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Estimating Perceptual Range of Female Monarch Butterflies (*Danaus plexippus*) to Potted Vegetative Common Milkweed (*Asclepias syriaca*) and Blooming Nectar Resources

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Abstract

Habitat loss in the summer breeding range contributes to eastern North American monarch (Lepidoptera: Nymphalidae) population decline. Habitat restoration efforts include increasing native prairie plants for adult forage and milkweed (*Asclepias* spp.) for oviposition and larval development. As the monarch is a vagile species, habitat establishment at a grain that matches the monarch perceptual range will facilitate efficient movement, decrease fitness costs of dispersal, and increase oviposition. We released 188 experimental monarch females 5, 25, 50, and 75 m downwind from potted milkweed and blooming forbs in 4–32 ha sod fields. Perceptual range was estimated from monarchs that flew towards and landed on the milkweed and forbs. Flight patterns of 49 non-experimental monarchs that landed on the resources were also observed. In our experimental, resource-devoid setting, wind-facilitated movement occurred most frequently. Monarchs performed direct displacement as evidenced by shallow turn angles and similarity of Euclidian and total distances traveled. We hypothesize similar monarch flight behavior when traveling over other resource-devoid areas, such as crop fields. Although the majority of experimental monarchs flew downwind, eight experimental and 49 non-experimental monarchs were observed flying upwind toward, and landing on, the potted resources from distances ranging from 3 to 125 m (mean = 30.98 m, median = 25 m, mode = 25 m). A conservative estimate of the perceptual range is 125 m, as longer distances cannot be precluded; however, the majority of observations were ≤50 m. Our findings suggest establishing habitat patches ~ 50 m apart would create functional connectivity across fragmented agricultural landscapes.

Key words: perception, olfaction, random-walk, direct displacement, insect search behavior

Discerning animal movement patterns in fragmented landscapes is foundational to understanding population dynamics, persistence, and distribution (Hanski 1998, Barton et al. 2009, Stevens et al. 2012, Schultz et al. 2017, MacDonald et al. 2019). As animals disperse across a fragmented landscape, they are guided by olfactory and visual cues. As such, animal movements are a function of resource patch grain across the landscape and the animal's perceptual range, which is the maximum distance individuals are able to detect stimuli with their sensory organs (Levins 1962, Wiens 1989, Ranta et al. 2000, Silver et al. 2000, Baguette and Van Dyck 2007, Öckinger and Dyck 2012, MacDonald et al. 2019). Efficient movement of individuals occurs when the grain of resource patches matches the

spatial scale of the animal's perception (Baguette and Van Dyck 2007, Delattre et al. 2010). Alternatively, when resources are spaced at distances beyond an animal's perceptual range, fitness costs are increased. Specifically, search time to find suitable habitat and the risk of predation are increased (Delattre et al. 2010). Knowing the distance at which animals can detect suitable habitat has implications for creating functional connectivity across fragmented landscapes (Baguette and Van Dyck 2007, Delattre et al. 2010).

For insects, the perceptual ability is based on the detection of visual and olfactory stimuli and subsequent processing in the central nervous system (Matthews and Matthews 2010). When attractive stimuli are detected, a positive taxis behavioral response toward

stimuli is performed. Butterfly perceptual ability is likely dominated by vision at a fine scale (e.g., within 5 m of a resource; Garlick 2007). At larger distances, olfactory stimuli are likely determinative, with insects flying upwind, in and out of an odor plume, until they reach the source of the attractant chemical signal (Bell 1991, Cardé and Willis 2008).

At a landscape scale, observational studies typically assume measurement of an organism's positive taxis to suitable habitat is a surrogate for perceptual range (Lima and Zollner 1996, Zollner and Lima 1997). Lepidopteran species are exceptional models for observational studies, as butterflies can be released at varying distances from habitat edges, and their behaviors can be monitored within the limits of observers' visual limits of detection (MacDonald et al. 2019). Perceptual ability is variable across species. For example, Merckx and Van Dyck (2007) reported a perceptual range of up to 100 m for the speckled wood (*Pararge aegeria*), while Fahrig and Paloheimo (1987) reported perceptual ranges of ≤1 m for cabbage white butterflies (*Pieris rapae*). Fender's blue butterflies (*Icaricia icariolides*) released in open areas 15 m from a habitat boundary successfully found habitat patches within prairies, open woods, and dense woods (Schultz et al. 2012).

Estimates of female monarch butterfly perceptual range are needed to support habitat restoration plans that create functional connectivity and facilitate egg-laying behavior across the fragmented agricultural landscapes of the Midwest US. The eastern North American monarch butterfly is at risk of quasi-extinction and classified as a candidate species for listing under the Endangered Species Act (USFWS 2020) due, in part, to the loss of their obligate host plant, milkweed (*Asclepias* spp.), in agricultural landscapes (Hartzler 2010, Pleasants and Oberhauser 2013, Pleasants 2017). Agent-based modeling of monarchs ovipositing as they travel through a landscape with various habitat types suggests establishing small habitat patches at distances within the female monarch's perceptual range (i.e., functional connectivity) will support increased egg densities in the landscape (Zalucki et al. 2016, Grant et al. 2018). While sensitivity analyses indicate an assumed perceptual range of 50 m produces realistic simulations of monarch butterfly movement paths (Grant et al. 2018), to date, empirical estimates of the perceptual range are unavailable.

Here, we aimed to advance understanding of breeding-season female monarch perceptual range to habitat that supports oviposition and foraging behaviors. As suitable habitat for monarchs is often isolated milkweed stems or small milkweed/forage patches surrounded by fields of maize (*Zea mays*), soybean (*Glycine max*), or cool-season, non-native grasses (Zalucki and Kitching 1982a, Pleasants 2015, Baker and Potter 2019), we designed a field-scale study to quantify perceptual range toward isolated stimuli. We released monarch females 5–75 m downwind from potted common milkweed (*Asclepias syriaca*) and blooming forbs in 4–32 ha mixed Kentucky bluegrass (*Poa pratensis*) and fescue (*Festuca*) sod fields and observed flights that terminated on the resource plants. This study provides empirical evidence to support estimations of female monarch butterfly perceptual range to isolated stimuli, and related movement behaviors, in a resource-devoid habitat.

Methods

Insects

Adult monarchs were caught in restored prairies and roadsides during the breeding season (late June–early August) in Story, Dallas, and Linn County, Iowa, USA. Females were collectively housed with field-captured males in a soft-sided mesh cage for 1–5 d before use

in an experiment to ensure they were gravid. Because collected monarchs that were starved overnight were lethargic upon release in an experimental trial the following day (unpublished observations), Gatorade (Chicago, IL) was provided ad libitum as a sugar source. On the morning of a trial, monarch females were transferred to individual glassine envelopes and transported to the field site in a cooler with an ice pack wrapped in a cloth.

Radio telemetry was employed to aid in relocating monarch females that traveled beyond the limits of visual detection. As described in Fisher et al. (2020), individuals were fitted with a 220 mg LB-2X transmitter (Holohil Systems Ltd, Ontario, Canada) using superglue. Additional females released without radio tags (untagged) or with watch batteries attached (sham-tagged; 300 mg; Energizer AZ10DP, St. Louis, MO) were used to evaluate potential effects of the transmitters on flight behavior (also see Fisher et al. 2020).

Perceptual Range of Experimental Monarchs to Potted Resources

To estimate the perceptual range of monarch females to isolated stimuli at distances beyond those possible under controlled laboratory conditions, we aimed to mimic a wind tunnel design (Baker and Linn 1984) under field conditions. Perceptual range was estimated by observing radio-tagged, sham-tagged, and untagged females flying upwind toward and landing on milkweed and/or nectar resources. This behavioral response was assumed to indicate detection of attractive stimuli and elicitation of a positive taxis response. Additional support for perceptual range was gained by observing females flying upwind toward (bearing ±45° of the resources) but not landing on the milkweed and/or nectar resources, as this behavior may indicate detection of the resources, but a determination they were unsuitable.

Experimental sites were 4–32 ha mixed Kentucky bluegrass and fescue sod fields in Story, Dallas, and Linn County, Iowa, USA, maintained at 5 cm in height by weekly mowing. These experimental sites, which were surrounded by maize and soybean fields, were selected to minimize the presence of confounding visual and olfactory stimuli. Two milk crates containing five greenhouse-reared vegetative-stage common milkweed plants (*Asclepias syriaca*) and one milk crate containing five greenhouse-reared blooming *Liatris* sp., which served as oviposition and feeding resources, respectively, were placed near the center of the sod fields. A potted, vegetative-stage *Monarda fistulosa* or *Liatris* sp., which did not provide ovipositing or forage resources, was positioned either 5, 25, 50, or 75 m downwind of the potted resources to serve as a release plant (Fig. 1). Plants were reared in Iowa State University greenhouses with no exposure to soil or foliar-applied insecticides.

Release distance (5, 25, 50, 75 m) was selected for a given day; release plants and potted resources remained at the same positions for all experiments on a given day. Consequently, because the wind direction occasionally shifted during the day, resources were not always directly upwind of the release plant. Additionally, monarchs released at 50 and 75 m were near sod field boundaries and would often enter the surrounding maize and soybean fields (e.g., 45% of monarchs released at 75 m flew into crop fields and were not recovered). Therefore, more monarchs were released at distances closer to the resource plants.

In 2017 and 2018, on clear or mostly clear days from 700 to 1500 h with the temperature between 20 and 33°C and wind speed below 16 kph, 112 radio-tagged, 60 sham-tagged, and 16 untagged female monarchs (*n* = 188 total) were anesthetized with dry-ice (Fisher et al. 2020) and released individually. Before the release of each monarch, temperature and wind speed were measured with an

Fig. 1. Example experimental set-up at a 32-ha sod field in central Iowa in which experimental monarchs were released on a vegetative stage *Monarda fistulosa* plant 5 m downwind from two milk crates containing vegetative stage *Asclepias syriaca* and one milk crate containing blooming *Liatris* sp.

anemometer (Extech Instruments; Nashua, NH; mini thermo-anemometer 45118), and wind direction was recorded. Monarchs were considered unresponsive if they did not move from the release plant within 10 min, and the observation period was terminated, similar to Zalucki and Kitching (1982b). For monarchs that moved from the release plant, behavior was observed for 0.2–52 min.

Technicians were positioned at least 25 m from the release plant and were equipped with a butterfly net, flagging tape, a Trimble Geo7x (Sunnyvale, CA), and radio telemetry equipment, which included a directional 3-element Yagi antennae (frequencies ranging from 164.000–166.000 MHz) connected to a VHF receiver (Alinco DJ-X11; Toyama, Japan) with a 0.9-m coax cable (Johnson's Telemetry, El Dorado Springs, MO). Sequential locations where monarchs landed between flight steps were flagged and georeferenced. Monarchs were caught by technicians as they approached the boundary of the sod field or when they were relocated with handheld radio telemetry in the surrounding agricultural fields.

Perceptual Range of Non-experimental Monarchs to Potted Resources

The potted milkweed and blooming *Liatris* sp. in the sod field were isolated stimuli in an otherwise resource-deprived landcover and, perhaps not surprisingly, attracted monarchs flying in the area. We opportunistically observed monarchs directly flying upwind to the potted resources. To estimate the perceptual range of these monarchs, investigators laid a flag at the monarch's location where direct flight was first observed, and the distance from the flag to the resources was measured. These observations were made under the same environmental conditions as described previously.

Statistical Analyses

We observed no significant differences in the frequency of unresponsive individuals in the tagged (24/112), sham-tagged (13/60), and untagged (3/13) groups (Table 1; $\chi^2=0.067975$, df=2, $P = 0.9666$), based on the number of days captive (Table 2; $\chi^2=3.6211$, df=4, $P = 0.4597$), or release distance from potted resources (Table 3; $\chi^2=7.1975$, df=3, $P = 0.06586$) with a χ^2 test of independence. Consequently, radio-tagged, sham-tagged, and untagged individuals, regardless of days in captivity, across 2017 and 2018, were combined for statistical analyses. Statistical analyses were performed in RStudio version 4.0.3 (R Core Team 2020, RStudio Team 2020).

The Euclidian bearing of movement (i.e., the bearing from release to capture) and the bearing of the monarch's first step were calculated in degrees using the movement.pathmetrics package in Geospatial Modeling Environment (GME; Spatial Ecology LLC, www.spatialecology.com/gme/) using the coordinates taken by technicians in the field. The direction of movement across all distances from the release site to the potted resources was compared, taking into account different daily wind directions. To compare directions for all released butterflies, the resource positions were all normalized to 0°; i.e., the bearing of movement was a relative measure. The bearings of movement were subtracted from the bearing of the release plant to the potted resources. To ensure the bearing fell between 0° and 360°, 360° was added to calculated bearings <0°, while 360° was subtracted from calculated bearings >360°.

Table 1. The number of individuals that flew or were unresponsive (UNR; no movement within 10 min of release) based on attachment treatment ($\chi^2 = 0.067975$, df = 2, $P = 0.9666$)

Treatment	Flew	UNR	Total	% UNR
Radio-tagged	88	24	112	21.4
Sham-tag	47	13	60	21.7
Untagged	13	3	16	18.8
Overall	148	40	188	21.3

Table 2. The number of individuals that flew or were unresponsive (UNR; no movement within 10 min of release) based on the number of days held in captivity before an experimental trial ($\chi^2 = 3.6211$, df = 4, $P = 0.4597$)

Days Captive	Flew	UNR	Total	% UNR
1	71	15	86	17.4
2	53	20	73	27.4
3	17	3	20	15.0
4	2	0	2	0.0
5	5	2	7	28.6
Overall	148	40	188	21.3

Table 3. The number of individuals that flew or were unresponsive (UNR; no movement within 10 min of release) based on the release distance to the potted resources ($\chi^2 = 7.1975$, df = 3, $P = 0.06586$)

Release Distance	Flew	UNR	Total	% UNR
5 m	94	30	124	24.2
25 m	32	3	35	8.6
50 m	11	6	17	35.3
75 m	11	1	12	8.3
Overall	148	40	188	21.3

Because collected data are based on angular orientation (e.g., degrees), circular statistics were employed (Jammalamadaka and SenGupta 2001, Notar and Thomas 2014, Thomas et al. 2017). Bearings relative to the resource position were analyzed at each release distance with the R package 'circular' (Lund 2017). Relative Euclidian bearings and relative bearings of the first step for all individuals at a release distance were analyzed with the Rayleigh Test of Uniformity (rayleigh.test) to determine whether movements exhibited a unimodal pattern (e.g., all movements were in the same direction) (Jammalamadaka and SenGupta 2001). Release distances with monarch movements that were statistically unimodal were then analyzed to determine whether the movement bearing was in the direction of the resources (0°) or the direction of the wind (180°) using the Watson-Williams Test for Homogeneity of Means (watson.williams.test) to ascertain if either stimulus facilitated direction of movement (Jammalamadaka and SenGupta 2001).

Perceptual range was quantified with the flight step lengths associated with landing on the resources. The number of individuals that landed on the resources was analyzed by release distance with χ^2 tests of independence. Additionally, we assumed monarchs that flew towards the resources (i.e., relative Euclidian bearings ± 45° of the potted milkweed and blooming *Liatris* sp,), but did not land on the plants, reflected a positive taxis behavioral response; although these flights may not have been in response to the resources. The number of individuals with relative Euclidian bearings ± 45° of the resources were analyzed by release distance with χ^2 tests of independence. Wind speeds and temperatures during observations of individuals whose relative Euclidian bearings were ± 45° of the resources (i.e., moving toward the resources) and individuals whose relative Euclidian bearings were < –45° or >45° of the resources (i.e., moving away from the resources) were analyzed separately with two-sample t-tests to determine whether there were differences in the distributions of wind speed or temperature that could help interpret the direction of movement.

To gain insights on overall monarch movement patterns in the sod fields, additional parameters were calculated with the movement.pathmetrics package in GME, including straight line distance from release to capture (Euclidian distance), step lengths between two consecutive georeferenced locations, and turn angles created from three consecutive georeferenced locations. Total distance traveled was calculated by summing all of the step lengths for an individual. Flight step lengths within the sod field and step lengths associated with exiting the sod field were also quantified.

Results

Experimental Monarch Perceptual Range to Potted Resources

When monarchs were released 5 or 25 m downwind of the potted resources, Euclidian bearings relative to the resources displayed a highly significant unimodal pattern (Fig. 2a; 5 m: *n* = 94, test statistic = 0.3242, *P* = 1e-04; 25 m: *n* = 32, test statistic = 0.6212, *P* = 0).

The pattern of relative Euclidian bearings from release point to capture for monarchs released 50 m downwind from the resources was marginally unimodal (Fig. 2a; *n* = 11, test statistic = 0.5309, *P* = 0.0412). Relative Euclidian bearings from release to capture when monarchs were released 75 m downwind of the resources did not show a directional pattern (*n* = 11, test statistic = 0.4391, *P* = 0.1193). The first steps of movement paths followed a similar trend. Unimodal movement was observed when resources were placed 5, 25, and 50 m from the release site; however, a directional pattern was not observed at 75 m (Fig. 2b; 5 m: test statistic = 0.2449, *P* = 0.0054; 25 m: test statistic = 0.6368, *P* = 0; 50 m: test statistic = 0.6442, *P* = 0.0074; 75 m: test statistic = 0.301, *P* = 0.3778).

The unimodal flight patterns following releases at 5, 25, and 50 m were not oriented upwind toward the potted resources (Table 4). Rather, the monarchs predominately flew downwind and away from the resources (Table 5). There was no difference in the distributions of wind speed at the time of release for individuals whose Euclidian bearings were ± 45° of the resources (toward the resources) and individuals whose Euclidian bearings were < –45° or >45° of the resources (away from the resources; *t* = 1.778, df = 18, 120, *P* = 0.08679). Likewise, there was no difference in the distributions of temperature at the time of release for individuals whose Euclidian bearings were ± 45° of the resources (toward the resources) and individuals whose Euclidian bearings were < –45° or >45° of the resources (away from the resources; *t* = –0.72856, df = 18, 120, *P* = 0.4733).

Although the Euclidian relative direction of monarch movement was predominately downwind and away from the
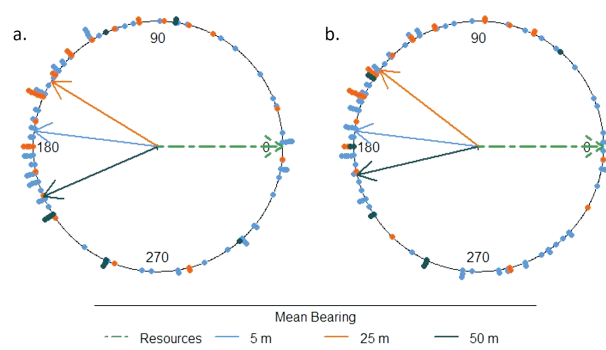
Fig. 2. Bearings of the Euclidian distance from release point to capture (a) and of the first flight step from release point to first stationary location (b) of 148 responsive, experimental monarchs released at distances that displayed a unimodal movement pattern (5, 25, and 50 m from the potted resources). All Euclidian bearings are represented as dots colored to correspond with their release distance. The mean bearing of monarch movement at each release distance is displayed as colored, solid arrows. As a frame of reference, the dot-dash green arrow is pointing from the release site to the resources; all directions of movement were normalized to the resources to be positioned at 0°.

Table 4. The unimodal movement patterns displayed at 5, 25, and 50 m releases were not oriented toward the resources as determined by the Watson–Williams Test for Homogeneity of Means (watson.williams.test). Resource positions were all normalized to 0°; a mean Euclidian bearing 0° and a *P* > 0.05 would indicate the butterflies traveled toward the resources

Release Distance	Mean Euclidian Bearing Relative to Resources	F	df	p
5 m	172.8	249.24	1, 174	< 2.2e-16
25 m	146.5	323.13	1, 74	< 2.2e-16
50 m	203.5	68.62	1, 20	6.77E-08
75 m	No unimodal movement pattern observed			

resources, the relative Euclidian bearing of 19 individuals was upwind and within ±45° of the resources (see Fig. 2; 16 from 5 m, two from 25 m, zero from 50 m, and one from 75 m). There was no significant effect of release distance on the number of landings on the resources ($\chi^2 = 8$, df = 6, $P = 0.2381$) or the frequency of relative Euclidian bearings orientated toward the resources ($\chi^2 = 12$, df = 9, $P = 0.2133$). Of the 94 monarchs that flew from the 5 m release point, 16 flew upwind toward the resource, and seven of them landed on the potted *Liatris* sp. Two of the 39 monarchs that flew from the 25 m release point had Euclidian movement upwind, but neither of them landed on the resources. None of the monarchs released at 50 m (0/11) had Euclidian movements in the direction of the resources. One of the 11 individuals that flew from the 75 m release point moved toward the resources and landed on the potted *Liatris* sp. Across all the release points, eight monarchs landed on the *Liatris* sp. The lengths of flight steps that terminated on the *Liatris* sp. were 3.5, 3.8, 4.9, 5.0, 5.0, 5.0, 5.0, and 59.7 m. Monarchs that flew toward, but did not land on the resources, terminated their flights on grass within the sod field, in the weedy field edge, or in the surrounding crop field.

To further characterize the movement in a resource-devoid habitat, regardless of orientation with the wind or towards the resources, ancillary data for all 148 individuals that flew from the 5, 25, 50, and 75 m release points were collected. The mean Euclidian distance (± SD) was 114.0 ± 182.5 m, with a maximum Euclidian distance of 1,479.6 m. The mean total distance was 114.8 ± 182.0 m, with a maximum of 1491.8 m. The essentially identical Euclidian and total distances indicate highly directional flight (Fig. 3). Consistent with the similar Euclidian and total distances, the majority of turn angles were close to zero (Fig. 4), which also indicates the monarchs moved in nearly straight lines. Step lengths that were summed to calculate the total distance traveled were a mean of 53.9 ± 117.2 m, with a maximum step length of 800.6 m (Fig. 5a). Flight steps within the sod field were a mean of 18.4 ± 24.1 m (Fig. 5b). The 46 steps associated with exiting the sod field ranged from 13.8 to 800.6 m with a mean of 292.8 ± 192.5 m (Fig. 5c).
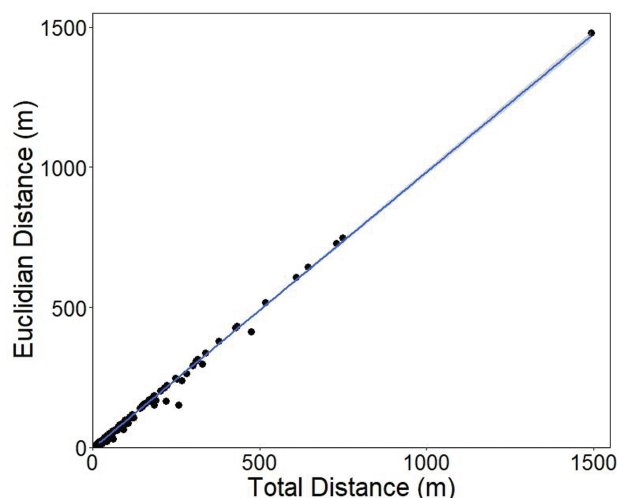

Fig. 3. Scatter plot displaying the similarity of Euclidian and total distance traveled ($R^2 = 0.9956$) for 148 experimental monarchs released on vegetative stage *Monarda fistulosa* 5, 25, 50, or 75 m from potted vegetative stage *Asclepias syriaca* and blooming *Liatris* sp in sod fields of 4–32 ha.
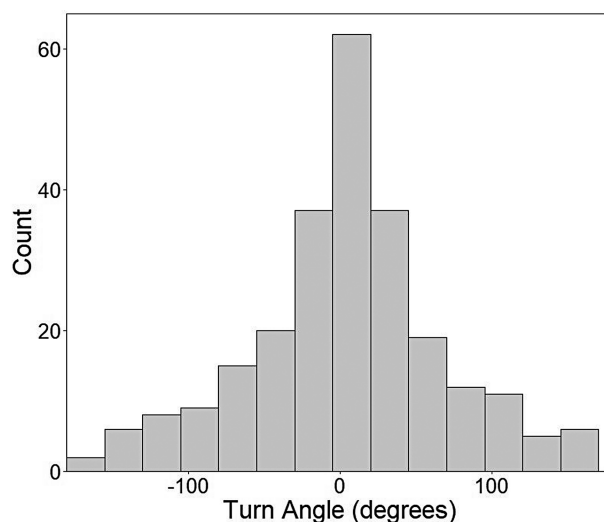

Fig. 4. Histogram of turn angles estimated from three consecutive georeferenced locations along flight paths of 99 experimental monarchs released on vegetative stage *Monarda fistulosa* 5, 25, 50, or 75 m from potted vegetative stage *Asclepias syriaca* and blooming *Liatris* sp in sod fields of 4–32 ha. Forty-two percent of the calculated turn angles (105/250) were between ± 25°, which indicates monarch flight patterns were highly directional.

Non-experimental Monarch Perceptual Range to Potted Resources

In 2017 and 2018, 86 non-experimental monarchs (sex unknown) were attracted to and landed on the potted blooming *Liatris* sp. Forty-nine of these monarchs were observed flying directly upwind for 3–125 m to the resources (Fig. 6). Four monarchs flew directly to the resources from <5 m, 27 monarchs flew between 5 and 29 m, and 18 monarchs flew between 30 and 125 m.

Discussion

Perceptual range of breeding season female monarchs was quantified with flight step lengths that terminated on our potted resources. Visual perception likely guided flight steps of seven experimental and four non-experimental monarchs from <5 m to the potted *Liatris* sp. (see Garlick 2007). Olfaction likely guided experimental and non-experimental monarchs with upwind flight steps that terminated on the potted *Liatris* sp. from 30 to 125 m (see Rutowski 2003, MacDonald et al. 2019). Sensory motivation could not be speculated for the other 27 non-experimental monarchs with upwind steps of 6 to 29 m that terminated on the potted *Liatris* sp. Additional insights on the perceptual range were gleaned from Euclidian bearings of experimental monarch flights upwind within ±45° of the potted resources that did not terminate on the resources. Eleven experimental monarchs flew upwind ±45° of the resources but did not land on the resources (nine from the 5 m release and two from the 25 m release).

In summary, a conservative estimate of the maximum perceptual range is 125 m; given our study design, longer distances cannot be precluded. Shorter flight steps to resources were, however, more common. Flights steps < 30 m were observed for 66.7% (38/57) of the total observations of experimental and non-experimental monarch flights that terminated on the potted resources, and flight steps ≤50 m were observed for 84.2% (48/57) of the total observations of experimental and non-experimental monarch flights that terminated on the potted resources. All of the Euclidian bearings of flight steps
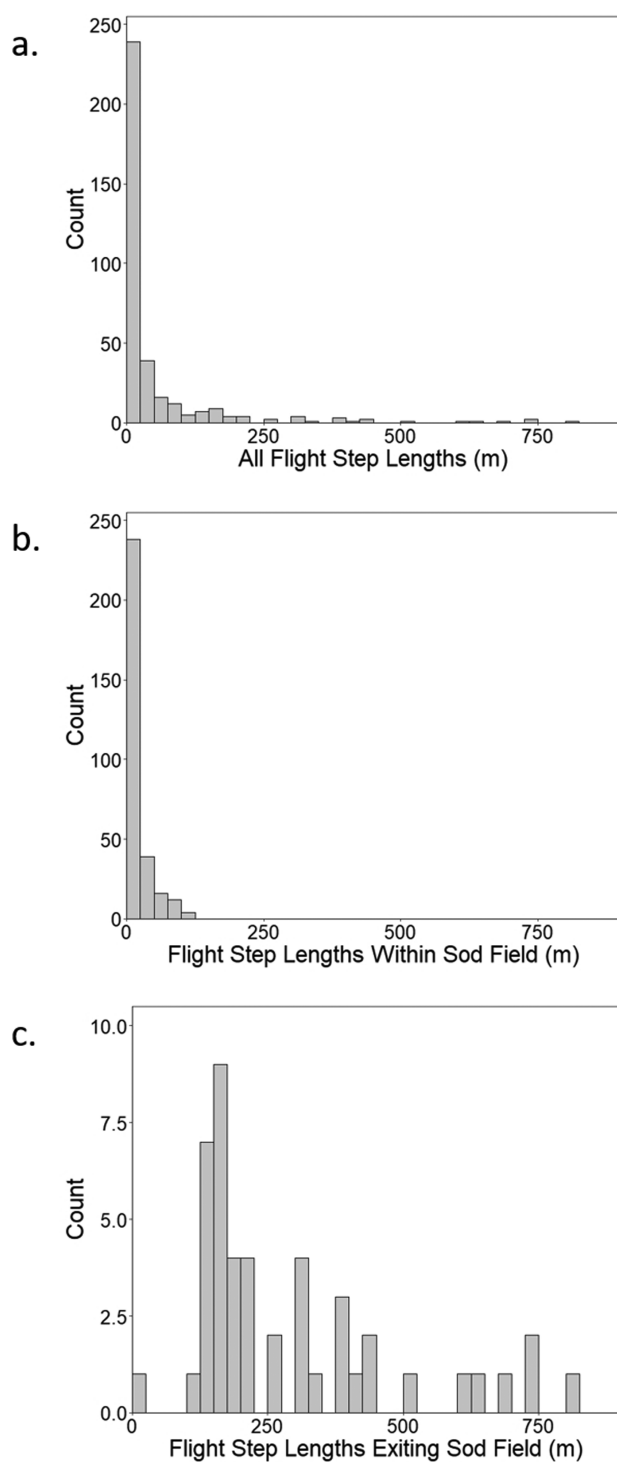
Fig. 5. Histograms with 25 m bins of all 418 flight steps (a), 372 flight steps within the sod field (b), and 46 flight steps associated with exiting the sod field (c) taken by 148 monarchs released on vegetative stage *Monarda fistulosa* 5, 25, 50, or 75 m from potted vegetative stage *Asclepias syriaca* and blooming *Liatris* sp in sod fields of 4–32 ha. Note the *y*-axis scale is 10 m rather than 250 m for flight steps exiting the sod field (c).

that were toward but did not terminate on the resources were ≤25 m (100%; 9/9).

As noted in the analyses of relative Euclidian bearings and relative bearings of the first step, approximately 90% of the experimental monarchs were not attracted to the potted resources

and instead flew downwind, away from the common milkweed and *Liatris* sp. However, 86 non-experimental monarchs were observed feeding on the potted *Liatris* sp., indicating the isolated and limited number of plants we provided were suitable nectar sources. These observations with the non-experimental monarchs are consistent with reports of monarchs finding isolated milkweed stems or small milkweed/forage patches in areas surrounded by fields of maize, soybean, or cool-season, non-native grasses (Zalucki and Kitching 1982a, Pleasants 2015, Baker and Potter 2019). The divergent behavior of most of the experimental monarchs could be because they: 1) were not motivated to feed or oviposit; 2) perceived the sod field as unfavorable and initiated dispersal behavior; and/or 3) required a larger olfactory or visual stimulus to elicit positive taxis behavior (i.e., a larger number of potted blooming forbs and milkweed).

The behavior exhibited by individual insects results from the physiological status of the nervous, endocrine, digestive, and/or reproductive systems (Matthews and Matthews 2010). Under our experimental conditions, if the female monarchs were not gravid or were satiated, they may not have been motivated to seek milkweed or a nectar source. Although not tested empirically, we assumed the field-collected females were gravid, or mated with field-collected males, because females and males were held in the same cage during the time in captivity before flight trials. Notwithstanding the reproductive status of the experimental female monarchs, during the two years of experiments, we never observed experimental or non-experimental monarchs oviposit on our potted vegetative stage common milkweed, suggesting that our plants may not have provided a suitable signal to elicit oviposition behavior. With regard to nutritional status, the experimental monarchs were provided Gatorade ad libitum as a sugar source while in captivity. Consequently, the monarchs may have been satiated upon release and not motivated to find forage. If we had provided milkweed plants that previously elicited oviposition by monarchs and/or if the experimental monarchs were starved before trials, there may have been a greater drive to engage in resource-finding behavior. However, as noted previously, we found that starved females were lethargic and typically failed to fly.

Alternatively, the experimental monarchs may have perceived the sod field as unfavorable habitat and were motivated to find cover. Although we occasionally observed non-experimental monarchs resting in the sod field early in the morning, when temperatures were typically below 19°C, during our flight trials non-experimental monarchs were typically observed flying over the sod fields. Experimental monarchs released 5, 25, and 50 m from the resource plants flew downwind for an average total distance of 115 m and typically terminated their flights and remained stationary after landing in weedy fence rows, roadsides, trees, or crop fields. Likewise, monarchs released 75 m downwind of the resources were approximately equidistant from the potted resources and a sod field boundary. Five of the 11 monarchs (45%) released at 75 m sought cover provided by the surrounding maize or soybean fields, while only one flew to and landed on the potted resources. Locating monarchs that found cover outside of the sod field and beyond the limits of visual detection was possible using radio telemetry.

MacDonald et al. (2019) estimated the perceptual range of great spangled fritillaries (*Speyeria cybele*) and Atlantis fritillaries (*Speyeria atlantis*) by releasing individuals from a boat on a lake 30, 40, 50, or 60 m from a target island. These investigators reported 20–80% of released butterflies navigated to the target island. The higher success rate reported by MacDonald et al. (2019) compared to our findings may reflect, in part, a difference in butterflies motivated to survive (fritillaries) versus butterflies motivated to find cover (monarchs).

Table 5. The unimodal movement patterns displayed at 5, 25, and 50 m releases were in the same direction as the wind, as determined by the Watson–Williams Test for Homogeneity of Means (watson.williams.test). Wind directions were all normalized to 0°; a mean Euclidian bearing 0° and a $P > 0.05$ would indicate the butterflies traveled downwind

Release Distance	Mean Euclidian Bearing Relative to Wind	F	df	p
5 m	2.5	0.13216	1, 172	0.7167
25 m	13.2	3.0201	1, 74	0.0864
50 m	26.0	0.61499	1, 20	0.4421
75 m	No unimodal movement pattern observed			
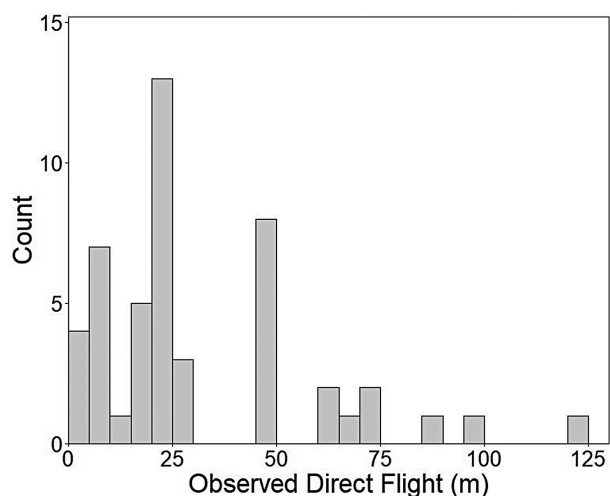
Fig. 6. Histogram with 5 m bins of opportunistically observed direct flights upwind to potted *Asclepias syriaca* and *Liatris sp.* by 49 non-experimental monarchs in 4–32 ha sod fields in 2017 and 2018.

Finally, the low number of experimental monarchs that flew upwind could be due to insufficient stimuli to elicit a positive taxis response, although previous studies suggest isolated milkweed stems or small milkweed/forage patches surrounded by fields of maize, soybean, or cool-season, non-native grasses are used by females (Zalucki and Kitching 1982a, Pleasants 2015, Baker and Potter 2019). To explore if a natural habitat patch with a greater number of resource plants would elicit a positive taxis response, we released 11 monarchs in an early-season soybean field 40, 60, or 80 m downwind of a 4-ha restored prairie (unpublished data that supplements the current study). Consistent with results for experimental monarchs released in the sod fields, two individuals flew to the prairie (18%), while the others flew downwind and away from the prairie and resource-devoid soybean field. The consistent downwind flight away from resources suggests experimental monarchs were more motivated to disperse from resource-devoid settings rather than seek blooming forbs or milkweed; however, this behavior could be enhanced to the extent the monarchs were satiated or not gravid.

To improve our study design, we recommend 1) ensuring monarchs selected for trials are pre-disposed to oviposit and 2) undertaking flight trials in more diverse settings that include resource plants. Females observed mating or ovipositing in captivity would indicate a gravid status. Alternatively, newly-emerged, starved monarchs reared and mated in captivity may be more motivated to oviposit or forage. However, from our experience, we found colony-reared and starved individuals were lethargic upon release and were not responsive. To avoid the potentially confounding effect of the resource devoid landcover, we are currently conducting studies in which monarchs are released in areas containing natural covers, such as tall grass-dominated fields and crop fields, and quantifying

the step lengths of flights that terminate on naturally occurring oviposition and forage resources.

In addition to providing the means to estimate perceptual range, our experimental setting facilitated observations of dispersal within and outside of the sod fields. Insect dispersal is a three-step process: emigration, matrix navigation, and immigration (Kral-O'Brien and Harmon 2021). Dispersal entails an insect leaving their occupied patch, navigating resource-devoid habitats to locate a new patch, and ultimately selecting a new, suitable habitat patch. Movement characteristics and behavior during this process often vary with land cover classification. While navigating the matrix, butterflies typically fly via direct displacement with shallow turn angles, i.e., correlated random walks (Zalucki and Kitching 1982b, Zalucki 1983, Delattre et al. 2010, Evans, Oliver, et al. 2020a, Evans, Sibly, et al. 2020b). Direct displacement is presumed to maximize survival probabilities and time and energy efficiencies (Heinz and Strand 2006, Schtickzelle et al. 2007, Barton et al. 2009). Over favorable habitats, tortuous movement and feeding behaviors are dominant (Bell 1991, Delattre et al. 2010, Evans, Oliver, et al. 2020a). Tortuous flight patterns are presumed to maximize the chance of finding resources (Bell 1991, Delattre et al. 2010).

Observations of the experimental female monarchs studied here combined with results reported by Fisher et al. (2020), who tracked female monarch movement over a restored prairie, provides an opportunity to quantify movement characteristics and behaviors while monarchs traverse within the matrix and favorable habitat. While traversing the sod field, a representative of the matrix, experimental monarchs performed direct displacement, as evidenced by shallow turn angles and the similarity between Euclidian distance and total distance traversed. Similar observations of straight flights by monarchs moving through a grass matrix among small patches of milkweed were also described by Zalucki and Kitching (1982b). In contrast, when monarchs were observed flying in a restored prairie, the majority of turn angles were approximately 180° and the Euclidian distance was significantly smaller than the total distance traversed, consistent with torturous movements (Fisher et al. 2020). In both studies, large flight steps exiting the focal areas were observed, which could indicate evidence of emigration and initiation of unguided direct displacement. In the current study, we observed 46 flight steps exiting the sod fields ranging from 13.8 to 800.6 m with a mean of 292.8 ± 192.5 m. Fisher et al. (2020) reported four individuals exited the restored prairie with flight steps ranging from 75 to 213 m. Similar observations of direct displacement through the matrix and torturous movement over favorable habitat are reported with other Lepidopteran species. Fender's blue butterflies have been observed to stop less frequently, perform more directed flights, and have a lower deviation in turn angle when they traverse the matrix than when they are within a habitat patch (Schultz and Crone 2001). Likewise, silver-studded blue butterflies (*Plebejus argus*) have been observed to exhibit faster and straighter flights over poor habitats than over high-quality habitats (Fernández et al. 2016).

Knowing the distance at which animals can detect suitable habitat and the behavioral attributes of movement have

implications for creating functional connectivity across fragmented landscapes (Lima and Zollner 1996, Zollner and Lima 1997, Baguette and Van Dyck 2007, Delattre et al. 2010). Our empirical evidence suggests the perceptual range of breeding season female monarchs to host plants and nectar forage resources can be up to or exceed 125 m. Large flight steps exiting the sod field of up to 800.6 m indicate emigration and initiation of unguided direct displacement behavior while searching for a new suitable habitat. Based on our observation that the majority of flight steps that terminated on a resource plant were ≤50 m, we suggest establishing new habitat patches ~50 m apart to encourage facilitated movement rather than unguided displacement behavior. Establishing spatial arrangements of habitat patches ~50 m apart in marginal cropland, grass-dominated sites, and road rights of ways will ensure the grain of resource patches matches the spatial scale of the female monarch's perception.

Data Availability

Data, metadata, and R code pertaining to this manuscript are publicly available through GitHub: https://github.com/kelseyefisher/estimating-monarch-butterfly-perception-of-potted-resources.

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