Oikos

o20805

Kummel, M., Brown, D. and Bruder A. 2013. How the aphids got their spots: Predation drives self-organization of aphid colonies in a patchy habitat. – Oikos 122: 896–906.

Appendices 1–6

Table of contents

Appendix 1

Nonstructural carbohydrates do not appear to play a role in determining aphid colonization and aphid population growth rates

In May 2010 we collected the terminal flower bud from each of 210 inflorescences at our US Air Force site. This included 33 inflorescences that were colonized by aphids and 177 inflorescences that were not. The buds were stored at –15°C prior to laboratory analysis. We selected buds from the 33 colonized inflorescences, and selected 33 buds from un-colonized inflorescences from the remaining 177 samples using a random number generator. The selected buds were oven dried at 45°C for 48 h, and pulverized in liquid nitrogen using mortar and pestle.

Our chemical analysis of non-structural carbohydrate concentration employed a slight modification of Chow and Landhausser's (2004) method. Using an analytical balance, 0.01 g of each sample was weighed out and extracted for 10 min at 90°C in 0.5 ml of 90% ethanol. The supernatant was decanted following a 2 min centrifugation at 14 000 rpm, and the sample was re-extracted with additional 0.5 ml of ethanol. The process was repeated again for the total of three extractions per sample. The supernatants from the three extractions were combined into a single test tube and the ethanol was evaporated at 45°C for 48 h, leaving a residue of the extracted carbohydrate in the test tube. Subsequently, the residue was re-constituted in 1 ml of 90% ethanol, and 0.1 ml of this ethanol solution was combined with 0.5 ml of 2% phenol and 2.5 ml of concentrated sulphuric acid. After 30 min incubation in the refrigerator at 4°C we measured absorbance of the mixture at 485 nm using a spectrophotometer. The instrument was calibrated with 50:50 mixture of sucrose and glucose at five different concentrations. Using the calibration curve and the absorbances we calculated the concentration of non-structural carbohydrate in each sample.

The results of our analysis of non-structural carbohydrates indicated that the concentration of non-structural carbohydrates did not play a significant role in either determining which plants were colonized, or in determining the daily population growth rate of aphids. The concentration of non-structural carbohydrates of colonized plants was not statistically distinguishable from that of randomly selected non-colonized plants at our field site ($t = 0.883$, $DF = 64$, $p = 0.381$). Likewise, the concentration of non-structural carbohydrates did not predict the per-capita population growth rate of aphids when used in linear regression as the sole independent variable ($R^2 = 0.013$, $n = 33$, $p = 0.525$), or when used in combination with population size ($\text{beta}_{\text{non-structural carbohydrates}} = 0.042$, $p = 0.845$). The best predictor for daily per capita population growth was population size ($R^2 = 0.175$, $n = 33$, $p = 0.014$).

Next we wanted to determine whether the nitrogen status of the plant played a significant role in determining aphid colonization and population growth rates. In particular, we hypothesized that aphid alates will preferentially colonize plants that are richer in nitrogen, and the plants richer in nitrogen will support higher aphid population growth rates. To test this hypothesis we determined the nitrogen content (% by weight) of our yucca samples using an elemental analyzer. The instrument was standardized using a high purity atropine standard. We used the remaining pulverized yucca flower bud samples from the nonstructural carbohydrate assay that had enough material for the elemental analysis ($n = 30$ colonized plants, $n = 32$ non-colonized plants). Contrary to our hypothesis, plant nitrogen content did not differ significantly between the colonized and un-colonized plants ($t = 1.742$, $DF = 60$, $p = 0.087$). Moreover the difference was in the direction opposite to what we hypothesized: the un-colonized plants had a slightly higher percentage of nitrogen (2.81% average), compared to the colonized plants (2.66% average). Nitrogen content was not a significant predictor of the aphid population growth rate when entered singly ($R^2 = 0.006$, $n = 30$, $p = 0.689$), or in a multiple regression together with aphid population size (Beta%N = 0.026, $p = 0.689$).

Reference

Chow P. S. and Landhausser, S. M. 2004. A method for routine measurements of total sugar and starch content in woody plant tissues. – Tree Physiol. 24: 1129–1136.

Appendix 2

Functional response of coccinellid predators

To parameterize the functional response of coccinellid predators we first determined the probability that a coccinellid was feeding as a function of aphid abundance. We selected 34 yucca racemes (aphid patches) in the field that ranged in aphid abundance from 0 to 3700 aphids. At the initiation of the experiment each raceme had 10–13 *Hyppodamia convergens* and 4–6 *Coccinella semptempunctuata*. We visited each plant 15 times at 10-min intervals and each time recorded the number of coccinellids of each species that were feeding as well as the total number of coccinellids. Using the data we calculated the probability of coccinellid feeding in each aphid patch as the number of coccinellids observed feeding in the patch summed across the 15 visits, divided by the total number of coccinellids in the patch summed across the 15 visits. We plotted the probability of feeding as a function of aphid abundance, and fitted Holling type II functional response curve to the data using maximum likelihood estimation procedure in Mathematica.

The results show that the two species differed in their parameter values. The half saturation constant for *C. semptempunctuata* was 196 aphids, and for *H. convergens* 238 aphids. Probability of feeding saturated at 0.155 and 0.132 for *C. semptempunctuata*, and *H. convergens* respectively.

Next we converted the probability of feeding into a feeding rate. Our observations showed that coccinellids do not feed at night, and that their feeding activity resumes soon after sunrise, at mid June this translates into 14 h of potential feeding time at our latitude. Given 14 h day we estimated that, *C. semptempunctuata* should spend the maximum of

14 × 0.155 = 2.17 h feeding. An analogous calculation reveals the maximum daily feeding time for *H. convergens* of 14 × 0.132 = 1.848 h

To turn the number of feeding hours into feeding rate we needed to estimate the number of aphids eaten during that time. We measured the amount of time that it took individuals of each coccinellid species to consume a single aphid twenty times and taking an average we estimated the handling time for *C. semptempunctuata* to be 63.5 s and for *H. convergens* to be 86.5 s (the difference is statistically significant $t = 4.312$, $DF = 38$, $p < 0.0005$). Given these results, the maximum feeding rate for *C. semptempunctuata* is 122.9 aphids day^{-1} and the maximum feeding rate for *H. convergens* is 77.1 aphids day^{-1}.

Both of the species were present during the 30-day study at an average ratio of 53 *C. semptempunctuata* to 47 *H. convergens*. Assuming that the two species forage independently (i.e. there is no direct interference), we combined the two sets of parameter estimates for the two species into a single set by weighted averaging. Thus the final half-saturation constant $h = 0.53 \times 196 + 0.47 \times 238 = 215.74$ aphids, and the maximum feeding rate $m = 0.53 \times 122.9 + 0.47 \times 77.1 = 97.84$ aphids day^{-1}.

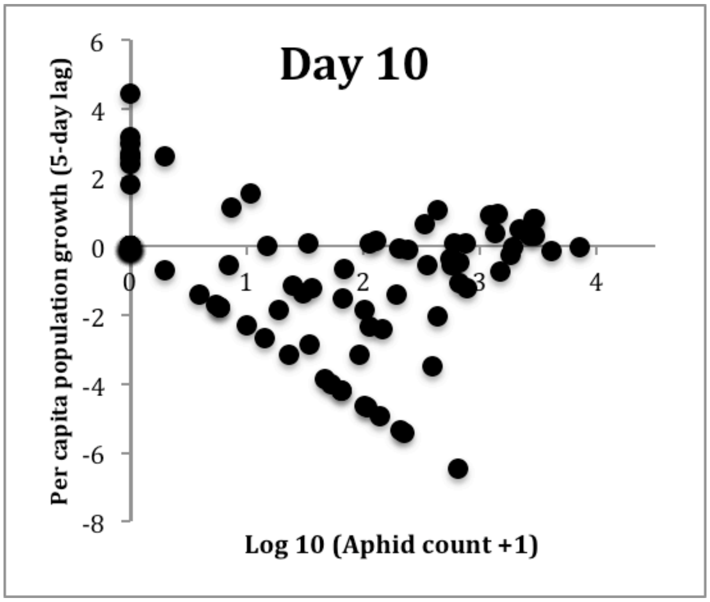
Appendix 3

The frequency distribution of non-zero aphid population sizes for each day in the 30 day time series (Supplementary material Table A3).

Day	Shapiro–Wilk statistic	n	p	Skewness	SE Skewness
1	0.974	40	0.489	–0.503	0.374
2	0.982	57	0.541	–0.271	0.316
3	0.971	61	0.157	–0.385	0.306
4	0.958	68	**0.022**	–0.581	0.291
5	0.974	68	0.169	–0.223	0.291
6	0.977	72	0.205	–0.106	0.283
7	0.981	68	0.401	–0.104	0.291
8	0.969	69	0.085	–0.291	0.289
9	0.976	65	0.242	–0.184	0.297
10	0.977	64	0.263	–0.087	0.299
11	0.963	62	0.057	–0.036	0.304
12	0.955	68	**0.015**	0.251	0.291
13	0.963	58	0.072	0.172	0.314
14	0.942	56	**0.009**	0.209	0.319
15	0.904	62	**<0.0005**	0.455	0.304
16	0.891	60	**<0.0005**	0.701	0.309
17	0.881	59	**<0.0005**	0.944	0.311
18	0.897	69	**<0.0005**	0.932	0.306
19	0.884	69	**<0.0005**	1.055	0.289
20	0.872	70	**<0.0005**	1.209	0.287
21	0.882	62	**<0.0005**	1.173	0.304
22	0.916	72	**<0.0005**	1.036	0.283
23	0.914	60	**<0.0005**	1.027	0.309
24	0.909	72	**<0.0005**	1.041	0.283
25	0.911	56	**0.001**	0.974	0.319
26	0.893	54	**<0.0005**	1.078	0.325
27	0.901	47	**0.001**	1.010	0.347
28	0.933	45	**0.012**	0.533	0.354
29	0.962	32	0.316	0.360	0.414
30	0.974	24	0.316	0.218	0.758

Appendix 4

Supplementary Figure A4. Per capita population growth rate based on a 5-day time lag.



Representative sample of a graph of per capita population growth rate (based on a 5-day lag) as a function of aphid population density. Notice that small populations tended to grow, medium populations tended to decline and large populations tended to stagnate. The aphid colonies on the straight lower edge are colonies that went extinct in the five day period: the per capita population growth rate is calculated as $\ln(A_{t+5} + 1 / A_t + 1)$, thus the equation for the extinct colonies is $\ln(1 / A_t + 1) = \ln(1) - \ln(A^t + 1)$, which is a straight line when plotted against $\log_{10}(A_t + 1)$.

Appendix 5

Power function describes the relationship between coccinellids and aphids (Supplementary material Table A4)

This table presents daily regressions of $\log_{10}$(coccinellid count) vs $\log_{10}$(aphid count) for the 30-day time-series. Entries in bold indicate that the value for the slope or intercept was statistically significantly different from zero. The log-log regression and thus the power function were statistically significant (i.e. a non-zero slope) on all days except for day 3 and day 23. Note that n varies because not all plants were colonized on any given day. We also included log-log coccinellid-aphid regressions from 2009 and 2010 fieldwork. The coccinellid and aphid abundance data for 2009 and 2010 came from single-day population censuses. The slopes across years are in fairly close agreement, however the intercepts of the 2009 and 2010 seasons are substantially higher. In 2009 and 2010 we conducted behavioral ecology observations on coccinellids and chose sites that were especially high in coccinellid abundance. The y-intercept of the log-log plot indicates the number of coccinellids on a plant with a single aphid. The higher intercept on the 2009 and 2010 data originates from the high overall coccinellid abundance at these field sites. (Table is located on the following page)

Day	Intercept	Slope	R^2	n	p for slope
1	0.021	**0.04498**	0.09594	85	0.0035
2	-0.008179	**0.070568**	0.2365	87	<0.0005
3	0.005453	0.016505	0.03614	89	0.0710
4	-0.01473	**0.05331**	0.1144	89	0.0010
5	0.01398	**0.12235**	0.2695	91	<0.0005
6	0.03899	**0.0985**	0.1714	92	<0.0005
7	0.02348	**0.14098**	0.2711	92	<0.0005
8	0.05237	**0.141**	0.3791	92	<0.0005
9	0.05534	**0.12457**	0.3315	93	<0.0005
10	**0.07694**	**0.08431**	0.1772	97	<0.0005
11	**0.06706**	**0.15381**	0.4726	98	<0.0005
12	**0.08567**	**0.17075**	0.4659	100	<0.0005
13	**0.11503**	**0.16449**	0.4526	101	<0.0005
14	**0.13866**	**0.15954**	0.4066	101	<0.0005
15	**0.11576**	**0.23416**	0.5677	102	<0.0005
16	**0.13346**	**0.26243**	0.6137	102	<0.0005
17	**0.09889**	**0.16748**	0.3409	103	<0.0005
18	0.05452	**0.17431**	0.3763	103	<0.0005
19	0.02118	**0.15502**	0.3377	102	<0.0005
20	**0.06362**	**0.15541**	0.3281	102	<0.0005
21	0.04303	**0.16275**	0.332	100	<0.0005
22	**0.12692**	**0.12041**	0.2208	100	<0.0005
23	**0.14893**	0.04014	0.02876	100	0.0884
24	**0.18098**	**0.11919**	0.1798	101	<0.0005
25	**0.12015**	**0.13671**	0.2261	101	<0.0005
26	**0.09009**	**0.18797**	0.4048	101	<0.0005
27	**0.11008**	**0.19062**	0.3533	102	<0.0005
28	**0.08684**	**0.20683**	0.4268	102	<0.0005
29	**0.11004**	**0.18652**	0.3042	101	<0.0005
30	**0.09793**	**0.21005**	0.3357	102	<0.0005
2007Average	**0.07578**	**0.141855**			

Additional observations from field seasons of 2009 at three field sites (F1–3) and 2010 at one field site

2009 F1	**0.657**	**0.152**	0.220	19	0.037
2009 F2	0.922	0.031	0.008	29	0.644
2009 F3	**0.172**	**0.212**	0.264	30	0.004
2010	**0.755**	**0.143**	0.200	31	0.012

Appendix 6

Mathematica code for spatial analysis.

The code starts on the following page.

Spatial Analysis via Resampling

This notebook contains the spatial analysis of interplant distances for our manuscript. We identify certain plants with high aphid populations as "centers", then compare their locations in space to those of randomly chosen samples from the population.

Center - Plant Distances

This section looks at whether the average distances from high - population ("center") plants to neighbors are different from analagous distances for randomly chosen plants.

```
SetDirectory["Desktop/aphids"]
```

```
/Users/dbrown/Desktop/aphids
```

```
dist = Flatten[Import["distances.xls"], 1];
(* table of distances from each plant to its 1st,
2nd,...,10th nearest neighbor *)
```

```
plants = Length[dist] (* total number of plants in the population *)
```

```
107
```

```
centers = {50, 54, 69, 78, 86, 97, 99, 100};
(* identities of the 8 "original" center plants,
i.e. those with highest average aphid populations *)
```

```
centers = {50, 69, 78, 86, 97, 99, 100};
(* 7 plants with average aphids > 1000 *)
```

```
centers = {15, 20, 50, 51, 54, 69, 72, 78, 80, 86, 93,  97, 99, 100, 101, 103};
(* 16 plants with average aphids > 500 *)
```

Note : pick one of the three lines above to define the "center" plants.

```
n = Length[centers]; (* the number of center plants *)
```

```
actualdist = Table[Mean[Table[dist[[centers[[i]], j]], {i, 1, n}]], {j, 1, 10}]
 (* avg distance from center plants to 1st, 2nd,...,10th nearest neighbor *)
```

```
{0.883632, 1.74635, 2.97863, 3.25981,
 3.53609, 3.82753, 4.30472, 4.57159, 4.86972, 5.22364}
```

```
pvals = {}; (* this will store the p-values for testing distances to 1st,
2nd, etc. neighbors *)
meanrandomdist = {}; (* this will store the average over all
 samples of the mean distances to 1st, 2nd, etc neighbors *)
trials = 10 000; (* how many times to resample the data *)
randomdist = Table[0, {j, 1, 10}, {i, 1, trials}];
(* this will store average distances from randomly chosen plants to 1st,
2nd, etc neighbors, repeatedly over many random samples;
ramdomdist[[j,i]] is the average distance to the jth neighbor
 (in the whole population)  from sample plants in sample i *)
```

```
Do[
  randomcenters = RandomSample[Range[plants], n];
  (* choose a random set of n plants from the overall population,
  to be compared to the actual centers *)
  Do[
   blah = Mean[Table[dist[[randomcenters[[i]], j]], {i, 1, n}]];
   (* compute the average distance to the 1st, 2nd,
   etc. nearest neighbors from the randomly chosen plants *)
   randomdist[[j, i]] = blah;,
   {j, 1, 10}],
  {i, 1, trials}]

 Null
```

```
Do[randomdist[[j]] = Sort[randomdist[[j]]];
 (* sort the average distances to neighbor j, using all samples *)
 p = 1. - Flatten[Position[randomdist[[j]],
       Nearest[randomdist[[j]], actualdist[[j]]][[1]]] / trials][[1]];
 (* compute the p-value by finding the location of each actual
   mean in the sortest list of sample means *)
 pvals = Append[pvals, p];
 meanrandomdist = Append[meanrandomdist, Mean[randomdist[[j]]]];
 , {j, 1, 10}]
```

- **8 original centers :**

```
pvals

{0.0054, 0.0978, 0.0014, 0.0369, 0.0514, 0.0641, 0.0535, 0.1233, 0.1083, 0.0891}

actualdist

{1.1611, 1.86845, 3.1853, 3.4837,
 3.67959, 3.97558, 4.38034, 4.57172, 4.86287, 5.2324}

meanrandomdist

{0.667625, 1.48326, 1.93572, 2.50531,
 2.76912, 3.07093, 3.38772, 3.77822, 3.99199, 4.25312}
```

- **7 centers with mean aphids > 1000 :**

```
pvals

{0.0324, 0.1285, 0.0012, 0.0488, 0.065, 0.0665, 0.0534, 0.1196, 0.1089, 0.0839}

actualdist

{1.04314, 1.83925, 3.32012, 3.49259,
 3.69642, 4.03065, 4.46197, 4.64895, 4.9319, 5.34765}

meanrandomdist

{0.665857, 1.48532, 1.9424, 2.51653,
 2.78041, 3.0792, 3.39623, 3.78594, 4.0014, 4.26418}
```

- **16 centers with mean aphids > 500 :**

```
pvals
```

```
{0.0405, 0.0957, 0., 0.017, 0.0189, 0.0256, 0.0117, 0.049, 0.0368, 0.0247}
```

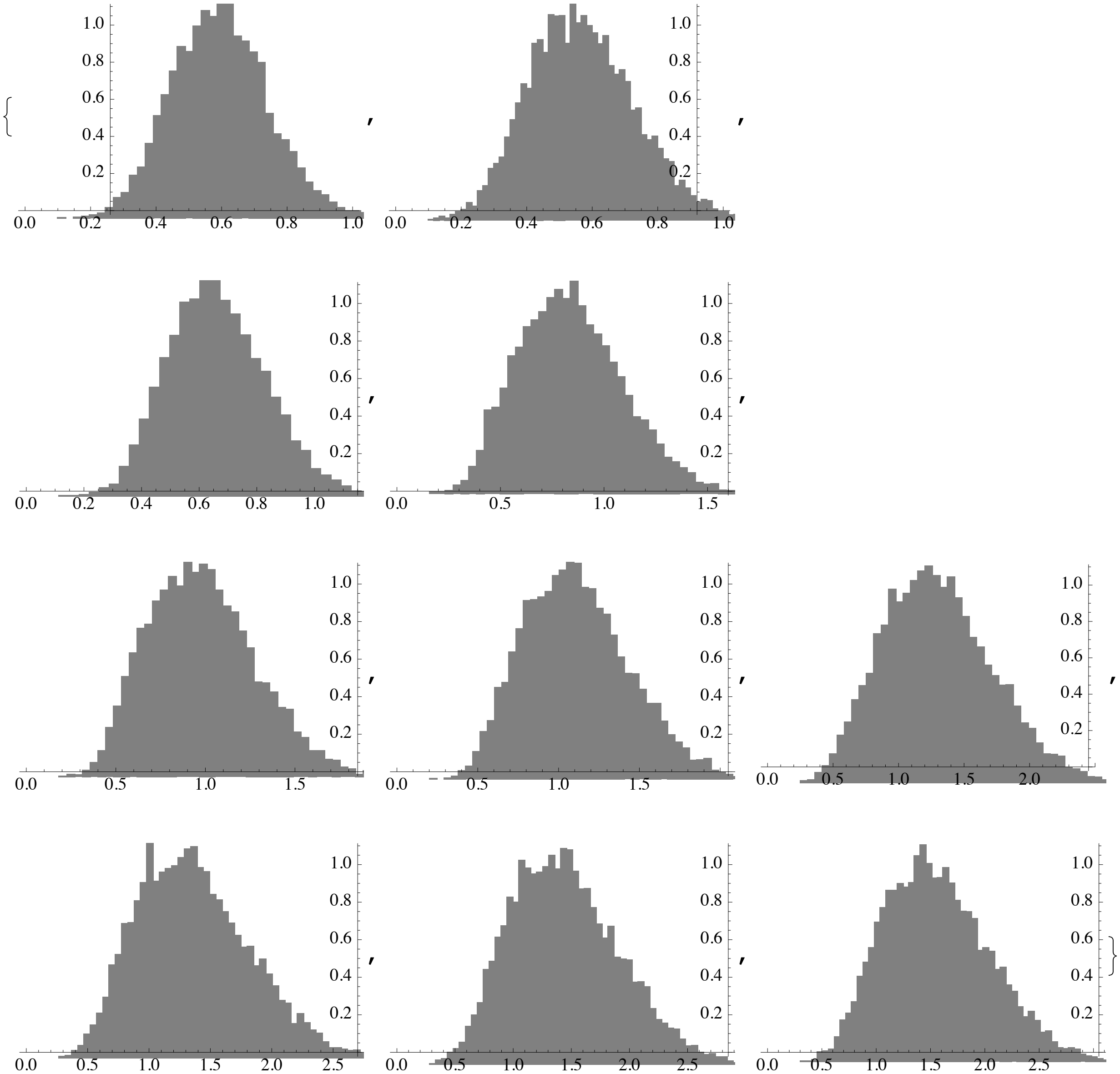
```
actualdist
```

```
{0.883632, 1.74635, 2.97863, 3.25981,
 3.53609, 3.82753, 4.30472, 4.57159, 4.86972, 5.22364}
```

```
meanrandomdist
```

```
{0.667123, 1.48283, 1.93729, 2.50302,
 2.76664, 3.0677, 3.38603, 3.77433, 3.98872, 4.24883}
```

```
Table[Histogram[randomdist[[j]]], {j, 1, 10}]
```



Center - Center Distances

This section looks at whether high population "center" plants are more evenly distributed than would be expected by chance. It compares both means and standard deviations of distances between centers to statistics from randomly chosen plants.

```
xycoords = Flatten[Import["xycoordinates.xls"], 1];
(* spatial locations of all of the plants in the population *)
```

```
centers = {50, 54, 69, 78, 86, 97, 99, 100};
(* identities of the 8 "original" center plants *)
```

```
centers = {50, 69, 78, 86, 97, 99, 100};
(* 7 plants with average aphids > 1000 *)
```

```
centers = {15, 20, 50, 51, 54, 69, 72, 78, 80, 86, 93,  97, 99, 100, 101, 103};
(* 16 plants with average aphids > 500 *)
```

Note : choose one of the three lines above to define the center plants.

```
n = Length[centers]; (* number of center plants *)
```

```
centercoords = Table[xycoords[[centers[[i]]]], {i, 1, n}];
(* table of the spatial locations of the center plants only *)
```

```
centerdists = Table[
   EuclideanDistance[centercoords[[i]], centercoords[[j]]], {i, 1, n}, {j, 1, n}];
(* table of distances between all pairs of center plants *)
```

```
sorteddists = Table[Sort[centerdists[[i]]], {i, 1, n}]; (* same as centerdists,
but with distance sorted from smallest to largest for each focal plant *)
```

```
avgdistances = Table[Mean[Table[sorteddists[[i, j]], {i, 1, n}]], {j, 2, 4}]
 (* mean distances from center plants to 1st,
 2nd, and 3rd nearest center plant *)
```

```
{3.75906, 5.97164, 8.18956}
```

```
stdevdistances =
 Table[StandardDeviation[Table[sorteddists[[i, j]], {i, 1, n}]], {j, 2, 4}]
 (* standard deviations of distances from center plants to 1st,
 2nd, and 3rd nearest center plant *)
```

```
{2.17116, 2.38276, 2.81816}
```

```
pvals = {}; (* this will store the p values for means *)
stdevpvals = {};(* this will store the p values for standard deviations *)
meanrandomdist = {};
(* this will store the average over all samples of the mean distances to 1st,
2nd, etc neighbors within the sample *)
stdevrandomresults = {}; (* same as above but standard deviations *)
trials = 10 000; (* how many samples to use *)
randomdist = Table[0, {j, 1, 3}, {i, 1, trials}];
(* this will store average distances from randomly chosen plants to 1st,
2nd, etc neighbors in the random sample,
repeatedly over many random samples; ramdomdist[[j,i]] is
 the average distance to the jth neighbor within sample i *)
stdevrandomdist = Table[0, {j, 1, 3}, {i, 1, trials}];
(* same as above but standard deviations *)
```

```
Do[
  randomcenters = RandomSample[Range[plants], n];
  (* choose a random sample of n plants *)
  centercoords = Table[xycoords[[randomcenters[[i]]]], {i, 1, n}];
  (* record their locations *)
  centerdists = Table[EuclideanDistance[centercoords[[i]], centercoords[[j]]],
    {i, 1, n}, {j, 1, n}];
  (* make a table of interplant distances in the sample *)
  sorteddists = Table[Sort[centerdists[[i]]], {i, 1, n}]; (* sort the
   distances from smallest to largest for each focal plant in the sample *)

  Do[
   blah = Mean[Table[sorteddists[[i, j]], {i, 1, n}]];
   randomdist[[j - 1, i]] = blah; (* compute and record the average distances
    to the jth neigherst neighbor over the n plants in the sample *)
   blah2 = StandardDeviation[Table[sorteddists[[i, j]], {i, 1, n}]];
   stdevrandomdist[[j - 1, i]] = blah2;
   (* same as above, but standard deviations insteead of mean *)
   ,
   {j, 2, 4}],
  {i, 1, trials}]
```

```
 Null
```

```
Do[randomdist[[j]] = Sort[randomdist[[j]]];
 stdevrandomdist[[j]] = Sort[stdevrandomdist[[j]]];
 (* sort the mean and standard deviation data from the random samples *)
 p = 1. - Flatten[Position[randomdist[[j]],
       Nearest[randomdist[[j]], avgdistances[[j]]][[1]]] / trials][[1]];
 pvals = Append[pvals, p]; (* compute p-values by finding the location
   of the actual statistic in the sorted list of sample statistics *)
 meanrandomdist = Append[meanrandomdist, Mean[randomdist[[j]]]];
 stdevp = 1. - Flatten[Position[stdevrandomdist[[j]],
       Nearest[stdevrandomdist[[j]], stdevdistances[[j]]][[1]]] / trials][[1]];
 stdevpvals = Append[stdevpvals, stdevp];
 stdevrandomresults = Append[stdevrandomresults, Mean[stdevrandomdist[[j]]]];
 , {j, 1, 3}]
```

- **8 original centers :**

```
pvals
```

```
{0.8756, 0.861, 0.8335}
```

Conclusion: The average distance from a true center to the 1 st, 2 nd, 3 rd nearest center is not significantly different than for randomly chosen plants.

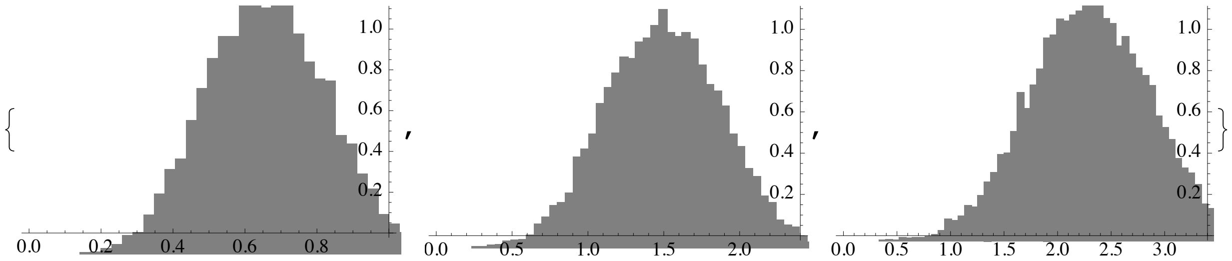
```
avgdistances
```

```
{3.20442, 5.68865, 8.02575}
```

```
meanrandomdist
```

```
{4.52179, 7.32568, 9.80923}
```

```
Table[Histogram[randomdist[[j]]], {j, 1, 3}]
```



```
stdevpvals
```

```
{0.9277, 0.9568, 0.8156}
```

Conclusion : The standard deviation of distance from a true center to the 1 st, 2 nd, 3 rd nearest center is not significantly different than for randomly chosen plants.

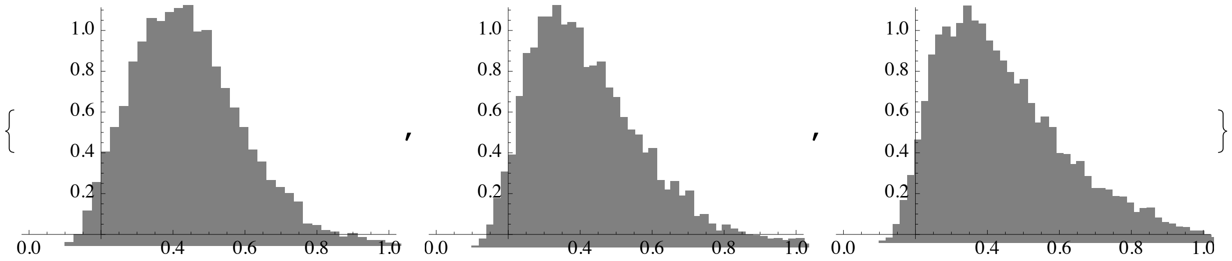
```
stdevdistances
```

```
{1.30377, 1.26094, 2.07363}
```

```
stdevrandomresults
```

```
{2.92726, 3.38519, 3.76222}
```

```
Table[Histogram[stdevrandomdist[[j]]], {j, 1, 3}]
```



- **7 centers with mean aphids > 1000 :**

```
pvals
```

```
{0.8152, 0.8855, 0.8839}
```

```
avgdistances
```

```
{3.75906, 5.97164, 8.18956}
```

```
meanrandomdist
```

```
{4.9303, 8.04427, 10.8506}
```

```
stdevpvals
```

```
{0.7152, 0.7119, 0.6718}
```

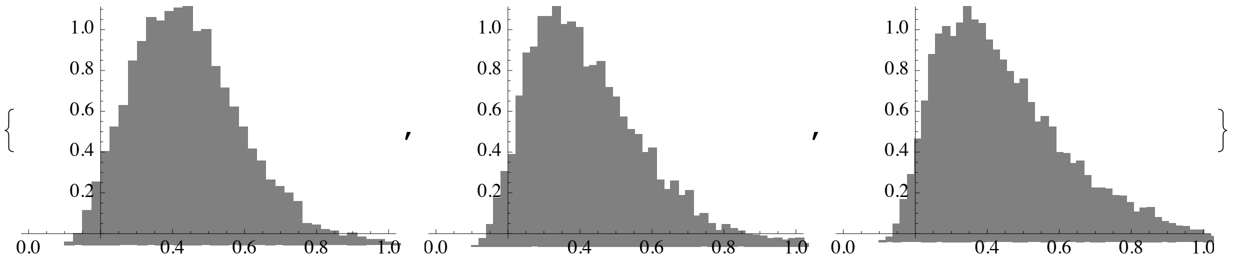
```
stdevdistances
```

```
{2.17116, 2.38276, 2.81816}
```

```
stdevrandomresults
```

```
{3.06943, 3.5903, 4.00469}
```

```
Table[Histogram[stdevrandomdist[[j]]], {j, 1, 3}]
```



- **16 centers with mean aphids > 500 :**

```
pvals
```

```
{0.3311, 0.852, 0.6773}
```

```
avgdistances
```

```
{3.16934, 4.01324, 5.7455}
```

```
meanrandomdist
```

```
{2.94168, 4.72746, 6.12642}
```

```
stdevpvals
```

```
{0.3974, 0.6076, 0.9275}
```

```
stdevdistances
```

```
{2.27654, 2.25221, 1.55274}
```

```
stdevrandomresults
```

```
{2.14594, 2.48209, 2.72619}
```

```
Table[Histogram[stdevrandomdist[[j]]], {j, 1, 3}]
```