## Fossil Safari – Practical 2 – Some Weirdoes

Today we will meet some rather peculiar organisms whose taxonomic affinity remains obscure. We will see how some straightforward mathematical approaches can help us to reconstruct how the organisms lived and grew, and thus where they might fall in the tree of life.

The practical will focus on two Ediacaran (Precambrian, c. 555 Ma) organisms, *Dickinsonia* and *Fractofusus*. In view of the limited amount of fossil material available, groups in TR1 should start with the *Dickinsonia* exercise, whereas groups in TR2 should begin with the *Fractofusus* exercise and move on to *Dickinsonia* in the second half of the practical.
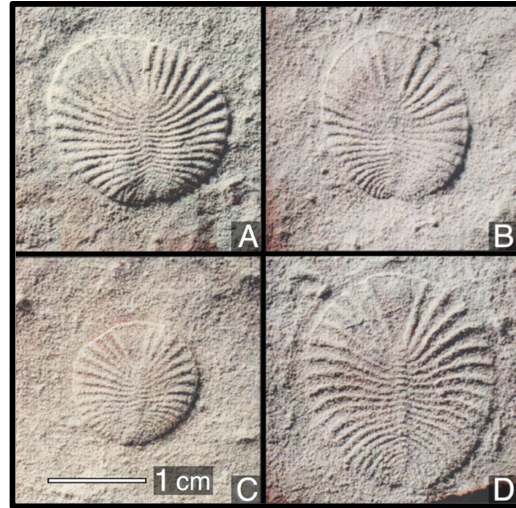
Part I. The affinity of *Dickinsonia* [75 mins]

Fossil preservation [15 mins]

Dickinsonia is one of the more abundant Ediacaran fossils, with perhaps a few thousand specimens known worldwide. As the fossils are difficult to extract from their outcrop, common practice is to take a mould of the fossil in the field, which can be used to make resin or plaster casts for study once back in the lab. You will be working with just such a cast today; the colour of the resin has been selected to match the original rock as accurately as possible.

Before we think about the anatomy of the organism, let's think about how it is preserved. What can you say about the rock that it was deposited in?
First make observations as best you can from the cast (particularly consider its grain size), then evaluate the possible depositional setting (How strong were the currents? Was oxygen likely to have been available?)

✎

Coarse-grained sandstone implies moderate currents, possibly facilitating mixing with oxygen-rich surface waters. Porous sandstone would be 'well ventilated'. Significance: non-mineralized tissue typically only preserves in dysoxic conditions, necessitating stagnant water and low porosity (i.e. clay-rich mudstones).

Now think about how the fossil was preserved. It might help to know that the sediment overlying this fossil had a similar composition to what you can see in the cast. (There was a bit of a clue in last week's lecture…)

Questions you might want to address include:

- Is any of the original organism (its skeleton or cell walls) preserved, and how?

- Are we looking at the upper surface of the organism, its lower surface, or a shrivelled version of an originally larger organism?

- Might the organism have had additional features that were lost to preservational processes?

✎

This is mouldic preservation; we have preserved an impression of the ?top of the organism, but nothing of the organism itself remains. As such we don't see any internal features, but can be reasonably confident that we see the entire external anatomy.
Dickinsonia has a distinctive yet simple morphology which makes it difficult to assign to any of the modern groups; it lacks any anatomical features that would allow it to be unambiguously placed within any living animal phylum. Those few morphological features that it does have (e.g. bilateral symmetry) may be unreliable if they are linked to function: similarities may represent convergent evolution towards an optimal solution to a physical problem, rather than an inheritance from a common ancestor. (You will remember from last week that Rugose corals overprinted a bilateral arrangement on their originally radial bodyplan).

A more reliable approach can be to look at how organisms grew: once a growth trajectory is established, it is very difficult to modify, as each stage of development draws on the one that came before it. Let's therefore try to reconstruct how *Dickinsonia* grew (i.e. how its morphology changed as it got bigger).

Before we go further, what are our options? Draw cartoons to illustrate at least three different possibilities.

✎ *Possibilities include:*
Growth by inflation: Each segment expands uniformly
Marginal accretion: Each segment grows at its end
Growth by terminal addition – new segments added at 'fat end' fully grown
Ternimal addition and inflation – segments added at 'narrow end' and continue to grow as organism does
Intercalary growth: new segments inserted between existing ones
Division of segments to become two segments
Moulting: shed robust skeleton then grow a new one, whose morphology is only loosely constrained by previous form
'Conveyor belt': new 'big' elements added at one end as little ones are shed from other end

Check that students have thought of at least one possibility that involves segment number constant during growth, and ideally two different means of adding segments

Now let's meet some baby *Dickinsonia* fossils (figured). We can safely assume that the organism on our cast grew from juveniles such as these. How does the number of 'segments' in the organism change as it grows? Is this consistent with any of the hypotheses you sketched in the previous question?
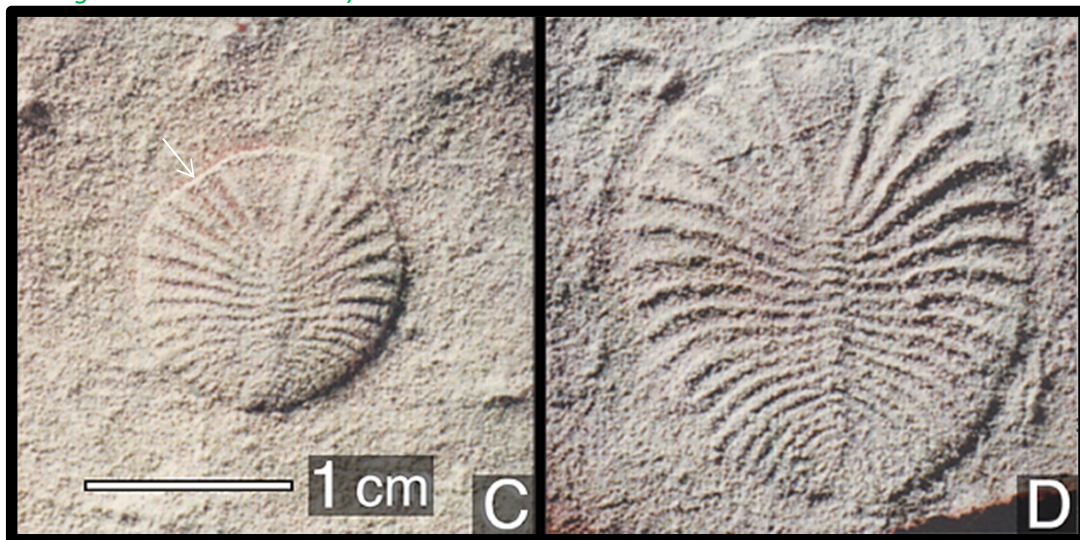
✎

Increase in segment number through growth discounts inflation & marginal accretion

If C was a juvenile of D, which of D's segments would the segment arrowed in C "grow up" to become?

✎

Would it be the second from the head (new segs added at back) or the 7th from the head (new segments added at head)?

Measurement [15 mins]

Now let's get quantitative and measure the fossils to reconstruct the growth sequence represented by fossils C, D and your cast. Use Vernier callipers to measure the radial length and maximum width of each segment in the cast, and record it on the next page. (You may want to work in groups of three or four to divide this labour, perhaps with one person calculating areas as others measure. You don't each need to transcribe the data.) Please **take care not to scratch the casts**, which are fragile!

Vernier callipers allow precise measurement. The zero on the Vernier scale points to the measurement on the main scale, here just over 2.1 cm. The alignment point gives the second decimal place: here line number **three** of the Vernier scale aligns with a line on the main scale, so the second decimal place is a three: the final reading is 2.13 cm.
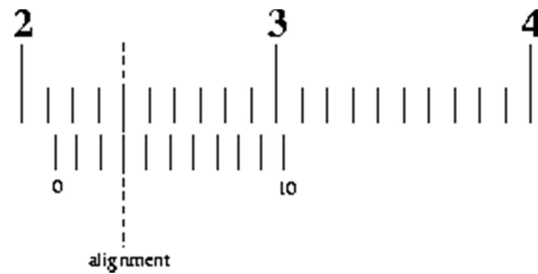
If you are short of time, you could measure every second (or even every third) segment. Stop when segments become too small to measure accurately.
Students may ask which end of the fossil counts as "segment 1". The answer is that it doesn't matter: we'll draw graphs on tracing paper so they can easily see what happens under both the "1 and the front" and "1 at the back" scenarios.

Reconstructing growth [25 mins]

Before we can reconstruct how *Dickinsonia* grew, we need to evaluate which segment corresponds to which in specimens of different sizes.

Use your data from the **fossil cast** to plot a graph on the graph paper below, with segment number on the x-axis, and the y-axis representing **segment length**. Draw a trend line through your points. (A trend line attempts to 'smooth out' noise from preservation and measurement error, and does **not** need to go through every point.)
*Remind students to plot with **+** not **×**, for greater accuracy: first referring to y axis to draw a horizontal bar in about the right place, then looking at the x axis to add the vertical bar.*

Then lay a sheet of **tracing paper** over the axes you have drawn, and plot the data given for **Specimen C** (photographed above) onto this sheet of tracing paper, drawing a trend line. Repeat this exercise on a **separate sheet of tracing paper** using the data for **Specimen D**.

Now slide the two sheets of tracing paper and see whether you can match any parts of the curves. Sliding the tracing paper along the x axis is equivalent to exploring different hypotheses of segment homology (i.e. 'the newest segment is situated at the front / back end of each specimen'). The alignment of the curves should allow you to answer the following questions:

What would be the biological interpretation of sliding the tracing paper up the y axis?

✎ *The same length is added to each segment: if curves align when shifted up the y axis, each segment has increased its length by the same amount.*

Segment number	C (mm / mm)			D			Fossil cast		
	Length	Width	Area	Length	Width	Area	Length	Width	Area
0	4.55	-1.9	-8.65	7.67	-2.51	-19.25			
1	5.04	1.37	6.9	8.56	2.09	17.89			
2	5.55	1.29	7.16	9.68	1.94	18.78			
3	6.22	1.34	8.33	10.08	2.13	21.47			
4	6.9	1.42	9.8	9.71	2.25	21.85			
5	6.69	1.48	9.9	10.2	2.18	22.24			
6	6.61	1.25	8.26	11.12	1.81	20.13			
7	6.61	1.19	7.87	10.2	1.96	19.99			
8	6.38	1.07	6.83	9.89	1.68	16.62			
9	6.06	0.97	5.88	9.53	1.51	14.39			
10	5.69	0.79	4.5	8.9	1.51	13.44			
11	5.31	0.78	4.14	8.44	1.4	11.82			
12	4.49	0.76	3.41	7.85	1.12	8.79			
13	3.92	0.59	2.31	7.6	1.19	9.04			
14	3.72	0.47	1.75	7.12	0.97	6.91			
15	3	0.55	1.65	6.36	0.99	6.3			
16	2.18	0.53	1.16	5.97	0.69	4.12			
17	1.25	0.62	0.78	5.47	0.91	4.98			
18	0.52	0.6	0.31	4.62	0.75	3.47			
19				3.51	0.44	1.54			
20				2.72	0.59	1.6			
21				2.03	0.63	1.28			
22				1.28	0.67	0.86			
23									
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All measurements in mm / mm²

Is 'segment zero' the oldest or the youngest segment? Where will the next new segment be added to the specimen? Are segments ever 'lost'?

✎ *The curve for C should overlie the curve for D with a shift of zero along the y axis and around 3.5 mm on the y axis: so 'segment zero' is the oldest segment; the smallest segments are the newest.*

Does the length of individual segments ever change / ever stop changing?

✎

Linear growth of each segment implied by shift of curve in y-direction; increase in length not proportional to age/size of segment (isn't that interesting – not the case in leaves, which stop growing, or in other taxa where growth rate is proportional to size).

Now that you have established the homology of segments, we can use **segment area** to drill down into exactly how Dickinsonia grew. On a separate sheet of graph paper (no need for tracing paper this time, as we know which segment corresponds to which) plot segment number against segment area for each of the three specimens.

How does the area of each segment change as the animal grows? How does this correspond to any changes in **length** as the animal grew?

Possibilities: (i) segments were secreted at a fixed shape and size; (ii) segments started small and grew to a fixed size; (iii) segments started small and kept on growing; (iv) segments exhibit non-linear growth (get bigger then smaller); (v) can you think of any alternatives?

✎

The area graph shows that segment growth is continuous. Segment area is further apart in successive growth stages the older each segment is.   The area (and probably volume) of each segment increases faster the larger each segment already is – a product of linear increases in both length and width as time goes on.
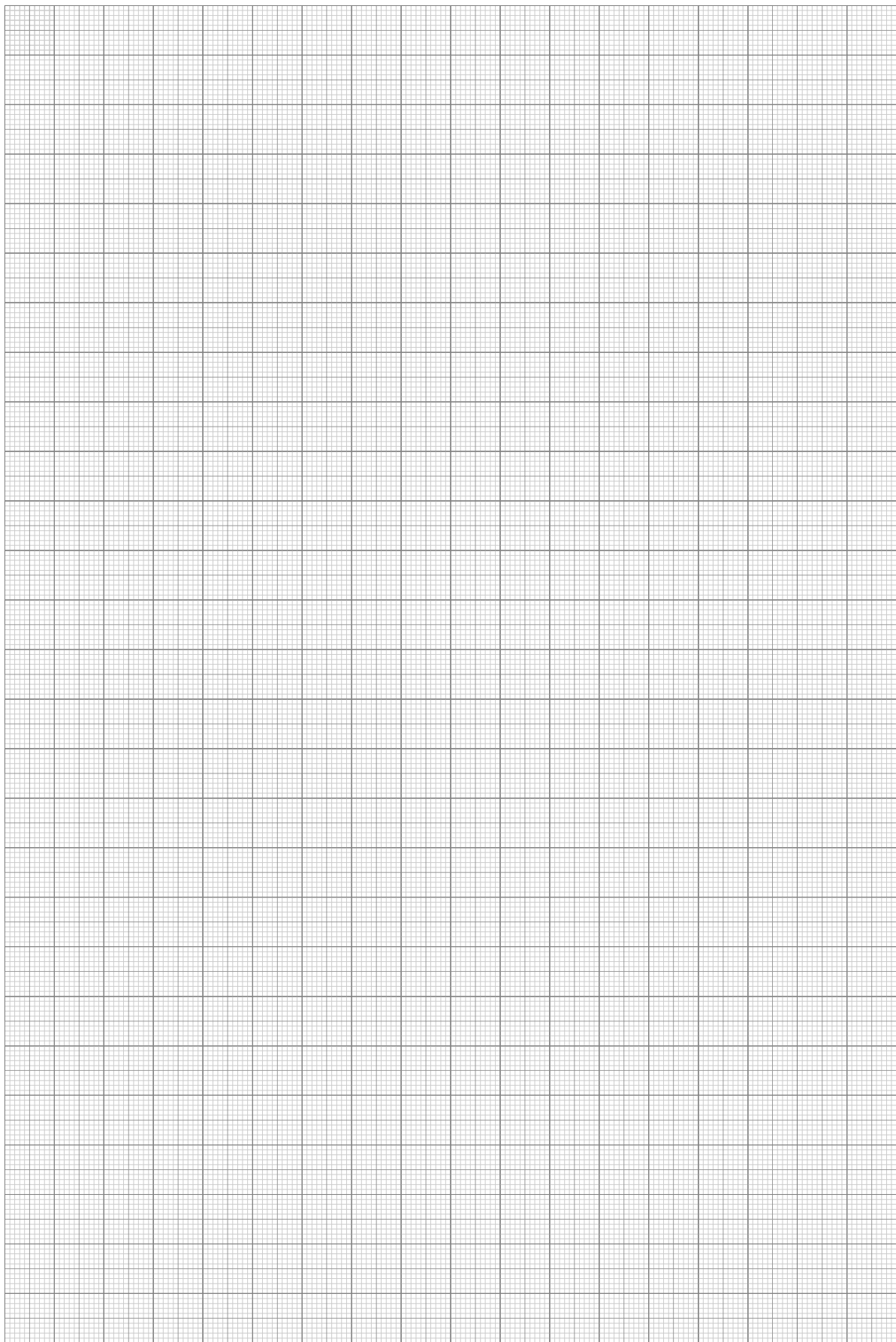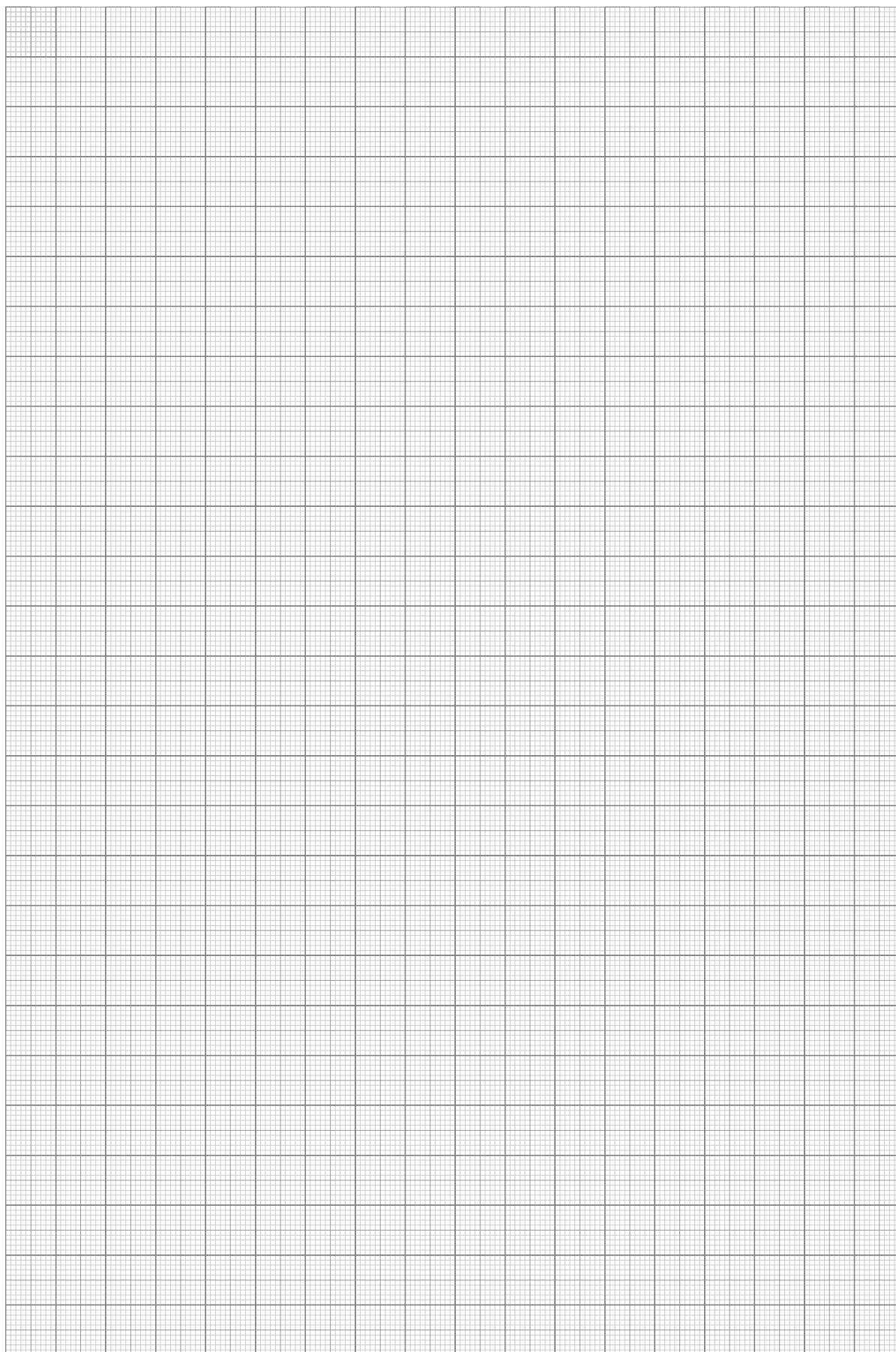
Affinity [10 mins]

Large organisms constructed from iterated units have evolved a number of times independently. Different growth modes are characteristic of different groups. The figure to the right represents some typical representatives, oriented such that new elements are added at the bottom of the figure.
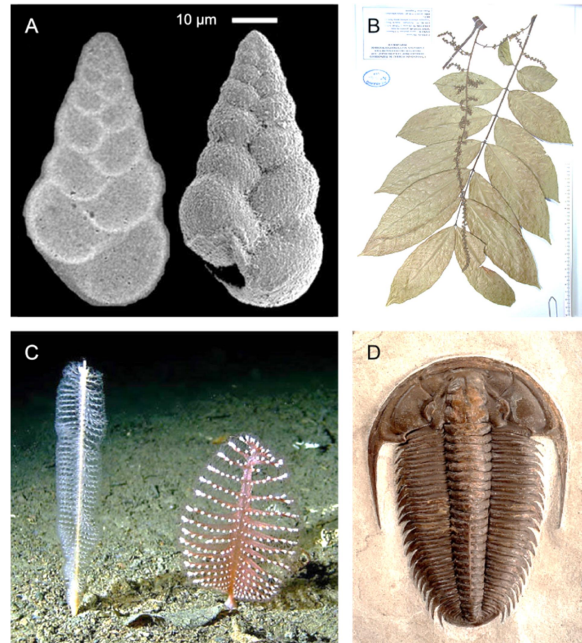
Foramanifera are single celled organisms, some of which secrete a chambered test ('shell') (**A**). New, larger chambers added at the end; once secreted, the chambers do not grow further.

Serial repetition has evolved three times in the 'algae': in brown and red algae (seaweeds), and in land plants (**B**). In each case, leaves stop growing once they reach a certain size, even if the axis continues to elongate.

Certain non-mineralizing cnidarians (sea pens [**C**], hydrozoans) grow in colonies, where each branch of the colony represents one or several zooids. In these taxa, new branches are added to the base (not the tip) of the stem, and continue to slowly grow as the organism gets larger. *To which animal group does phylum Cnidaria belong? Which fossils have you met that belong to it?*

✎ *Radiata – radial symmetry of **individuals** the norm – colonies can have bilateral arrangement. Last week we met the **Corals**.*

Finally, the ancestral bilaterian grew by the posterior addition of new segments; although many bilaterians have added complications to this basic growth pattern, some groups still reflect this ancestral condition (nicely displayed in trilobite **D**). In these taxa, serially repeated elements typically continue to grow as the primary axis (i.e. body) gets longer.

Consider what pattern these four growth trajectories would take if plotted on the graphs you produced above. On the basis of this ontogenetic ('growth history') data, is *Dickinsonia* a protist, plant or animal?

✎ *The growth data indicate a bilaterian-like growth trajectory.*

This practical exercise reproduces, in a slightly less sophisticated form, a recent study in Evolution & Development. *You should look up this paper when you get home, and see whether you reached the same conclusions as the authors.*

Gold D.A., Runnegar B., Gehling J.G., Jacobs D.K. 2015. Ancestral state reconstruction of ontogeny supports a bilaterian affinity for *Dickinsonia*. *Evol. Dev*. 17:315–324.

Part II. Growth and reproduction in *Fractofusus* [45 mins]

Fractofusus is an unusual spindle-shaped Ediacaran (565 Ma) fossil with a distinctive fractal arrangement – each branch is a smaller version of its parent branch, and is made up of smaller branches that are miniature copies of itself. As in *Dickinsonia*, no organs are preserved, so morphology can't tell us much about how it lived (besides its lack of mobility) or what it was related to.


The fossils were formed when the deep-water turbidite deposits on which they were living were smothered by a layer of volcanic ash – they thus represent an intact ecosystem frozen in time. Large numbers of *Fractofusus* occur on extensive surfaces exposed on the Newfoundland coast, revealing how the organisms were distributed on the ancient sea floor. We're going to reproduce a recent study in the top scientific journal *Nature*[*], which used this data to provide a new insight into how this organism lived.

The diagram below depicts the distribution and size of *Fractofusus* individuals on a single bedding surface. A pattern may not leap off the page, but let's see what we can find.


* Mitchell E.G., Kenchington C.G., Liu A.G., Matthews J.J., Butterfield N.J. 2015. Reconstructing the reproductive mode of an Ediacaran macro-organism. *Nature.* 524:343–346.

First we'll examine the sizes of the individuals. The largest is 32 cm long. Sketch how common you would expect *Fractofusus* individuals to be in increasing size categories, assuming size to be **normally distributed**.
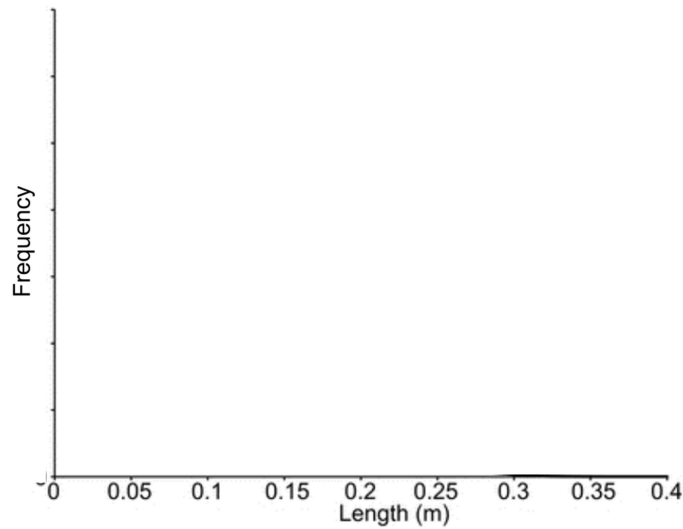
Now, example the plot to the right, showing the data from the H14 surface that was depicted on the previous page.

What are the key features of this size-frequency plot? Provide a biological interpretation of its shape.

✎
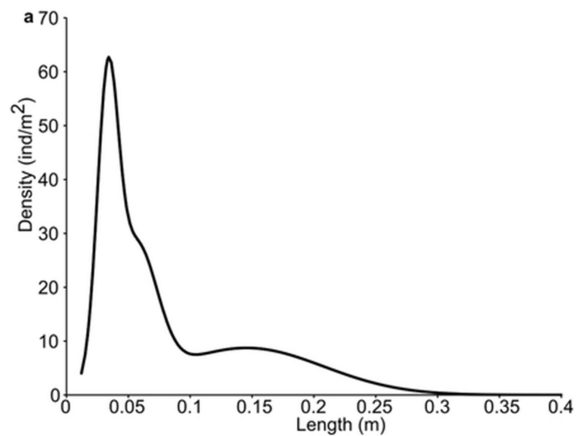
The graph denotes three overlapping normal distributions. There is a small population (mean length: c. 4 cm), a medium population (mean length: 7 cm), and a large population (15 cm). These could be three species, but more likely represent 'babies', 'kids' and 'adults'.

One interpretation of this curve provides a basis for subdividing the specimens into three separate categories – large, medium and small. The below figures show the distribution of large (left), medium (right) and small (bottom) *Fractofusus* individuals on the bedding surface; the red squares delimit regions of about ¼ m².

Label each ¼ m² region with the number of **large** and of **medium** individuals it contains.
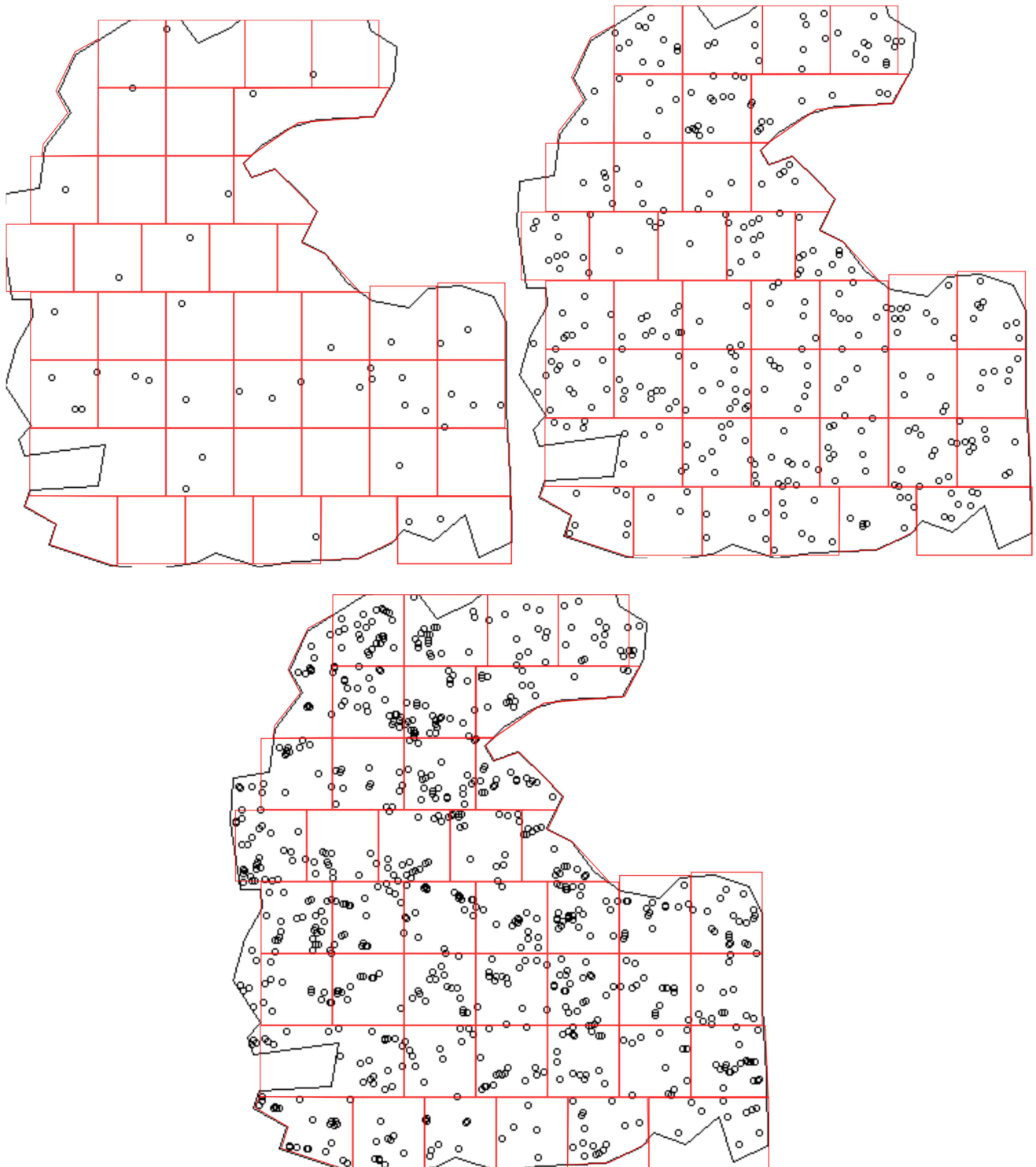
Complete the table below, then plot histograms of the distribution of large and medium *Fractofusus* individuals on the axes below.

n	0	1	2	3	4	5	6	7	8	9	10	11	12	13	…
Number of regions containing *n* **large** individuals	20	15	4	1	2	1									
Number of regions containing *n* **medium** individuals	0	0	2	2	3	7	3	3	3	4	5	7	1	1	2
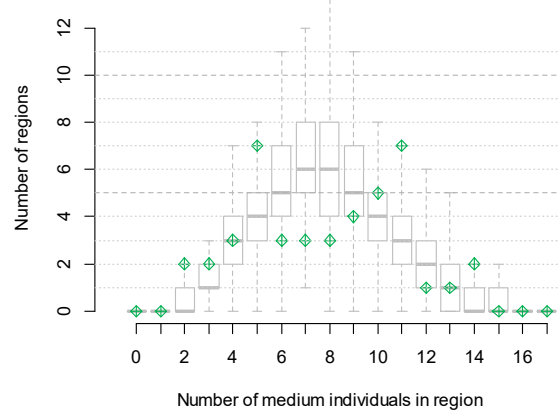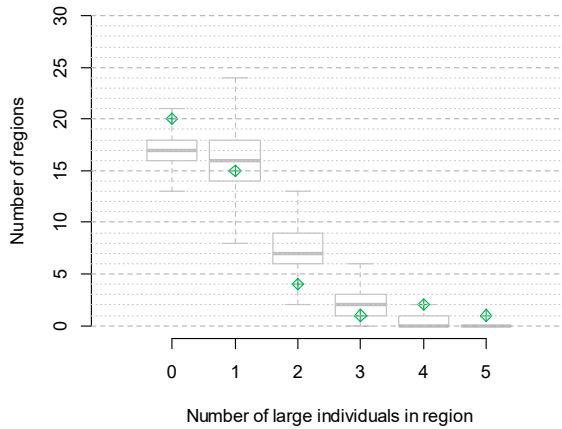
To help you interpret your histograms, the box-and-whisker charts below summarise the results expected if individuals are distributed entirely at random (the box denotes one standard deviation; the whiskers extend to the 95% confidence interval).
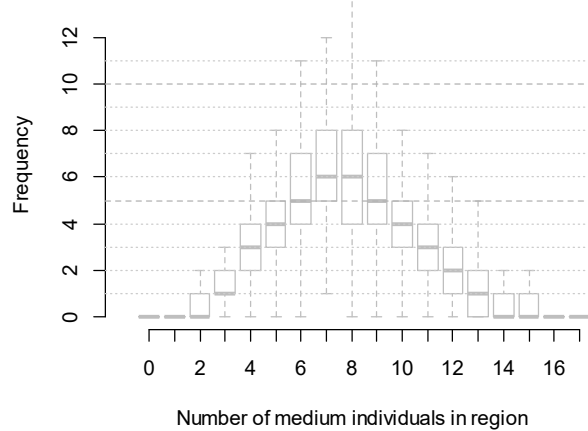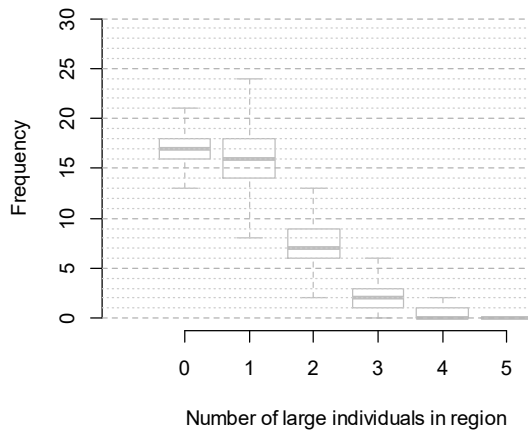
A given distribution can be thought of as being 'even' or 'clumpy'; the clumpiness can be measured with a metric called the Pair Correlation Function. PCF = 1 means that spacing is entirely random; PCF = 0 when individuals exhibit perfectly regular spacing, like bees in a honeycomb; PCF > 1 means that individuals are clumped together, like apples fallen from trees. For example, if a cluster has a PCF of 7, then the density within the cluster is 7× higher than the mean density over the region.
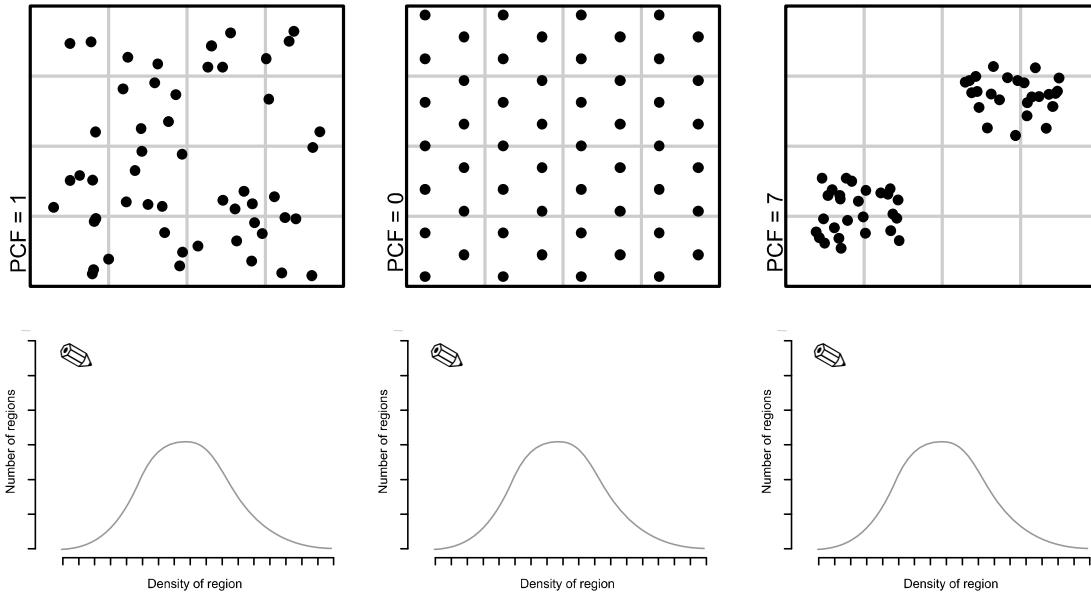
For reference, the axes above contain a bell-shaped curve corresponding to the frequency distribution that would be observed if individuals were randomly distributed. **Add** to each plot the frequency distribution expected at the corresponding PCF (1, 0 or 7, respectively).

Based on the histograms you made from the ¼ m² regions superimposed on the H14 surface, is the PCF for large and for medium *Fractofusus* individuals less than, close to, or greater than 1?

Large: 1; medium: > 1

What do your histograms tell you about the distribution of large and medium individuals?

Large individuals are randomly distributed. Medium individuals show two peaks, around 5/region and 11/region. This means that some boxes are fuller and others emptier than would be expected by chance. Medium individuals show a degree of clustering.

The PCF is a function of region size. Imagine apples clustered underneath trees: if your region size is about the size of an apple, then each region will contain either zero or one apples, and the spatial distribution of the apples is not captured: the PCF will be indistinguishable from 1. If the region is about the size of a tree, then regions that include a tree will be disproportionately full, and regions that are not near a tree will be much emptier

than expected by random. The histogram will have a U shape, and the PCF will be large.
But if the region size is so large that each region contains lots of apple trees and lots of empty
space, then most regions will contain a similar number of apples: the histogram will be bell-
shaped and the PCF will approach 1.

The graph below shows how the PCF varies for large, medium and small individuals of
Fractofusus when different region sizes are used ('Distance', *x*-axis)*. The grey area shows
the range of values that might be encountered if the distribution is entirely random. Look
first at the large individuals: does this graph confirm your earlier interpretation?

✎ *Yes – large individuals are randomly distributed*

Now look at medium individuals. How do you interpret the wiggle at a 0.05 m region size?

✎ *PCF > 1 at distance < 0.1 m; medium individuals display clustering*

Finally, look at the small individuals, which display two peaks – one around 0.03 m, one
around 0.1 m. How are the small individuals distributed? (Hint: think how ants might be
distributed around an apple tree…)

✎ *Two peaks means "clusters of clusters" – as ants cluster around apples, which themselves cluster around apple trees*
What does this distribution suggest
about how *Fractofusus* reproduced?

✎ *Large individuals washed in to colonise new surfaces– perhaps from sexual spores?*
Subsequent two generations from stolons, like a spider plant; presumably asexual.
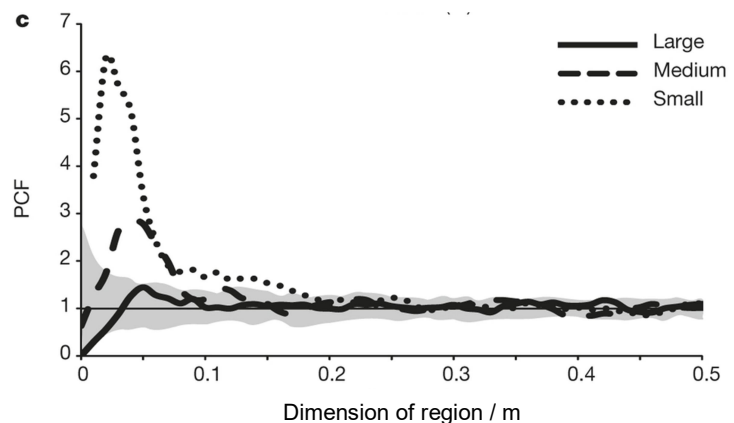
* Actually the PCF is a bit more complicated than this. I hope you'll forgive the simplification. Find out how it
really works by reading the study on which this exercise is based: Mitchell et al 2015, Nature 524:343–346.