Mathematical Supplement to "*Organ-level quorum sensing directs regeneration in hair stem cell populations*" by C-C Chen et al.

Estimating the length scale of the distressor from the data

We begin by considering the idea that plucked hairs release a "distressor" that spreads within skin, and neighboring follicles respond by regenerating if and only if the concentration of distressor they experience, $\boldsymbol{c}$, exceeds some threshold $\boldsymbol{\tau}$. If we had a direct way of measuring c, or knowing τ, we could easily find the distressor length scale, i.e. the characteristic distance over which it diffuses before its concentration decays by a factor of $1-\frac{1}{e}$. However, not knowing either piece of information, we seek to extract the length scale by fitting data on patterns of regeneration.

We will begin by making the assumption that there is a single distressor, and it spreads according to the laws of diffusion. This does not necessarily mean that the distressor is a diffusing molecule. It could, for example, consist of spreading macrophages and the molecules they secrete. As long as spreading can be modeled as an unbiased random walk, however, the dynamics of spreading should be mathematically equivalent to diffusion. Because follicles effectively lie in a plane, we may model this as a two - dimensional problem, where the loss of the distressor (whether by destruction or diffusion out of the plane) is represented with a constant loss term.

Ideally, we should treat a plucked region as a collection of regularly spaced point sources. However to develop some basic intuition, we'll also consider a disc-shaped plucked region to be a single spatially uniform source, the production rate at any point in space being proportional to the plucking density within the disc. In this simplification, the problem may be reduced to a one-dimensional problem in radial coordinates.

Continuum Model

Making the assumption of a disc-shaped plucked region that acts as a spatially uniform source of a diffusible distressor, and letting β stand for the radius of the plucked disc, Fick' s laws state that, at steady state, the balance between production, diffusion and loss will obey the following equations :

$$0 = \frac{D}{r}\frac{\partial}{\partial r}\left(r\,\frac{\partial c(r)}{\partial r}\right) - k\,c(r) + v,\ \ r \leqslant \beta, \qquad 0 = \frac{D}{r}\frac{\partial}{\partial r}\left(r\,\frac{\partial c(r)}{\partial r}\right) - k\,c(r), \qquad r > \beta \tag{1}$$

where D is an effective diffusion coefficient, $c(r)$ is the concentration of the distressor at location r, v is the rate of production of distressor, and k is the rate constant of distressor loss. Subject to the boundary conditions of radial symmetry-- $c'(0)=0$ and zero distressor as $r \to \infty$ --we find that the solution for these equations is

$$c(r) = \begin{cases} \nu\left[1 - \frac{\beta}{\lambda} I_0\left(\frac{r}{\lambda}\right) K_1\left(\frac{\beta}{\lambda}\right)\right], & r < \beta, \\ \nu\,\frac{\beta}{\lambda} I_1\left(\frac{\beta}{\lambda}\right) K_0\left(\frac{r}{\lambda}\right), & r > \beta \end{cases} \tag{2}$$

where λ stands for $\sqrt{\frac{D}{k}}$ and ν for $\frac{v}{k}$, and where I_α and K_α are the modified Bessel functions of the first and second kind, respectively. The term λ corresponds to a characteristic distressor length scale, while ν represents a natural unit of concentration, which may be seen as the concentration that distressor reaches in the center of an arbitrarily large disc when the distressor production rate is v. Let us take $\nu_{\max}$ to be the value of ν associated with the maximum production rate (i.e. that achieved by plucking every hair). Then we may normalize both $c(r)$ and ν to νmax, i.e. define $C = c/\nu_{\max}$ and $\varphi = \nu/\nu_{\max}$:

$$C(r) = \varphi\left[1 - \frac{\beta}{\lambda} I_0\left(\frac{r}{\lambda}\right) K_1\left(\frac{\beta}{\lambda}\right)\right], \quad r < \beta, \qquad \varphi \frac{\beta}{\lambda} I_1\left(\frac{\beta}{\lambda}\right) K_0\left(\frac{r}{\lambda}\right), \quad r > \beta \tag{3}$$

In this formulation, we note that φ is simply the "plucked fraction", i.e. the fraction of total hairs plucked in a region. We may simplify this expression further by normalizing lengths to λ, i.e. defining B and R as β/λ and r/λ respectively. Thus:

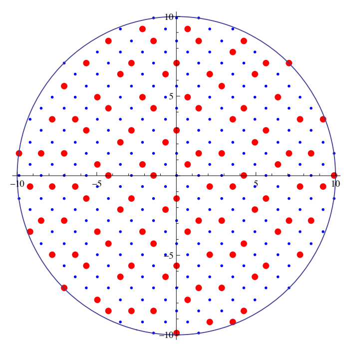
$$C(R) = \varphi[1 - B\, I_0(R)\, K_1(B)], \quad R < B, \qquad \varphi\, B\, I_1(B)\, K_0(R), \quad R > B \tag{4}$$

Thus, the concentration of distressor depends in a straightforward way upon disc radius B, location from the center for the disc R, and plucked fraction φ, and the distressor length scale λ.

Figure 2A shows a series of concentration curves for the distressor for various values of B (i.e. β/λ, or radius of plucked region in units of λ). The red symbols show the distressor concentration at the boundary of the plucked region for each curve. As plucked regions get larger, the value at the boundary asymptotes to 1/2 (in units of ν). By the time the plucked region has a radius of 3λ or greater, the distressor concentration at the boundary is already within less than 20% of this asymptotic value.

Discrete Model

Here we consider a discrete model of follicles arrayed on a hexagonal grid, plucked at various densities. For example, the picture below plots Cartesian coordinates of all follicles (blue) and plucked follicles (red) for an experiment in which the the plucked region had a radius of 10 interfollicular distances (assuming an interfollicular distance of 0.15 mm, which corresponds to the observed value of ~44 hairs$/$mm^2, that would mean a 3 mm diameter disc), and a plucking density of 0.33 (i.e. on average 1/3 of follicles were plucked).



We then allow each plucked follicle to act as a point source for a substance diffusing in two dimensions, with constant loss. According to Fick's law, the distressor profile from each hair will form a steady state gradient of declining exponential form

$$c_{ij}(x, y) = a\, e^{-\sqrt{(x-x_i)^2+(y-y_j)^2}\,/\lambda}$$

where $x - x_i$ and $y - y_j$ give the Cartesian distance to plucked follicle ij, λ is the distressor length scale (again equal to $\sqrt{D/k}$), and a is a constant proportional to the rate of distressor production at each follicle. To obtain the distressor gradient for an entire region, we simply sum over $c(x,y)$ evaluated at all those positions $\{x,y\}$ that were plucked, i.e.

$$c_{\text{total}}(x, y) = \sum_{plucked} a\, e^{-\sqrt{(x-x_i)^2+(y-y_j)^2}\,/\lambda} \tag{5}$$

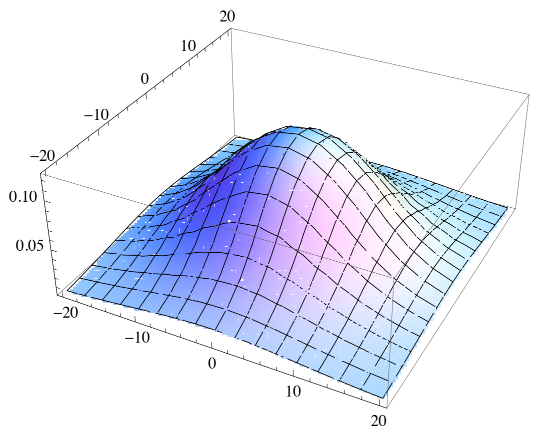
It is straightforward to show that, as the radius of a plucked region gets arbitrarily large, the distressor value in the center of a fully plucked region should approach 2 a $\pi\, (\lambda/\xi)^2$, where ξ is the average distance between follicles. This is because the total amount of distressor associated with every point source is $\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} a\, e^{-\sqrt{x^2+y^2}/\lambda}\, dx\, dy = 2\pi a \lambda^2$, whereas the area per point source is ξ^2. Accordingly, if we express $c(x,y)$ in terms of the value that $c(x,y)$ would take on in an infinitely large fully plucked region -- so that it is normalized in the same way as we did for the continuum model -- we get

$$C(x, y) = \frac{1}{2\pi(\lambda/\xi)^2} \sum_{plucked} e^{-\sqrt{(x-x_i)^2+(y-y_j)^2}/\lambda} \tag{6}$$

Corrections to the Continuum Model

The continuum model has advantages in terms of being able to find best fits to data. But intuition suggests that the appropriateness of the continuum approximation should depend upon the size of the plucked region being large relative to the length scale of the distressor. Here we investigate the magnitude of the error when that isn't the case.

As stated above, the empirically observed value of ξ, the interfollicular distance, is ~0.15mm. Thus, a disc of 1.5 mm radius corresponds to 10 plucked hairs. Below, we use the discrete formula (equation 6) to calculate C(x,y) when 25% of the hairs are plucked in a circular region with a radius of 10 hairs. Using an interfollicular distance, ξ, of 0.15 mm, and a value of λ/ξ=6 (i.e. λ=0.9 mm), we obtain the following steady state profile for the distressor (the x- and y-axes are expressed in units of ξ, and the center of the plucked disc as at {0,0}.



In the leftmost plot below, we show a cut through the center of the above shape (red line), and compare it with the shape obtained from the continuum model (i.e., equation 3; shown in dashed lines). In the middle and rightmost graphs, we make a similar comparison, but increase the radius of the plucked region to 20 and 30 interfollicular distances. In all cases the plucked fraction was 0.25.

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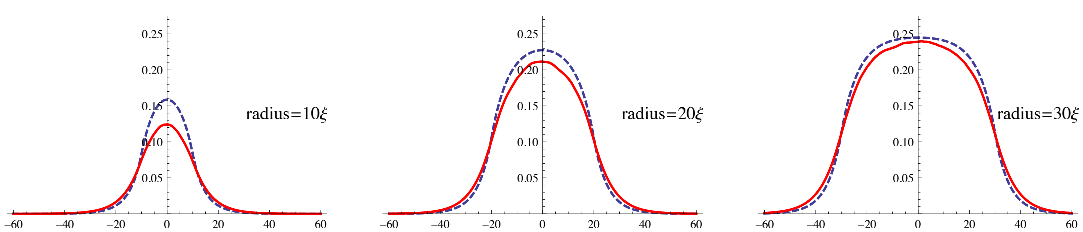

As expected, the error in the continuum model is greatest when the radius of the disc gets close to the value of λ. By calculating the error for a variety of value of disc radius, β, plucked fraction, and λ, we find firstly that this error is essentially independent of the plucked fraction, and secondly that it is closely approximated by a declining exponential function of β/λ. In particular, at the center of a plucked disc, the value obtained by the continued model can be corrected by multiplying it by a factor of $1 - e^{-\beta/\lambda}$. At the very edge of a plucked disc, the value obtained by the continued model can be corrected by multiplying it by a factor of $1 - e^{-1.35\,\beta/\lambda}$

Fitting Data

- **There are three ways in which we will fit data on regeneration after plucking of circular regions, with the goal of obtaining values of τ and λ**
- **METHOD 1:**

First, we notice that, in the continuum model, at *R*=0 (where *C* is always at its highest), equation 4 reduces to

$$C = \varphi[1 - B\,K_1(B)] \quad (7)$$

Applying the correction factor determined above for the center of a plucked region, we get

$$C = \varphi\left(1 - e^{-B}\right)(1 - B\,K_1(B)) \quad (8)$$

Thus, the criterion for whether regeneration of unplucked hairs can occur at least somewhere within a plucked region is simply

$$\varphi\left(1 - e^{-B}\right)(1 - B\,K_1(B)]) > \tau$$

Note that because *C* is expressed normalized to *v*max, implicitly so is τ. So if there is to be any regeneration at all, τ must be less than unity, that is to say the threshold for regeneration must be lower than the maximum attainable value of *C*. Accordingly, we may state that, at the threshold of plucking density and region size at which we just begin to see regeneration, the following relationship must hold (here we'll restore the disc radius B to its non-dimensionalized form):

$$\frac{1}{\varphi} = \frac{1 - e^{-\beta/\lambda}}{\tau}\left[1 - \frac{\beta}{\lambda}K_1\left(\frac{\beta}{\lambda}\right)\right]$$

What this tells us is that, in a plot of β versus $1/\varphi$, λ and τ are just proportional scaling factors for the two axes. In other words, if we start with a set of data with different plucking densities and different region sizes, and plot region size versus inverse plucking density, we need only ask by what two constants we need multiply the abscissa and ordinate data to maximize their agreement with the following curve.

For example, the composite graph shown in Figure 2C, experimental data are presented. The abscissa shows the radii of plucked regions in mm (β), and the ordinate shows $1/\varphi$, the inverse of the plucked fraction. The points are colored green if regeneration was observed, and red if it was not.

Choices of λ and τ that position the curve of $\frac{1-e^{-\beta/\lambda}}{\tau}\left[1 - \frac{\beta}{\lambda}K_1\left(\frac{\beta}{\lambda}\right)\right]$ midway between the red and green data points are $\lambda \approx 1.1$ mm and $\tau \approx 0.1$ (i.e. for a very large disc, plucking density should exceed 10% in order to induce regeneration) However, the curve can still be made to fall between the points for values of λ as low as 0.6 (with τ=0.2) or as high as 1.6 mm (with τ=0.06). Thus the estimate of λ from this data set is 1.1 ± 0.5mm.

It is interesting to note that, without the added correction factor of $\left(1 - e^{-\beta/\lambda}\right)$, the quality of the fit to data is noticeably poorer (not shown). This makes sense, since the smallest plucked regions (1.0 mm radius) or of a size similar to λ.

- **METHOD 2**

Here, instead of address whether regeneration occurred at all within a circular plucked region, we look at how far regeneration took place just *outside* of a plucked region, as an indication of how far the distressor spreads.

From the continuum model, equation 4, the value of *C* at some distance *D* (also expressed in units normalized to λ) beyond the edge of a plucked region is:

$$C = \varphi\,B\,I_1(B)\,K_0(B + D) \quad (9)$$

In the case, the appropriate correction to the continuum model is that estimated at the edge of a plucked region, i.e.

$$C = \varphi\left(1 - e^{-1.35\,\beta/\lambda}\right) B\, I_1(B)\, K_0(B + D) \tag{10}$$

Putting this back in terms of dimensionalized units of measurement, we find that the absolute distance δ outside a plucked region that we can expect regeneration to extend to is given by the following equation:

$$\frac{1}{\varphi} = \frac{1 - e^{-1.35\,\beta/\lambda}}{\tau}\,\frac{\beta}{\lambda}\, I_1\left(\frac{\beta}{\lambda}\right) K_0\left(\frac{\beta}{\lambda} + \frac{\delta}{\lambda}\right) \tag{11}$$

With sufficient data on the relationship between β, d and φ, we should be able to fit λ and τ simultaneously. Figure 2C shows the surface represented by this equation that best fits data from 13 experiments in which disc size and plucking density were varied, and the spread of regeneration outside the plucked region was measured.

Doing a least-squares best fit of the data yields estimated values of λ and τ of 1.0 mm and 0.125 respectively.

METHOD 3

Here we consider a series of experiments in which relatively small non-circular regions were plucked, in multiple animals, at a plucking fraction of 0.5 (i.e. every other hair was plucked). In one set of experiments a line of hairs, 100 interfollicular distances in length, was plucked (i.e. 50 hairs were plucked). In a second set, the region was a rectangle 4 x 24 interfollicular distances in size (48 hairs plucked). In a third set, the region was 10 x 10 interfollicular distances, in which 50 hairs were plucked. Figure 2 D, E and F illustrate, in cartoon form, these three scenarios.

In the first case (line), it was observed that regeneration was never seen in any animal. In the second case (rectangle), many individuals showed no regeneration, but a few did. In the third case (square) regeneration was robust in every individual. We may therefore ask what values of λ and τ would be consistent with such data.

Since all of these experiments involve relatively short distances (relative to our estimates of λ from other experiments), and since the regions are not circular, we turn directly to the discrete model, rather than the continuum one, for predictions. In Figure 2D'-F', we plot predicted results for the two-dimensional profile of C, for the above experiments (x- and y- axes are in units of interfolicular distance), and dots mark the locations of plucked hairs. The dots are colored red if they fall above the shaded gray plane that marks $C=\tau$, i.e. red dots are placed at those hairs whose distressor concentration is predicted to fall above the threshold for regeneration. Notice that the model clearly predicts that maximal values of C will be higher for the square than the rectangle or line, in agreement with the experimental observations. In order to agree with the observation that the rectangular region is very close to the threshold at which regeneration just begins to be seen, parameter exploration shows that acceptable values are not far away from those estimated using the previous two methods. For example, in the plots shown in Fig. 2D'-F', the value of λ was taken to be 6ξ, i.e. 0.9mm and τ was 0.087. Reasonably good fits can be obtained with values of λ as low as 0.66 mm (with τ=0.125) or as high as 1.2 mm (with τ=0.06).

SUMMARY

Depending upon the method used, we estimate a length scale for the distressor close to 1 mm. The threshold for triggering regeneration is estimated to be between 6% and 13% of the level of distressor that would be reached in the center of a fully plucked region of arbitrarily large size.