Do turning biases by the 7-spot ladybird, *Coccinella septempunctata*, increase their foraging efficiency?

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(Accepted: 21 December 2006)

Summary

The hypothesis that foraging male and female *Coccinella septempunctata* L. would exhibit a turning bias when walking along a branched linear wire in a Y-maze was tested. Individuals were placed repeatedly in the maze. Approximately 45% of all individuals tested displayed significant turning biases, with a similar number of individuals biased to the left and right. In the maze right-handed individuals turned right at 84.4% of turns and the left-handed individuals turned left at 80.2% of turns. A model of the searching efficiency of *C. septempunctata* in dichotomous branched environments showed that model coccinellids with greater turning biases discovered a higher proportion of the plant for a given number of searches than those with no bias. A modification of the model to investigate foraging efficiency, by calculating the mean time taken by individuals to find randomly distributed aphid patches, suggested that on four different sizes of plants, with a variety of aphid patch densities, implementing a turning bias was a significantly more efficient foraging strategy than no bias. In general the benefits to foraging of implementing a turning bias increased with the degree of the bias. It may be beneficial for individuals in highly complex branched environments to have a turning bias slightly lower than 100% in order to benefit from increased foraging efficiency without walking in circles. Foraging bias benefits increased with increasing plant size and decreasing aphid density. In comparisons of two different plant morphologies, one with a straight stem and side branches and one with a symmetrically branched morphology, there were few significant differences in the effects of turning biases on foraging efficiency between morphologies.

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Behaviour 144, 143-163
Also available online - www.brill.nl/beh

Keywords: cropping strategy, outline tracing, handedness, foraging efficiency simulation model.

Introduction

Predatory insects use a number of different foraging patterns in order to optimise their patch discovery rate. Search orientation can be divided into two phases, search strategies and search tactics. A search strategy is ‘a set of basic rules of scanning and locomotion that results in the effective encountering of a specific distribution of food’. A search tactic is ‘an adaptive change in scanning or locomotion occurring once a predator has arrived in a specific area where prey are available’ (Smith, 1974).

Insects typically search for food, hosts or sexual partners in geometrically complex environments (Casas & Aluja, 1997), for example, aphidophagous coccinellids often oviposit, and feed on aphids, on a wide range of plant species (Majerus & Kearns, 1989). The majority of studies of foraging behaviour of coccinellids have been conducted on their larvae (Dixon, 2000); however, Kindlmann & Dixon (1999) suggest that the search strategies of adult coccinellids selecting suitable patches for oviposition are likely to be the most influential in determining the animal’s fitness.

When a coccinellid is in a branched plant environment selection will favour individuals that use the most efficient mechanism to find aphids on a plant. Physical cues and plant architecture can greatly effect coccinellid foraging, for example a greater degree of overlap between leaves of adjacent plants greatly increases the rate at which adult convergent ladybird beetles *Hippodamia convergens*, move through vegetation (Kareiva & Perry, 1989). In experiments monitoring the movement of *C. septempunctata*, in different sized plots of broccoli, the spatial scale of plots and occurrence of patch boundaries modified individuals’ displacement and movement behaviour (Banks & Yasenak, 2003). Additionally, *C. septempunctata* displays area restricted search behaviour following the consumption of aphids, by increasing the frequency at which they turn (Nakamuta, 1985). This area restricted searching results in aggregation of predators (Karieva & Odell, 1987).

Some coccinellids can only detect aphids visually at very short distances; 7 mm in adult *C. septempunctata* (Nakamuta, 1984). There is some evidence to suggest that coccinellids are able to use aphid volatiles as cues to find their prey (Obata, 1986; Raymond et al., 2000; Sengonca & Liu, 1994), although other authors suggest that coccinellids are not able to detect volatiles from aphids alone (Nakamuta, 1984; Schaller & Nentwig, 2000; Ninkovic et al., 2001; Girling & Hassall, unpubl.), as is the case for the larvae of *Adalia bipunctata*, which are not attracted by the odour of aphids (Hemptinne et al., 2000). In a comprehensive review, Dixon (2000) concluded that we do not know the extent to which olfactory and visual cues are used by coccinellids to search for prey, but that when they are used they can only detect such cues over short distances. Therefore, there will be strong selection to evolve the most efficient basic rules for locomotion to maximise the probability of encountering prey patches.

One such search strategy is ‘cropping’. This is often used by animals that need to locate many food objects (Jander, 1977). Some ants search horizontal branches using a cropping strategy known as ‘outline tracing’. When searching on a branch an ant will make either a right or a left turn and will continue to turn in the same direction at every following junction it encounters on the branch. This ensures that the overall path length will be as short as possible (Jander, 1977). Outline tracing is based on an insect showing an innate bias to turn in one direction or another, often referred to as ‘handedness’.

Many experiments on insect foraging behaviour have been conducted in Y-tube olfactometers. In these types of experiments, any evidence that the insect is using a searching strategy of outline tracing would result in invalidation of the commonly assumed null hypothesis that the insect will follow each branch of the olfactometer 50% of the time. The present study was designed to investigate possible turning biases in a population of *C. septempunctata* on a stylised branch in a Y-tube olfactometer with no odour sources in either arm, i.e., a Y-maze. A simulation model was used to investigate whether turning more frequently in one direction would increase searching efficiency of an individual in a branching environment.

The specific hypotheses tested were: 1. Individual male and female *C. septempunctata* will show significant turning biases when walking on a stylised branch in a Y-maze, which was tested with laboratory experiments; and 2. Turning biases will alter the searching efficiencies of individual coccinellids foraging in complex branching environments, which was tested with a simulation model.

Material and methods

Study organisms

Coccinella septempunctata L. (Coleoptera: Coccinellidae) were collected as pupae from the University of East Anglia campus (Norwich, Norfolk) (52°36′N, 1°15′E) during June 2004. Pupae were kept separately in 9 cm Petri dishes at room temperature under a L16:D8 light regime and after hatching adults were fed three times a week on an excess of *Acyrthosiphon pisum* Harris (Homoptera: Aphididae), which had been reared on *Vicia faba* L. (Fabaceae) plants, grown under greenhouse conditions.

Design of Y-maze

Dual-choice tests were carried out in a Y-maze (Steinberg et al., 1992). A traditional glass Y-tube olfactometer was difficult to clean, especially if animals defecated whilst in the tube. Furthermore, due to the shape of the tubes, individual *C. septempunctata* would often circle within them and so take a long time to reach the Y-junction. A new design of Y-maze containing a linear wire for test specimens to walk along was therefore developed (Figure 1). This was made from two blocks of Perspex (17.8 × 29 × 1.3 cm) with a Y-shaped semi-circular channel, 1.9 cm in width, cut into one side of each block with arms 15 cm long and at an angle of 60°. When placed together the pieces made a cylindrical Y-shaped tube. On one block a channel 0.3 cm wide by 0.25 cm deep was cut 0.5 cm from the edge of both sides of the Y-channel containing a 0.3 cm diameter O-ring, to create an airtight seal when the two blocks were placed together. Three 0.6 cm holes were drilled through both blocks, one between the arms of the Y-junction and the other two either side of the main arm (Figure 1). 0.5 cm bolts fitted with wing nuts held the two blocks together. The Y-maze could then be opened easily for cleaning the entire internal surface.

A linear wire for the coccinellids to walk along, similar to that described by Sabelis & van de Baan (1983), was designed to fit inside the maze. The wire was made from 0.3-cm-diameter stainless steel rod, the main arm was 14.5 cm and the two shorter arms 12 cm. The three arms were carefully welded together, to produce as smooth a join as possible. 1 cm at the end of each arm was bent over to form supports to hold the wire away from the surface of the maze tube.

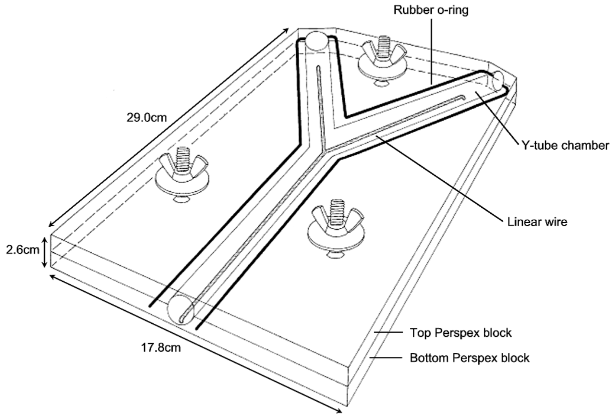

Figure 1. Details of design of Perspex Y-maze.

Air, without any volatiles introduced, was pumped through an activated charcoal filter, through Teflon tubing and divided in two by a T-junction. The two airflows then passed through separate flow meters, which regulated the flow rate at 400 ml min^{-1}. The air then passed into the arms of the Y-maze. All experiments were conducted in a constant temperature room at ca. 23°C. The majority of previous studies using Y-tube olfactometers have oriented them horizontally, e.g., Steinberg et al. (1992). Therefore, the Y-maze in this study was also oriented horizontally, so that the results could be directly comparable to those of previous studies. The Y-maze was lit from above by a fluorescent lamp, fitted with a prismatic filter, to ensure a completely even light distribution.

Laboratory test for bias in individual C. septempunctata

Individuals were placed one at a time on the wire at the stem end of the maze and given 5 minutes to enter either arm. When an individual entered one of the arms of the maze it was recorded as having made a turning decision. After each individual coccinellid was tested, the maze was dismantled, cleaned with tissue soaked in 100% ethanol and allowed to dry before the next test. For each individual several different linear wires were selected randomly to avoid any consistent physical asymmetry in the simulated branch. Each individual was tested between 14 and 16 times, aligning the maze to different points of the compass an equal number of times to negate the effects of any environmental gradients within the room. Each individual was tested at each compass point on more than one day to eliminate any random daily bias. Individuals were starved for approximately 24 hours before testing. Starving beetles for 18 hours resulted in maximum search activity (Frazer & Gilbert, 1976). A total of 42 individuals were tested: 17 male and 25 female. For each individual tested, the total number of left and right turning decisions was compared using a chi-square test.

Simulation model of searching in branched environments

A simple model was developed, using Microsoft Visual Basic 6.0© software, to investigate how turning biases affect the searching efficiency of *C. septempunctata* in dichotomous branched environments. The model was used to investigate the effect of turning bias on the rate at which a plant was explored. It was assumed that no other stimuli were used in searching. Searching behaviour was simulated for two different branch morphologies: i) a symmetrically branched plant (Figure 2a); and ii) a plant with a straight stem and side branches (Figure 2b). Four sizes of plants were used, with 7, 15, 31 and 63 branches, these numbers being derived by adding an extra layer of branches to the basic seven-branch symmetrical plant for each increment of size.

The model was based on the following rules: individuals 1) always start searching at the base of a plant; 2) always walk at the same speed along equal length branches; 3) walk in the same direction along a branch until they reach the end of it when they turn around (Frazer & McGregor (1994) demonstrated on leaf models that *C. septempunctata* most often left the leaf model by turning around and walking back down the stem); 4) when

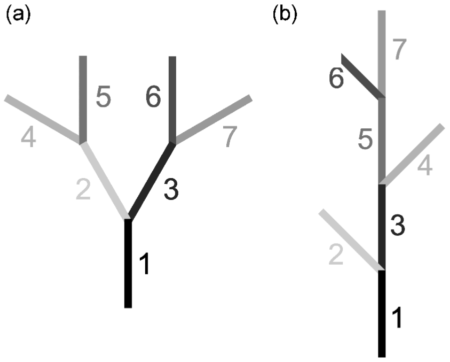

Figure 2. Seven-branch model plant morphologies: (a) symmetrical plant; (b) straight plant. Different shading indicates different individual branches.

reaching a junction always have to ‘select’ to turn either left or right, with a frequency determined by the turning bias with which the individual was programmed. The responses of individuals with three different turning biases were investigated: i) 50% left-turns (no bias, i.e., at each junction there was a 50% probability of the individual turning left or right); ii) 80% left-turning bias (the mean left-turning bias of the individuals that were significantly left-handed in the maze experiment); iii) 100% left-turning bias. Each movement an individual made from one branch to another was counted as one unit of search. Discovery of a branch was defined as the first time an individual walked on it. The percentage of the plant discovered was determined for different lengths of search, that is, for different numbers of search units, e.g., 5, 10, 25, 50, 100 up to 1000 in a search. Each length of search was run 100 times, simulating the responses of 100 individuals and responses averaged to give the mean search efficiency, defined as the mean percentage of the entire plant discovered for a given search length.

Additionally, a modification to the model was made to investigate the effects of turning bias’ on foraging efficiency, by calculating the mean number of searches required for modelled individuals to locate different numbers of randomly distributed aphid food patches. Aphid patches were randomly assigned to one of the plants branches (other than the starting basal branch), each branch could only be assigned one patch, and discovery of a patch was defined as the first time an individual walked on that branch assigned with a patch. The mean number of search units required for 500 simulated coccinellid individuals to discover an aphid patch was determined, for each of the 50%, 80% and 100% turning bias categories, at the following aphid patch densities: (a) One aphid patch randomly located on any branch of the plant, for each of the four plant sizes, of both plant morphologies; and (b) Multiple aphid patches randomly located, on both plant morphologies of only the 63 branch plants, with either 3, 6, 9 or 16 branches possessing an aphid patch (this corresponds to aphid patch densities of ca. 5, 10, 15 and 25% of branches, respectively). These densities were chosen because aphid numbers in nature fluctuate greatly (depending on the season, stage of infestation etc.) (Dixon, 1998) and at high aphid densities, the difference in foraging efficiency between an individual using a foraging strategy and one searching at random will likely be reduced, assuming that the aphid patches are randomly distributed on the plant. For example, if aphid densities reach >25% (i.e., more than one branch in four infested), then it is unlikely that a foraging strategy will significantly increase patch discovery rate over a random search strategy.

A series of Shapiro-Wilk tests showed that the data was not normally distributed. Furthermore, data could not be normalized by transformation. Therefore, Kruskal-Wallis tests were used to compare the mean number of search units taken to discover an aphid patch, by model coccinellids programmed with different turning biases, between all three turning bias categories, within each of the 16 combinations of plant size, plant morphology and aphid patch density. Additionally, Mann-Whitney tests were used to compare the mean number of search units taken to discover an aphid patch between individual pairs of different turning bias categories (e.g. 50% vs. 80%), within each of the 16 combinations of plant size, plant morphology and aphid patch density. Further Mann-Whitney tests were used to compare, between the two plant morphologies, the mean number of search units taken to discover an aphid patch, for pairs of matching turning bias categories, either within each plant size, for tests with plants assigned a single aphid patch (e.g., between the 50% bias category on the 7 branch symmetrical plant and the 50% bias category on the 7 branch straight plant), or within each aphid patch density category, for tests with plants assigned multiple aphid patches. All statistics were performed using SPSS v.10.

Results

Laboratory test results: Test for bias in individual C. septempunctata

19 of the 42 individuals tested (45%) displayed a significant turning bias (Table 1), 23.8% to the left and 21.4% to the right. 47% of males and 44% of females exhibited a bias. Of those that did exhibit a bias 75% of the males and 36% of females were biased to the left (Table 1). Overall, for the 42 individuals of both sexes tested, in all three groups: right-handed, left-handed and ambidextrous combined, the mean proportion of their turns that were to the left was 50.0 ± 3.7% and to the right were 50.0 ± 3.7%, confirming that there were no environmentally induced biases in the apparatus. Of those classified as left-handed 80.2 ± 2.0% of their turns were to the left and of those classified as right-handed 84.4 ± 2.7% of their turns were to the right (Table 1).

Table 1. Number of turns made by individual male and female *C. septempunctata* when repeatedly tested in a Y-maze.

Individual Number	No. of left turns	No. of right turns	% left turns	% right turns	χ^2	p	Handedness	Sex
1	13	1	92.9	7.1	10.29	<0.01	Left	♀
2	13	3	81.25	18.75	6.25	0.01	Left	♀
3	13	3	81.25	18.75	6.25	0.01	Left	♂
4	12	2	85.7	14.3	7.14	0.01	Left	♀
5	12	2	85.7	14.3	7.14	0.01	Left	♂
6	12	4	75	25	4	0.05	Left	♂
7	12	4	75	25	4	0.05	Left	♂
8	12	4	75	25	4	0.05	Left	♂
9	12	4	75	25	4	0.05	Left	♂
10	12	4	75	25	4	0.05	Left	♀
11	11	5	68.75	31.25	2.25	0.13	Ambidextrous	♀
12	11	5	68.75	31.25	2.25	0.13	Ambidextrous	♂
13	10	4	71.4	28.6	2.57	0.11	Ambidextrous	♀
14	10	6	62.5	37.5	1	0.32	Ambidextrous	♀
15	9	5	64.3	35.7	1.14	0.29	Ambidextrous	♂
16	9	7	56.25	43.75	0.25	0.62	Ambidextrous	♀
17	9	7	56.25	43.75	0.25	0.62	Ambidextrous	♀
18	9	7	56.25	43.75	0.25	0.62	Ambidextrous	♀
19	8	6	57.1	42.9	0.29	0.59	Ambidextrous	♀
20	8	6	57.1	42.9	0.29	0.59	Ambidextrous	♂
21	8	8	50	50	0	1	Ambidextrous	♂
22	8	8	50	50	0	1	Ambidextrous	♂
23	8	8	50	50	0	1	Ambidextrous	♂
24	7	9	43.75	56.25	0.25	0.62	Ambidextrous	♂
25	7	9	43.75	56.25	0.25	0.62	Ambidextrous	♀
26	7	9	43.75	56.25	0.25	0.62	Ambidextrous	♀
27	6	8	42.9	57.1	0.29	0.59	Ambidextrous	♀
28	6	8	42.9	57.1	0.29	0.59	Ambidextrous	♀
29	6	10	37.5	62.5	1	0.32	Ambidextrous	♀
30	6	10	37.5	62.5	1	0.32	Ambidextrous	♂
31	5	9	35.7	64.3	1.14	0.29	Ambidextrous	♀
32	5	11	31.25	68.75	2.25	0.13	Ambidextrous	♂
33	4	10	28.6	71.4	2.57	0.11	Ambidextrous	♀
34	4	12	25	75	4	0.05	Right	♀
35	4	12	25	75	4	0.05	Right	♂
36	3	13	18.75	81.25	6.25	0.01	Right	♀
37	3	13	18.75	81.25	6.25	0.01	Right	♀
38	3	13	18.75	81.25	6.25	0.01	Right	♀
39	2	12	14.3	85.7	7.14	0.01	Right	♀
40	2	14	12.5	87.5	9	<0.01	Right	♀
41	1	13	7.1	92.9	10.29	<0.01	Right	♀
42	0	16	0	100	16	<0.001	Right	♂

Table 1. (Continued)

Total left turns	Total right turns	Mean % left (± SE)	Mean % right (± SE)	
322	324	**50.0** ± 3.7	**50.0** ± 3.7	All individuals
123	31	**80.2** ± 2.0	**19.8** ± 2.0	Left-handed ($N = 10$)
22	118	**15.6** ± 2.7	**84.4** ± 2.7	Right-handed ($N = 9$)

Simulation results: Modelling searching on simple branched plants

On all sizes of both symmetrically and straight branched plants, individuals with greater turning biases discovered a higher percentage of branches for a given number of search units (duration of search) than those with smaller or no turning biases (data only shown for 63 branch plant, Figure 3a & b). The increased searching efficiency, resulting from larger turning biases, became most apparent after individuals had discovered between ca. 30% to 50% of the plant, depending on the size of plant. Even on the largest plant, where the effects of bias were greatest, when less than approximately 30% of the plant was discovered the turning biases of the individuals did not greatly affect their searching efficiency (Figure 3a & b).

Simulation results: Modelling foraging on different sized plants with one aphid patch

On simulated plants, assigned one randomly distributed aphid patch, there were significant differences in the mean number of searches taken to discover the patch, by simulated coccinellids programmed with different turning biases, between all three turning bias categories, on all symmetrical branching plants: 7 branch (K-W, $\chi^2 = 20.3$, df = 2, $p < 0.001$), 15 branch (K-W, $\chi^2 = 42.4$, df = 2, $p < 0.001$), 31 branch (K-W, $\chi^2 = 58.9$, df = 2, $p < 0.001$), 63 branch (K-W, $\chi^2 = 132.4$, df = 2, $p < 0.001$) (Figure 3c); and on all straight branching plants: 7 branch (K-W, $\chi^2 = 18.2$, df = 2, $p < 0.001$), 15 branch (K-W, $\chi^2 = 55.7$, df = 2, $p < 0.001$), 31 branch (K-W, $\chi^2 = 155.2$, df = 2, $p < 0.001$), 63 branch (K-W, $\chi^2 = 265.4$, df = 2, $p < 0.001$) (Figure 3d). Furthermore, in comparisons of the mean number of searches taken to discover the aphid patch between individual pairs of different turning bias categories, within each of the eight plant morphology and plant branch number combinations, all pairs were significantly different to at least $p = 0.05$ (Table 2). In all comparisons, coccinellids programmed with

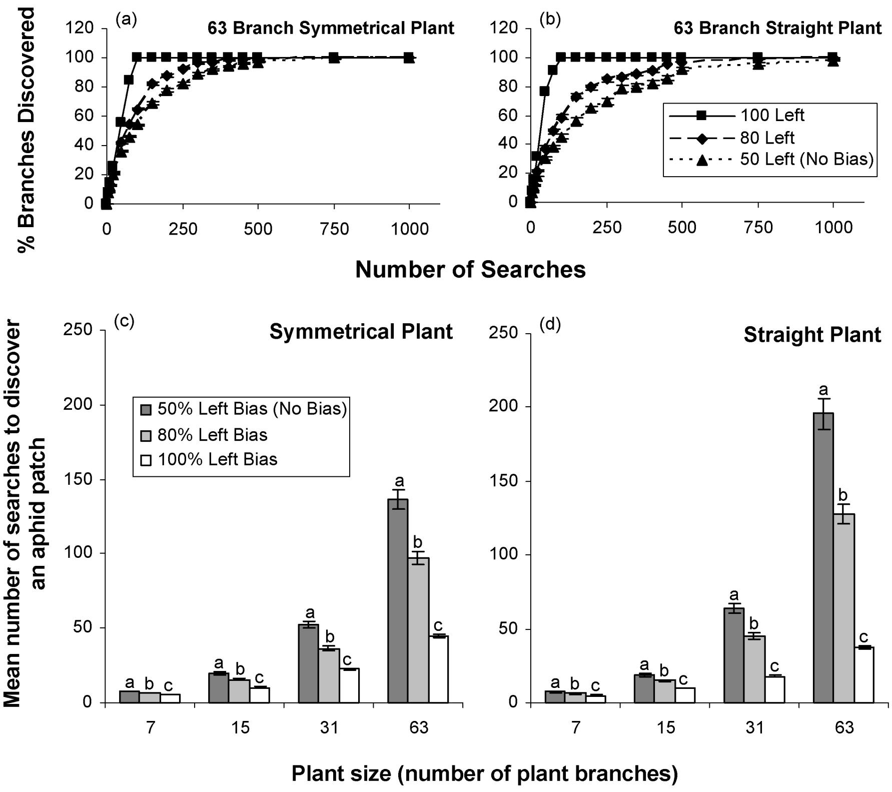

Figure 3. (a, b) Simulation model results of the mean number of branches discovered, as a percentage of the number of branches searched by 100 individuals programmed with each of three different left turning biases (100 individuals per turning bias) on 63 branch plants with (a) a symmetrical morphology or (b) a straight morphology. (c, d) Simulation model results of the mean number of search units (duration of search) taken to discover a randomly located aphid patch by individuals with three different left turning biases (500 individuals per turning bias) on (c) symmetrical or (d) straight plants of four different sizes: 7, 15, 31 or 63 branch plants. Bars marked by different letters, within a plant size and morphology category, are significantly different (Mann-Whitney, $p < 0.05$).

a 100% bias took significantly the fewest mean number of searches and those programmed with no bias significantly the greatest. These data suggest that, when there was only a single aphid patch, implementing a turning bias was a significantly more efficient foraging strategy than implementing no bias, for both plant morphologies at all four plant sizes. Furthermore, the benefits of implementing a turning bias increased as the degree of bias increased. Additionally, these data show a general trend where, as the plant branch number

Table 2. Results of Mann-Whitney tests, used to analyse simulation data, comparing the mean number of searches taken to discover a randomly distributed aphid patch, by simulated coccinellids programmed with one of three different turning biases, between pairs of different turning bias categories, within each of eight plant morphology and plant branch number combinations.

Turning bias categories		Symmetrical plant size (# branches)				Straight plant size (# branches)			
		7	15	31	63	7	15	31	63
50% vs. 80%	Z	−2.28	−1.96	−4.28	−3.60	−2.09	−2.48	−3.55	−4.93
	p	0.02	0.05	<0.001	<0.001	0.04	0.01	<0.001	<0.001
50% vs. 100%	Z	−4.56	−6.21	−7.79	−11.16	−4.30	−7.26	−11.97	−15.42
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
80% vs. 100%	Z	−2.12	−4.61	−3.06	−7.81	−2.12	−4.98	−8.77	−11.68
	p	0.03	<0.001	<0.01	<0.001	0.03	<0.001	<0.001	<0.001

increased so did the level of significance of comparisons of the mean number of searches taken to find a patch between different turning bias categories (Table 2). This indicates that the foraging benefits of employing a turning bias increase with increasing plant size.

In comparisons between the two plant morphologies, of the mean number of searches taken to discover the aphid patch by simulated coccinellids, for pairs of matching turning bias categories within each plant size, the only significant differences between morphologies were in the 100% bias category on the 31 (M-W, $Z = -6.50$, $p < 0.001$) and 63 (M-W, $Z = -4.67$, $p < 0.001$) branch plants, where there were significantly fewer searches on straight plants, and in the 50% (M-W, $Z = -3.59$, $p < 0.001$) and 80% (M-W, $Z = -2.00$, $p = 0.05$) bias categories on the 63 branch plants, where there were significantly fewer searches on symmetrical plants (Figure 3c & d). This suggests that there were very few differences in foraging efficiency between the two plant morphologies, for all turning bias categories, except at larger plant sizes where a 100% bias was more efficient on straight than symmetrical plants, and no bias or an 80% bias was more efficient on symmetrical than straight plants.

Simulation results: Modelling foraging on 63 branch plants with multiple aphid patches

On simulated 63 branch plants, assigned multiple randomly distributed aphid patches, there were significant differences in the mean number of searches taken to discover an aphid patch, by simulated coccinellids programmed with different turning biases, between all turning bias categories, on symmetrical branching plants with: 3 patches (K-W, $\chi^2 = 38.4$, df $= 2$, $p < 0.001$), 6 patches (K-W, $\chi^2 = 8.2$, df $= 2$, $p = 0.02$), 9 patches (K-W, $\chi^2 = 6.8$, df $= 2$, $p = 0.03$), but not 16 patches (K-W, $\chi^2 = 5.0$, df $= 2$, $p = 0.08$) (Figure 4a); and also on all straight branching plants with: 3 patches (K-W, $\chi^2 = 98.7$, df $= 2$, $p < 0.001$), 6 patches (K-W, $\chi^2 = 35.1$, df $= 2$, $p < 0.001$), 9 patches (K-W, $\chi^2 = 26.9$, df $= 2$, $p < 0.001$), 16 patches (K-W, $\chi^2 = 18.4$, df $= 2$, $p < 0.001$) (Figure 4b). Furthermore, in comparisons of the mean number of searches taken to discover an aphid patch between individual pairs of different turning bias categories, within each of the eight plant morphology and aphid patch density combinations, all pairs were significantly different to at least $p < 0.05$, except for comparisons between the 50% and 80% bias categories on symmetrical plants with 16 aphid patches, and the 80% and 100% bias categories on symmetrical plants with 6, 9 and 16 aphid patches (Table 3). In all comparisons, regardless of whether differences were significant or not, coccinellids programmed with a 100% turning bias took the fewest mean number of searches and those programmed with no bias took the greatest. These data suggest that implementing a turning bias was a significantly more efficient foraging strategy than implementing no turning bias at all aphid densities on both plant morphologies, except for the 80% bias category on the highest level of aphid patch density on symmetrical plants. Furthermore, on symmetrically branched plants possessing the three highest aphid patch densities there was no significant increase in foraging efficiency for coccinelids implementing a 100% turning bias over an 80% bias. These data also show a trend, although not strictly adhered to, that as aphid patch densities decreased the significance of comparisons of the mean number of searches taken to find a patch between different turning bias categories, increased (Table 3). This indicates that the foraging benefits of employing a turning bias increase with lowering aphid density.

In comparisons between the two plant morphologies, of the mean number of searches taken to discover the aphid patch by simulated coccinellids,

Table 3. Results of Mann-Whitney tests, used to analyse simulation data, comparing the mean number of searches taken to discover a randomly distributed aphid patch, by simulated coccinellids programmed with one of three different turning biases, between pairs of different turning bias categories, within each of eight plant morphology and aphid patch number combinations.

Turning bias categories		Symmetrical plant size (No. of aphid patches)				Straight plant size (No. of aphid patches)			
		3	6	9	16	3	6	9	16
50% vs. 80%	Z	−2.14	−2.56	−2.38	−1.12	−4.33	−2.08	−2.87	−2.10
	p	0.03	0.01	0.02	0.26	<0.001	0.04	<0.01	0.04
50% vs. 100%	Z	−6.04	−2.32	−2.12	−2.25	−10.06	−5.74	−5.22	−4.29
	p	<0.001	0.02	0.03	0.02	<0.001	<0.001	<0.001	<0.001
80% vs. 100%	Z	−4.07	−0.61	−0.33	−1.10	−5.31	−3.92	−2.21	−2.21
	p	<0.001	0.54	0.74	0.27	<0.001	<0.001	0.03	0.03

for pairs of matching turning bias categories within each plant size, the only significant differences between morphologies were in the 100% bias category on plants with 3 (M-W, $Z = -2.39$, $p = 0.02$) and 6 (M-W, $Z = -2.34$, $p = 0.02$) aphid patches, where there were significantly fewer searches on straight plants, and also in the 50% bias category on plants with 3 (M-W, $Z = -2.43$, $p = 0.02$) and 16 (M-W, $Z = -2.31$, $p = 0.02$) aphid patches and the 80% bias category on plants with 6 (M-W, $Z = -2.37$, $p = 0.02$) aphid patches, where there were significantly fewer searches on symmetrical plants (Figure 4). These results suggest that there were few differences in foraging efficiency between plant morphologies but that at the two lower aphid densities, implementing a 100% bias was more efficient on straight than symmetrical plants. Additionally, on the occasions when the 50 and 80% biases were significantly more efficient on one plant morphology than another it was always on the symmetrical branching plants.

Discussion

We found that nearly half of both male and female *C. septempunctata* exhibited turning biases when they encountered a single Y-junction (Table 1). On average left-handed individuals made 80.2% of turns to the left and right-handed individuals 84.4% of turns to the right. Previously handedness has

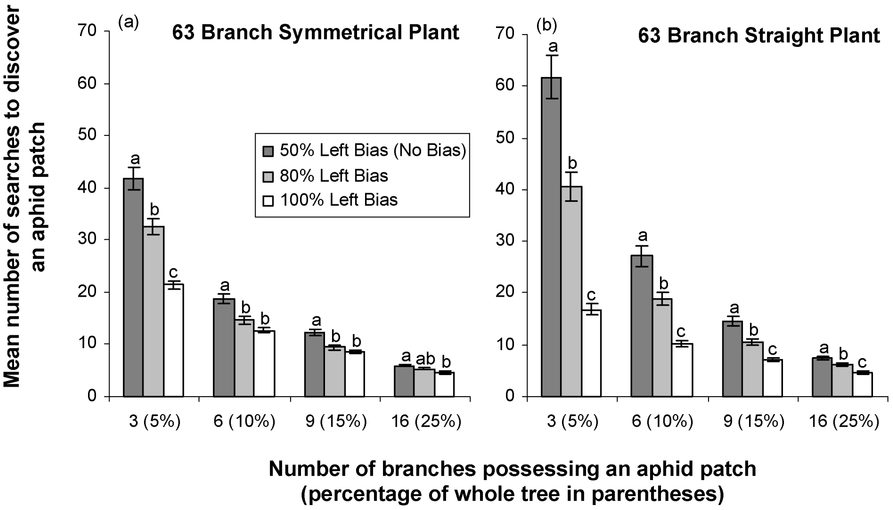

Figure 4. Simulation model results of the mean number of search units (duration of search) taken to discover randomly located aphid patches on a 63 branched plant, at four different aphid densities (3, 6, 9 and 16 aphid patches per plant), by individuals with three different left turning biases (500 individuals per turning bias) on plants with: (a) symmetrical branching morphology; and (b) straight branching morphology. Bars marked by different letters, within an aphid patch density category, are significantly different (Mann-Whitney, $p < 0.05$).

only been described for coccinellids in the unfed larvae of the 2-spot ladybird *Adalia bipunctata* (Banks, 1957), on flat surfaces. Bansch (1966) reported no evidence of handedness in adults or larvae of *A. bipunctata* when searching a 50-cm-high branching model tree, instead their behaviour was suggested to be controlled mainly by geotaxis. *C. septempunctata* did not display a turning bias in a traditional glass Y-tube olfactometer without wires (Schaller & Nentwig, 2000). We suggest that *C. septempunctata* behaved differently when walking along branched wires as these more closely resemble a branched plant stem than does the surface of a glass tube. We have not yet tested whether individuals of *C. septempunctata* would respond similarly on vertically orientated branches.

Other examples of turning biases in arthropods in Y-tube olfactometers and Y-mazes have been reported. The tropical ant *Azteca* sp., walking along a wire in a Y-tube olfactometer showed a similar turning bias (D. Edwards, pers. comm.) Turning biases have also been reported for *Aleochara bilineata* (Coleoptera) (Putnam, 1962), *Calcinus herbsteii* and *Clibinarius zebra* (two species of hermit crab) (MacKay, 1945, 1947) and *Heliodrilus* (Annelida) (Swartz, 1929). There have also been a few reports of turning biases on flat surfaces, e.g., desert ants (Wehner & Srinivasan, 1981) and *Pediculus humanus* (Anoplura) (Wigglesworth, 1941) and in other environments e.g. in a number of bumblebee species (Cheverton, 1982; Kells & Goulson, 2001). This suggests that turning biases may be a widely distributed trait, throughout the Arthropoda. However, the effects of turning biases on foraging behaviour are likely to be different between different feeding guilds, e.g., predators, seed predators, herbivores, etc.

The existence of biases in turning behaviour of *C. septempunctata*, complicates the use of linear wire Y-tube olfactometry as a bioassay because null hypotheses have to be adjusted after testing for handedness of each individual used. Using a linear wire however has the advantage of more closely resembling the field environment, where coccinellids forage in a complicated three-dimensional branching matrix not only on flat surfaces (Casas & Aluja, 1997).

The model of searching efficiency suggests that on all plant sizes of both plant morphologies, model coccinellids with stronger turning biases discovered a higher proportion of the plant for a given number of searches than for those with no bias (50% left turns), therefore turning biases increased searching efficiency (Figure 3a & b). A modification to the model to investigate the effects of turning biases on foraging efficiency suggested that on all plant sizes of both plant morphologies, with a variety of different aphid patch densities, implementing a turning bias was a significantly more efficient foraging strategy than no turning bias (Figures 3 & 4). In general, the benefits to foraging efficiency of employing turning biases increased with the degree of the bias.

The model also suggested that the benefits of implementing a turning bias increased with increasing plant size and also increased with decreasing aphid density, i.e., the more scarce the aphid patches were, the greater were the increases in foraging efficiency. Because the average development time of coccinellid larvae is similar to the average duration of existence of an aphid prey patch, adult female coccinellids must find prey patches to lay eggs in at an early stage in their development, to allow enough time for their larvae to complete their development before the aphid patch disappears (Hemptinne & Dixon, 1991). Therefore, because the model suggests that turning biases may increase foraging efficiency when aphid prey are scarce, turning biases may increase an individuals probability of discovering smaller prey patches early in their development. The distributions of aphid patches in the model were always random. Whether the results would differ for clumped distributions of aphid patches was not tested. We predict that clumping of patches would likely result in an increase in foraging efficiency of turning biases over no bias because clumping would essentially mean that the aphid patches were a more isolated and scarce resource. In the model there were few significant differences in the effects of turning biases on foraging efficiency between plant morphologies. The only consistent result was at lower aphid densities tested, where a 100% bias was more efficient on straight than symmetrical plants.

The morphologies of plants in this model were relatively simple. In more complex branched environments where the possibility of walking in a loop can occur, individuals with a 100% turning bias run the risk of repeatedly walking in circles. In these more realistic branched environments it may therefore be beneficial for an individual to have a turning bias slightly lower than 100%. The foraging efficiency of individuals with an 80% turning bias (as observed in the maze) was less than those with a 100% bias but those with an 80% bias would not run the risk of walking in circles and their efficiency was still significantly greater than that of individuals with no bias.

Handedness in branched environments has been shown to be an extremely efficient cropping mechanism (termed ‘outline tracing’) in ants cropping nectaries (Jander, 1977). Foraging ants that turn in the same direction at every successive junction they encounter, follow the edges of leaves and make a complete 180° turn when they reach a dead end visit each leaf of a plant only once with the shortest possible search path. Aphidophagous coccinellids spend much of their adult lives searching for patchily distributed individual food items and oviposition locations (Hassell & Southwood, 1978). Because *C. septempunctata* can detect aphids such as *Myzus persicae* only over very short distances (Nakamuta, 1984) and is mainly associated with herbaceous plants (Dixon, 2000), a search strategy such as ‘outline tracing’ could be a highly efficient method of searching a whole plant to maximise the chances of encountering prey close enough to detect them visually.

In the field, *C. septempunctata* and other ladybirds forage on leaf surfaces as well as branches. Adult *Hippodamia convergens* walk mainly on the stems and the edges of leaves and stipules of *Pisum sativum* (pea) (Eigenbrode et al., 1998). The larvae of the 10-spot ladybird *Adalia decempunctata*, follow surface irregularities of a leaf such as veins or the leaf rim (Dixon, 1959) while the larvae of the 14-spot ladybird, *Propylea quatuordecimpunctata* (Banks, 1957) and the larvae of *C. septempunctata* (Carter et al., 1984), have also been shown to search the edges of leaves. By following the principles of 'outline tracing', if individuals display turning biases they could benefit by increasing their foraging efficiency, as predicted by this model. Handedness on branches, combined with following the edges of small leaves and veins of larger ones would, therefore, result in an effective cropping strategy. However although turning biases may provide a foraging benefit they were not displayed by all individuals. There is a possibility that if handedness is a genetically inherited trait it may be in a linkage disequilibrium with another trait, selection for which may influence the presence of handedness within an individual. In the field many factors affect the foraging efficiencies of natural enemies on plants. For example, it has been shown that a number of aphid predators and parasitoids including coccinellids, forage more effectively, walk for longer, and fall less frequently on pea plants with reduced wax compared to those with normal wax and also may be more efficient at reducing aphid densities on reduced wax plants in the field (Eigenbrode et al., 1998; White & Eigenbrode, 2000a, b; Rutledge & Eigenbrode, 2003; Rutledge et al., 2003; Chang et al., 2004). Therefore, factors such as how efficiently a coccinellid can traverse a plant may have effects on the effectiveness of employing a turning bias in the field.

In conclusion, in laboratory experiments nearly half of the 42 *C. septempunctata* tested displayed turning biases. Therefore, we used these data to create a model to investigate whether turning biases could have an effect on foraging efficiency. From this, we predict that turning biases may result in selective advantages in foraging efficiency if *C. septempunctata* employs an 'outline tracing' cropping strategy, which would be most effective at lower aphid densities. The next stage of this research is to test these predictions with ladybirds foraging for aphids on real branched plants and monitoring differences in capture rates.

Acknowledgements

We thank Prof. A.F.G. Dixon, Prof. G. Poppy, Dr. B. Emerson, Dr. W. Powell, Dr. D. Yu and two anonymous referees for their comments, Brendan O'Brien for construction of the Y-maze, David Alden for maintenance of aphid cultures and Philip Judge for drawing the figures. This work was funded by a studentship to Dr. R. Girling from the Natural Environment Research Council and a small ecological project grant from the British Ecological Society.

References

Banks, C.J. (1957). The behaviour of individual coccinellid larvae on plants. — Anim. Behav. 5: 12-24.

Banks, J.E. & Yasenak, C.L. (2003). Effects of plot vegetation diversity and spatial scale on *Coccinella septempunctata* movement in the absence of prey. — Entomol. Exp. Appl. 108: 197-204.

Bansch, R. (1966). On prey-seeking behaviour of aphidophagous insects. — In: Ecology of aphidophagous insects (Hodek, I., ed.). Academia, Prague & Dr. W. Junk, The Hague, p. 123-128.

Carter, M.C., Sutherland, D. & Dixon, A.F.G. (1984). Plant structure and the searching efficiency of coccinellid larvae. — Oecologia 63: 394-397.

Casas, J. & Aluja, M. (1997). The geometry of search movements of insects in plant canopies. — Behav. Ecol. 8: 37-45.

Chang, G.C., Neufeld, J., Durr, D., Duetting, P.S. & Eigenbrode, S.D. (2004). Waxy bloom in peas influences the performance and behavior of *Aphidius ervi*, a parasitoid of the pea aphid. — Entomol. Exp. Appl. 110: 257-265.

Cheverton, J. (1982). Bumblebees may use a suboptimal arbitrary handedness to solve difficult foraging decisions. — Anim. Behav. 30: 934-935.

Dixon, A.F.G. (1959). An experimental study of the searching behaviour of the predatory coccinellid beetle *Adalia decempunctata* (L.). — J. Anim. Ecol. 28: 259-281.

Dixon, A.F.G. (1998). Aphid Ecology (2nd edn). — Chapman & Hall, London.

Dixon, A.F.G. (2000). Insect predator-prey dynamics: ladybird beetles & biological control. — Cambridge University Press, Cambridge.

Eigenbrode, S.D., White, C., Rohde, M. & Simon, C.J. (1998). Behavior and effectiveness of adult *Hippodamia convergens* (Coleoptera: Coccinellidae) as a predator of *Acyrthosiphon pisum* (Homoptera: Aphididae) on a wax mutant of *Pisum sativum*. — Environ. Entomol. 27: 902-909.

Frazer, B.D. & Gilbert, N. (1976). Coccinellids and aphids: a quantitative study of the impact of adult ladybirds (Coleoptera: Coccinellidae) preying on field populations of pea aphids (Homoptera: Aphididae). — J. Entomol. Soc. B.C. 73: 3-56.

Frazer, B.D. & McGregor, R.R. (1994). Searching behaviour of adult female coccinellidae (Coleoptera) on stem and leaf models. — Can. Entomol. 126: 389-399.

Hassell, M.P. & Southwood, T.R.E. (1978). Foraging strategies of insects. — Annu. Rev. Ecol. Syst. 9: 75-98.

Hemptinne, J.-L. & Dixon, A.F.G. (1991). Why ladybirds have generally been so innefective in biological control. — In: Behaviour and impact of aphidophaga (Polgar, L., Chambers, R.J., Dixon, A.F.G. & Hodek, I., eds). SPB Academic Publishing bv, The Hague, p. 149-157.

Hemptinne, J.-L., Gaudin, M., Dixon, A.F.G. & Lognay, G. (2000). Social feeding in ladybird beetles: adaptive significance and mechanism. — Chemoecology 10: 149-152.

Jander, R. (1977). Orientation ecology. — In: Grzimek's encyclopedia of ethology (Grzimek, B., ed.). Van Nostrand Reinhold, New York.

Kareiva, P. & Odell, G. (1987). Swarms of predators exhibit "preytaxis" if individual predators use area-restricted search. — Am. Nat. 130: 233-270.

Kareiva, P. & Perry, R. (1989). Leaf overlap and the ability of ladybird beetles to search among plants. — Ecol. Entomol. 14: 127-129.

Kells, A.R. & Goulson, D. (2001). Evidence for handedness in bumblebees. — J. Insect Behav. 14: 47-55.

Kindlmann, P. & Dixon, A.F.G. (1999). Strategies of aphidophagous predators: lessons for modelling insect predator–prey dynamics. — J. Appl. Entomol. 123: 397-399.

MacKay, D.C.G. (1945). Left-right tendency in the hermit crab, *Calcinus herbsteii*. — J. Comp. Psychol. 38: 131-133.

MacKay, D.C.G. (1947). Left-right tendency in the hermit crab, *Clibinarius zebra* Dana. — J. Comp. Psychol. 40: 421-425.

Majerus, M. & Kearns, P. (1989). Ladybirds, Naturalists' Handbooks 10. — Richmond Publishing, Slough.

Nakamuta, K. (1984). Visual orientation of a ladybeetle, *Coccinella septempunctata* L., (Coleoptera: Coccinellidae), toward its prey. — Appl. Entomol. Zool. 19: 82-86.

Nakamuta, K. (1985). Behavioral mechanism of switchover in search behavior of the ladybeetle *Coccinella septempunctata*. — J. Insect Physiol. 31: 849-856.

Ninkovic, V., Al Abassi, S. & Pettersson, J. (2001). The influence of aphid-induced plant volatiles on ladybird beetle searching behavior. — Biol. Control 21: 191-195.

Obata, S. (1986). Mechanisms of prey finding in the aphidophagous ladybird beetle, *Harmonia axyridis* [Coleoptera: Coccinellidae]. — Entomophaga 31: 303-311.

Putnam, C.D. (1962). The non-random behaviour of *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) in a Y-maze with neither reward nor punishment in either arm. — Anim. Behav. 10: 118-125.

Raymond, B., Darby, A.C. & Douglas, A.E. (2000). The olfactory responses of coccinellids to aphids on plants. — Entomol. Exp. Appl. 95: 113-117.

Rutledge, C.E. & Eigenbrode, S.D. (2003). Epicuticular wax on pea plants decreases instantaneous search rate of *Hippodamia convergens* larvae and reduces attachment to leaf surfaces. — Can. Entomol. 135: 93-101.

Rutledge, C.E., Robinson, A.P. & Eigenbrode, S.D. (2003). Effects of a simple plant morphological mutation on the arthropod community and the impacts of predators on a principal insect herbivore. — Oecologia 135: 39-50.

Sabelis, M.W. & van de Baan, H.E. (1983). Location of distant spider-mite colonies by phytoseiid predators — demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. — Entomol. Exp. Appl. 33: 303-314.

Schaller, M. & Nentwig, W. (2000). Olfactory orientation of the seven-spot ladybird beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae): Attraction of adults to plants and conspecific females. — Eur. J. Entomol. 97: 155-159.

Sengonca, C. & Liu, B. (1994). Responses of the different instar predator, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), to the kairomones produced by the prey and non-prey insects as well as the predator itself. — Z. Pflanzenk. Pflanzen. 101: 173-177.

Smith, J.N.M. (1974). The food searching behaviour of two European thrushes II: The adaptiveness of the search patterns. — Behaviour 49: 1-61.

Steinberg, S.M., Dicke, M., Vet, L.E.M. & Wanningen, R. (1992). Response of the braconid parasitoid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. — Entomol. Exp. Appl. 63: 163-175.

Swartz, R.D. (1929). Modification of behaviour in earthworms. — J. Comp. Psychol. 9: 17-33.

Wehner, R. & Srinivasan, M.V. (1981). Searching behavior of desert ants, Genus Cataglyphis (Formicidae, Hymenoptera). — J. Comp. Physiol. 142: 315-338.

White, C. & Eigenbrode, S.D. (2000a). Effects of surface wax variation in *Pisum sativum* on herbivorous and entomophagous insects in the field. — Env. Entomol. 29: 773-780.

White, C. & Eigenbrode, S.D. (2000b). Leaf surface waxbloom in *Pisum sativum* influences predation and intra-guild interactions involving two predator species. — Oecologia 124: 252-259.

Wigglesworth, V.B. (1941). The sensory physiology of the human louse *Pediculus humanus corporis* De Geer (Anoplura). — Parasitology 33: 67-109.