Pattern Development in Pollen Grains

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Pollen: A sphere with a surface pattern

Images courtesy of: A. Radja

M. O. Lavrentovich (UT Knoxville)
1. Pattern development on cell surfaces: pollen grains
2. Phase separation coupled to membrane undulations
3. Transitions to spatially modulated states
4. Evolutionary considerations
5. Liquid-crystalline analogs
6. Nucleation and growth on a sphere
Micron-sized patterning is observed across many taxa!


scale bars: 10 µm
Pollen exhibit a diverse set of patterns

images: Asja Radja

Asja Radja

Alison Sweeney
Each pollen pattern is robust in a species

images: Asja Radja
All seed plants have pollen. There are two basic types.
Pollen patterning is a complicated, multi-stage process.

The development follows a series of steps, with the following main features:

1. Material called glycocalyx is secreted at the pollen surface.
2. The plasma membrane undulates and glycocalyx forms domains.
3. The cell wall is dissolved, tough material is deposited.

Passion flower development

*Passiflora incarnata*

[Image of Passion flower plant and buds]
A phase transition in developing pollen?

Primexine Phase I

Primexine Phase Separation

Primexine Phase II

10 μm

[Diagram showing different phases of pollen development with labels for Cytoplasm, Plasma membrane, Primexine, and Callose Wall]
What is primexine?

1. Likely a complicated mix of polysaccharides: pectin, hemicellulose, cellulose (short chains, amorphous, possibly branched).

2. These compounds typically phase separate (A. J. MacDougall, N. M. Rigby, and S. G. Ring *Plant Physiol.* 114 353 (1997)).

3. Calcium may be used as cross-linker, inducing gelation.
What is primexine? A small test case.

Polysaccharide analysis done at the University of Georgia

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Early in development, the approximately spherical pollen membrane undulates at a wavelength $\lambda_0$. What sets $\lambda_0$?

We model the membrane as an undulating surface with bending modulus $\kappa$ and surface tension $\sigma$. Then, for nearly planar membranes:

$$\mathcal{H}_{\text{membrane}} = \frac{1}{2} \int d^2x \left[ \sigma(\nabla h)^2 + \kappa(\nabla^2 h)^2 \right]$$

Membrane prefers a flat configuration. Where is the length $\lambda_0$?
Phase separation at the plasma membrane

- A (glycocalyx) concentration $\Psi(x) = c(x) - c_0$ has a free energy

$$\mathcal{H}_\Psi = \int d^2x \left[ \frac{K_0}{2} (\nabla \Psi)^2 + V[\Psi] \right], \quad V[\Psi] = \frac{\tau_0}{2} \Psi^2 + \frac{\lambda_3}{3!} \Psi^3 + \frac{\lambda_4}{4!} \Psi^4$$

- There is a phase transition with $\tau_0$ the control parameter

Where is the characteristic $\lambda_0$? The ordered states coarsens:

Coupling the membrane undulations to phase separation

- How do we get a $\lambda_0$? Couple to membrane fluctuations:
  \[ \mathcal{H}_{\text{coupling}} = \mu \int d^2x \, \Psi(\nabla^2 h). \]
  \[
  |\mathbf{R}| = R_0 + h(\theta, \phi)
  \]

- Total free energy:
  \[ \mathcal{H} = \int d^2x \left[ \frac{\sigma}{2} (\nabla h)^2 + \frac{\kappa}{2} (\nabla^2 h)^2 + \frac{K_0}{2} (\nabla \Psi)^2 + V[\Psi] + \mu \, \Psi(\nabla^2 h) \right] \]

The fields $h(\Omega = (\theta, \phi))$ and $\Psi$ are expanded in modes $h^m_\ell$, $\Psi^m_\ell$:

$$h(\Omega) = \sum_{\ell=2}^{\infty} \sum_{m=-\ell}^{\ell} h^m_\ell Y^m_\ell(\Omega)$$

Integrating out the modes $h^m_\ell$, we are left with:

$$H_{\text{effective}} \approx \frac{1}{2} \sum_\ell \left[ \omega(\ell) + R^2 \tau_0 \right] |\Psi^m_\ell|^2 + H_{\text{int}},$$

where the interaction terms, $H_{\text{int}}$, couple modes. A non-zero unstable mode appears with $\ell_0 \approx 2\pi R/\lambda_0$!
The Brazovskii free energy: mean field

- The free energy $\mathcal{H} = \frac{1}{2} \sum_{\ell} \left[ \omega(\ell) + R^2 \tau_0 \right]|\Psi_\ell^m|^2 + \mathcal{H}_{\text{int}}$.
- The kinetic term $\omega(\ell)$ may be minimized by $\ell = \ell_0, \ell_0 + 1$:

$$
\omega(\ell)
$$

0 1 2 3 4 5
\ell

0 1 2 3 4 5
\ell

- The mean-field solution minimizes $\mathcal{H}$, taking into account the angular momentum coupling (Gaunt) coefficients

$$
\int \left[ \frac{\lambda_3}{3!} \Psi^3 + \frac{\lambda_4}{4!} \Psi^4 \right] \, d\Omega = \frac{\lambda_3}{3!} \sum_{\{\ell_i\}^3_{i=1}} \gamma_{m_1,m_2,m_3}^{\ell_1,\ell_2,\ell_3} \Psi_{m_1}^{\ell_1} \Psi_{m_2}^{\ell_2} \Psi_{m_3}^{\ell_3}
$$

$$
+ \frac{\lambda_4}{4!} \sum_{\{\ell_i\}^4_{i=1}} \gamma_{m_1,m_2,m}^{\ell_1,\ell_2,\ell_3,\ell_4} \gamma_{m_3,m_4,-m}^{\ell_3,\ell_4,\ell} \Psi_{m_1}^{\ell_1} \Psi_{m_2}^{\ell_2} \Psi_{m_3}^{\ell_3} \Psi_{m_4}^{\ell_4}
$$
The Brazovskii free energy: mean field

- Minimization with $2\ell_0 + 1$ (or $4\ell_0 + 4$) degrees of freedom $\bar{\Psi}_m^{\ell}$
- Solve using gradient descent, simulated annealing, etc.
- Once the states $\bar{\Psi}_m^{\ell}$ are found, it’s possible to calculate the stability:

$$\mathcal{H}[\bar{\Psi}_m^{\ell} + \delta\Psi_m^{\ell}] = \mathcal{H}_0 + \frac{\delta H}{\delta \Psi_m^{\ell}} \bar{\Psi}_m^{\ell} + \frac{1}{2} \frac{\delta^2 \mathcal{H}}{\delta \Psi_m^{\ell} \delta \Psi_m^{\ell'}} \bar{\Psi}_m^{\ell} \bar{\Psi}_m^{\ell'}$$

- Diagonalizing the Hessian, we find eigenvalues

$$\lambda = 2.9138, 1.0971, 1.7034, 3 \times 1.8269, 4 \times 1.6145, 3 \times 0$$

- There are always three 0-modes for the 3 rotations
The Brazovskii free energy: mean field results for one $\ell_0$
The Brazovskii free energy: mean field results for two $\ell_0$s

\[\ell_0 = 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, 11.5, 12.5, 13.5, 14.5\]

$\lambda_3 \neq 0$

$\ell_0 = 6.5, 14.5$
Mean field phase diagram

\[ \lambda_3 \]

\[ \ell_0 \]
An application of the mean field: viral capsids


- **Rotation group** $SO(3)$ breaks to a discrete subgroup

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What about fluctuations?

- Suppose we look at fluctuating fields away from the minimum, i.e., we expand

\[ \mathcal{F} = -k_B T \ln \int \mathcal{D} \Psi e^{-\beta \mathcal{H}[\Psi_0 + \delta \Psi]} \]

- In the planar case, there is an entire shell of momentum space in which fluctuations occur:

\[ \mathcal{H}_{\text{eff}} = \int \frac{d^2 k}{(2\pi)^2} \left[ \frac{1}{2} \left( (k - k_0)^2 + \tau \right) |\delta \Psi(k)|^2 \right] + \mathcal{H}_{\text{int}}. \]

- Fluctuations make an anomalously large contribution \((P \sim e^{-\beta \mathcal{H}})\)

\[ \langle \delta \Psi^2 \rangle \propto \int_0^{\Lambda} \frac{k \, dk}{(k - k_0)^2 + \tau} \approx \frac{\pi k_0}{\sqrt{\tau}} + \ln \left( \frac{\Lambda}{\sqrt{\tau + k_0^2}} \right) \]
Calculating fluctuation corrections on a sphere

- The general free energy $[\ell \equiv (\ell, m)]:$

$$\mathcal{H} = \sum_{\ell} \left[ \frac{(\ell - \ell_0)^2 + R^2\tau}{2} \right] |\Psi_{\ell}^m|^2 + \sum_{\{\ell_i\}_i=1}^3 \frac{\gamma^{(3)}}{3!} \prod_{i=1}^3 \Psi_{\ell_i}^{m_i} + \sum_{\{\ell_i\}_i=1}^4 \frac{\gamma^{(4)}}{4!} \prod_{i=1}^4 \Psi_{\ell_i}^{m_i}$$

- The propagator

$$\equiv \left\langle \Psi_{\ell_1}^{m_1} \Psi_{\ell_2}^{m_2} \right\rangle_0 = \frac{(-1)^{m_1} \delta_{\ell_1 - \ell_2} \delta_{m_1 + m_2}}{(\ell_1 - \ell_0)^2 + R^2\tau}$$

- The three point vertex with Gaunt coefficients $\Upsilon_{\ell_1,\ell_2,\ell_3}^{m_1,m_2,m_3}$

$$\equiv -\lambda_3 R^2 \Upsilon_{\ell_1,\ell_2,\ell_3}^{m_1,m_2,m_3} = -\gamma^{(3)}$$

- The four point vertex

$$\equiv -\lambda_4 R^2 \sum_{\ell} (-1)^m \Upsilon_{\ell_1,\ell_2,\ell}^{m_1,m_2,m} \Upsilon_{\ell_3,\ell_4,\ell}^{m_3,m_4,-m} = -\gamma^{(4)}$$

Fluctuations prevent a second order transition in any case

- When $\lambda_3 \neq 0$, there is a first-order transition

- If $\lambda_3 = 0$, fluctuations prevent a second-order transition at $\tau = \tau_c = 0$ from happening:

$$\tau \rightarrow t = \tau - \frac{\lambda_4 \ell_0}{4R\sqrt{t}} \coth(\pi R \sqrt{t}) > 0$$ for all $\tau$!
Fluctuations prevent a second order transition in any case. However, a first-order transition is induced for special modes \( \ell_i = (\ell_i, m_i) \) for which the quartic interaction flips sign

\[
\gamma^{(4)} \rightarrow \Gamma^{(4)} = \bigotimes - \bigotimes - \bigotimes - \bigotimes.
\]

The special modes have \( \ell_i = \ell_0 \) and pair cancellations \( m_i = -m_j \). In the planar case, these are standing wave patterns.

For the spherical case, the patterns have defects.
Computing the free energy of the states

- The patterned state has a free energy which must be expanded around a new minimum \( \Psi = \bar{\Psi} + \psi \), with modes \( \bar{\Psi}_m = \bar{a}c_m \). The \( \bar{\Psi} \) are the ordered states we observe.

- The patterned state modes are found by calculating the equation of state:

  \[
  H_{m0} = \frac{1}{4\pi R^2} \left\langle \frac{\partial \mathcal{H}[\bar{\Psi} + \psi]}{\partial \bar{\Psi}_m} \right\rangle_{\psi} = 0
  \]

- The change in free energy density between the two states is computed by integrating the mode amplitude \( \bar{\Psi}_m = ac_m \) from \( a = 0 \) to a final \( a = \bar{a} \):

  \[
  \Delta \Phi = \frac{1}{4\pi R^2} \int_{0}^{\bar{a}} \left\langle \frac{\partial \mathcal{H}}{\partial a} \right\rangle da
  \]
Results for the free energies $\Delta \Phi$ of the patterned states

We plot ordered states $\Psi = \sum_m \bar{a}_c m$. $\Delta \Phi$ only depends on $|c_m|^2$ in our approximation.
The process seems to be phase separation, occurring relatively rapidly (fixed volume). So, might expect conserved dynamics:

$$\partial_t \Psi = D \nabla^2 \left[ \frac{\delta \mathcal{H}[\Psi]}{\delta \Psi} \right] = D \nabla^2 \left[ (\nabla^2 + q_0^2)^2 \Psi + r \Psi + \frac{\lambda_3}{2} \Psi^2 + \frac{\lambda_4}{6} \Psi^3 \right]$$

This is the phase field model.

In flat geometries, if the wavelength selection is weak, foamy structures are formed (N. Guttenberg, N. Goldenfeld, and J. Dantzig *PRE* 81, 065301(R) (2010))
A similar structure may evolve on the sphere

Using a finite volume method to solve the equation on a tesselated sphere (radius $R = 18$):

$$\partial_t \Psi = \nabla^2 \left[ (\nabla^2 + 1)^2 \Psi - \Psi + \Psi^3 \right]$$
Three consequences of the theoretical modelling

1. Membrane undulations + phase separation $\rightarrow$ phase transition to a pattern with characteristic size

$$\lambda_0 \approx \frac{2\pi \sqrt{\kappa}}{\sqrt{\mu \sqrt{\sigma/K_0} - \sigma}} \sim 0.1 - 10\mu m$$

2. Spherical patterns have defects, and hence a much larger set of possible patterns than in the planar case

3. Introducing dynamics yields different possibilities for quenched patterns, including foam-like structures.
Comparing data to possible patterns

The field $\bar{\Psi}(\Omega)$ templates the places where the tough sporopollenin material is deposited.

The evolution of plants

40 × 10^6 years

- Not At Equilibrium, <1μm
- Not At Equilibrium, 1-3μm
- Not At Equilibrium, >3μm
- At Equilibrium
The evolution of plants: details

- Gymnosperms, basal, magnoliids
- Monocots
- Eudicots

- Not at Equilibrium, <1μm
- Not at Equilibrium, 1-3μm
- Not at Equilibrium, >3μm
- At Equilibrium
There is no apparent selective force toward patterning

1. Calculate transition rates $A \rightarrow B$ along evolutionary tree
2. Statistically significant rate in going from $A = \text{equilibrium pattern}$ to $B = \text{quenched/non-patterned}$
3. Significant rate in going from $A = \text{small } \ell_0$ to $B = \text{large } \ell_0$
4. “We don’t make mistakes, just happy little accidents.” - Bob Ross
1. Energy of a nucleated droplet of cholesteric

\[ E_{\text{drop}} = \sigma |\partial M| - c |M| \]

interface cost \hspace{1cm} condensation energy

2. Isoperimetric inequality for a surface of constant Gaussian curvature \( K \):

\[ |\partial M|^2 \geq 4\pi |M| - K |M|^2. \]

Critical droplet size and kinetics

The inequality is saturated for a geodesic disc. On a surface with constant $K$:

$$|\partial M|^2 = 4\pi |M| - K |M|^2$$

The droplet energy is thus

$$E = \sigma \sqrt{4\pi |M| - K |M|^2} - c|M|.$$ 

Assuming an over-damped dynamics

$$\frac{\partial |M|}{\partial t} = -\frac{1}{\tau} \frac{dE}{d|M|},$$

the critical radius is

$$R^* = \frac{1}{\sqrt{K}} \arctan \left( \frac{\sqrt{K} \sigma}{c} \right) \approx R_{flat}^* \left\{ 1 - \frac{K (R_{flat}^*)^2}{3} + O\left[ K (R_{flat}^*)^2 \right] \right\}$$

Corrections due to thermal fluctuations and geometry

The line tension $\sigma$ and condensation energies $c$ from the field theory:

$$\mathcal{H} = \int d^2 x \left[ \frac{\kappa}{2} (\nabla \psi)^2 + V[\psi] \right] \Rightarrow \mathcal{H} = \int ds [a + bk_g(s)]$$

where $a$, $b$ depend on $\Psi_0(\xi)$ and $k_g$ is the geodesic curvature of the interface. Adding fluctuations, we find (flat case)

$$\frac{\mathcal{F}}{k_B} = \frac{a}{k_B} 2\pi R_0 + T \ln \frac{\pi R_0^2}{A} + \frac{TN}{2} \ln \frac{aR_0}{2\pi k_BT} + \text{const.}$$

E. Horsley, M. O. Lavrentovich, and R. D. Kamien (in preparation)
Summary

- Developed model for pattern formation as a phase separation coupled to membrane undulations.
- Constructed general model, calculated mean field patterns by minimizing Brazovskii free energy.
- Showed pattern variety on the sphere relative to the planar case due to presence of defects.
- Added fluctuations showing that the transition always has a first-order character.
- Examined a cholesteric liquid crystal analog.
- Discussed how patterns nucleate and grow on a curved surface.

images: www.paldat.org