship between the statistical indices used (fractal dimension and amplitude–frequency slope) and the behaviour is not directly obvious. Also, when the observational technique can record behaviour on a finer scale than the effective scale of the animal, the ruler size at which linearity fails to hold suggests a natural length scale at which the animal interacts with its environment. The point at which the relationship needed to evaluate fractal dimension becomes nonlinear is often a subjective decision. In our data, at least, this problem did not occur in the use of Fourier transforms. Although powerful, both fractal dimension and Fourier transforms must be used with care before valid biological conclusions are possible. Overall, however, we feel that Fourier transforms provide the greatest benefit to assessing biological movement.

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