Experimental and Theoretical Studies of Elastic Instability in Growing Yeast Colonies and Thin Sheets

by

Baochi Thai Nguyen

B.S., University of California, Los Angeles, 1998

Submitted to the Department of Mathematics
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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Abstract

The thesis gives a comprehensive study of elastic instability in growing yeast colonies and thin sheets. The differential adhesion between cells is believed to be the major driving force behind the formation of tissues. The idea is that an aggregate of cells minimizes the overall adhesive energy between cell surfaces. We demonstrate in a model experimental system that there exist conditions where a slowly growing tissue does not minimize this adhesive energy. A mathematical model demonstrates that the instability of a spherical shape is caused by the competition between elastic and surface energies. The mechanism is similar to the Asaro-Tiller instability in prestressed solids. We also study the buckling of a highly constrained thin elastic plate under edge compression. The plate is clamped lengthwise on two edges and constrained by foam pieces along one of the shorter edges. The remaining edge is free. Applying uniform compression along the clamped edges generates a cascade of parabolic singularities. We apply the theories pioneered by Pogorelov, who showed that any zero gaussian curvature surfaces are solutions of the von Karman equations. When two such surfaces intersect, the adjoint surfaces remains a solution everywhere except at the boundary of intersection. However, for small plate thickness and the asymptotic limit, it is possible to construct a solution for the boundary. The total energy of the solution is then given as the sum of the energy of individual surfaces and the boundary energy. We demonstrate that by intersecting a cone and a cylinder the deformation of a parabolic singularity is entirely determined.

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Chapter 1

Introduction

Elastic instability occurs in many systems ranging from the growth of thin films on a solid substrate to plant development. This thesis focuses on experimental and theoretical studies of elastic instability in two systems - a growing yeast (Saccharomyces cerevisiae) colony and a highly constrained elastic plate under compressive load. In each of these problems we present an experimental observation and an accompanying theoretical investigation. The bulk of the thesis focuses on the yeast project.

Each of the following two chapters is self contained. In each chapter, an introduction describes the problem and motivation for the research project. An experimental section then provides details of the techniques, apparatus, sample preparation, and results. We then develop a mathematical model, and discuss its application to the experiments. The references are listed at the end of the thesis.

Chapter two investigates the role of elastic stress in shaping a growing yeast colony, a model system for tissue growth. The colony material is composed of cells, surface protein, and water from the agar substrate through which the nutrients diffuse. We choose Baker’s yeast as our model system because its adhesive protein is genetically controlled. Therefore, different strains of yeast can be created by expressing this protein at different levels. This surface protein creates bonds between the cells, and between cell and the substrate. Hence the system allows us to experimentally quantify the role of cell-cell adhesion and access its importance to tissue growth. The other advantage of using yeast is that the cells are immobile and spherical, and they are
too large to undergo brownian motion. We investigate a growing yeast colony that starts out from a single yeast cell and demonstrate experimentally that the contact angle of a yeast colony or yeast droplet obeys Young's law similar to that of a liquid droplet. When the colony is small, its equilibrium shape is spherical like the case of a liquid droplet. As the colony grows larger, its shape transitions to a non-spherical shape. It is well known that surface tension alone allows only spheres. Hence, other forces must act to cause the transition. We consider the case when the bulk elasticity of the colony is comparable to surface tension. The total energy of the system is then comprised of surface and elastic energies. Minimizing the total energy gives the mathematical model for the equilibrium shape of the colony. The model indicates that a spherical droplet on a solid substrate with fixed contact angle is unstable above a critical volume, quantitatively consistent with experiments. This could be the initial instability of the pattern founded by Fink at. el. [12]. In addition, the stress driven instability that we discover turns out to have an analogy to the planar instability of growing thin film on a substrate where the lattice constant of the film and of the substrate are different (The so-called Asaro Tiller instability [20]). Another example of an analogous effect in material science is the formation of an elliptical inclusion in an isotropic matrix. The spherical inclusion lowers the strain energy by transforming into an elliptical one [21]. The observed experimental nonlinear shapes are similar to the morphologies of drying colloidal drops in the experiments of Allain et. al. ??.

To our knowledge, the experiment and accompanying theoretical analysis that is reported in this chapter represents the first demonstration of the role elastic stresses can play in causing morphological transitions in growing tissue. This chapter is written with A. Upadhyaya, A. van Oudenaarden, and M.P. Brenner.

Chapter three studies the buckling of a highly constrained elastic plate. It is well known that thin elastic plates develop highly nonsmooth shapes under typical boundary conditions (Crumpled Paper). The surface then consists of smooth regions bounded by singularities in which there is significant elastic strain on the scale of the thickness of the plate. Three types of stress singularities have been identified: the developable cone [30, 36], ridges [26, 27, 28, 29, 32], and circular singularity [24]. The
first two types have lower energy than the last; therefore they should be more favorable in most systems. However the pattern of stress singularities that occurs during most situations (e.g. the crumpling of paper) is very complicated; Our study was designed to find a simple set of boundary conditions under which the stress concentration could be developed in a systematic and organized way. We demonstrate experimentally that under a certain simple set of constraints, the plate deforms into a network of parabolic singularities. In our experiment, the plate is clamped along its length. Part of the plate along one of the shorter edges is sandwiched between two foam pieces acting as elastic foundations. The remaining edge is free. Beyond a critical compressive load along the clamped edges, a curved singularity appears from underneath the foams and outlines a parabolic like region. As the applied load increases, the parabolic region travels towards the free end. Eventually the initial singularity escapes beyond the free edge and a new parabolic region appears from underneath the foams. This second parabola never escapes from the plate like the first one when the applied load increases. In response to further compression, more parabolic regions appear after the the second parabola and the plate organizes itself into a cascade of parabolic like curved singularities.

Our theoretical analysis of these experiments focuses on understanding a single parabolic singularity; this calculation will provide the foundation to the investigation of the complex cascade structure. A single parabolic singularity can be experimentally generated by first deforming a plate into a semi-cylinder then applying a normal load at one of the free ends. From this we can measure a relationship between the applied load, the amplitude of the deformation, \( \varepsilon \), and the length of the deformed region. Analytically, this relationship can be derived from geometrical constraints and energy minimization. We study the deformation using the idea of intersecting surfaces of zero gaussian curvature. A.V. Pogorelov [24] proved that such surfaces are solutions to the von Karman equations and intersecting the surfaces forms a new solution everywhere except at the boundary between the two different solutions\(^1\). In the asymptotic limit of small plate thickness, it is possible to construct a boundary

\(^1\)This idea was rediscovered independently in the course of writing this thesis.
solution between the different regimes. The total energy of the solution is then given as sum of the energies of the individual solutions plus the energy of the boundary. The parabolic regions of curved singularity can be generated by intersecting a cone and a semi-cylinder, both have zero gaussian curvature. The geometry of a cone is prescribed by its length and the opening angle, $\theta$. The other parameter of the problem is $\epsilon$, the deflection of the plate. The areas of the intersecting surfaces on the cone and the cylinder must equal. This constrain determines $\theta$ and energy minimization determines the remaining parameters. This chapter is written with M. Brenner, A. Kudrolli, and L. Mahadevan.
Chapter 2

Elastic Instability in Growing Yeast Colony

2.1 Introduction

The differential adhesion hypothesis states that cells in a growing tissue organize themselves to minimize the surface energy associated with the adhesion of different cells to each other. The hypothesis assumes that an aggregate of cells with different adhesive strengths is similar to a system of different liquids with different surface tensions. Over the years, many studies have provided evidence for this hypothesis via both experiments [1, 2, 3, 4, 5, 6, 7, 9] and simulations [10, 11]. The surface tension of certain embryonic tissues have been directly measured [6, 7], and as expected, mixtures of cells segregate to minimize the overall surface energy.

If the morphology of a growing tissue is dictated solely by surface energy minimization, then this has implications for not only the position of cells relative to each other but also for the overall shape of the tissues: in the absence of external forces, a tissue minimizing surface energy should be composed of spherical regions. The goal of the present work is to test this hypothesis within a particularly simple example: the shape of a “droplet” of a single cell type growing on a nutrient-enriched substrate. As for liquid droplets, the equilibrium shape of such a structure is a spherical cap.
with a contact angle given by Young's law,

\[ \gamma_{CC} \cos(\theta) + \gamma_{CS} = \gamma_{SA} \]  

(2.1)

which relates the equilibrium contact angle \( \theta \) of the colony at the agar substrate to the surface energies of the liquid and solid. Here, \( \gamma_{CC} \) is the adhesion energy of the cells to each other, \( \gamma_{CS} \) is the adhesion energy of the cells to the substrate, and \( \gamma_{SA} \) is the energy per unit area of the substrate. All of these quantities should change when the types of cell and substrate are varied.

Our experiments focus on colonies of Baker's yeast (\textit{Saccharomyces cerevisiae}), growing on an agar substrate. The advantage of this system is twofold. First, the gene expressing the adhesive protein (FLO11) is known, and thus the cell-cell adhesion \( \gamma_{CC} \) can be genetically controlled. Second, the adhesivity of the substrate \( \gamma_{CS} \) can be varied by changing the agar concentration. Third, yeast cells are spherical and have no mechanism for active motility. The experiments demonstrate that, (consistent with Young's law equation (2.1)), changing either the agar concentration or the expression of FLO11 modifies the local contact angle of the yeast droplet. Moreover, when the colony is sufficiently small, its shape is a spherical cap, consistent with surface energy minimization. However, above a critical (contact angle dependent) volume the spherical structure is unstable, and the colony develops a non-spherical shape. Since these shapes are inconsistent with surface energy minimization, the experiments demonstrate that there must be other forces acting on the tissue. The possible candidates in our experiments are gravity, adhesive gradients, growth stresses, and elastic stresses. We present a mathematical model suggesting that the change in tissue morphology arises from elastic deformations of the colony. The model demonstrates that a spherical elastic droplet on a solid substrate with fixed contact angle is unstable above a critical (contact angle dependent) volume, quantitatively consistent with experiments. The model reproduces both the instability threshold and the shape of the yeast droplets near the threshold, consistent with the experiments.
The organization of this chapter is as follows. In the next section we describe our experimental system and discuss the experimental results. A phase diagram is presented delineating the borderline between spherical shapes (where the colony shapes minimize surface energy), and non-spherical shapes (where other forces are acting). Section three derives a mathematical model for an elastic droplet on a solid surface, and analyzes the stability of the droplet to non-spherical perturbations. The instability threshold is computed and compared with experimental observations. We also present analytic calculations of droplet shapes beyond the transition. Finally, section four presents conclusions and directions for future work.

2.2 Experiment

2.2.1 Background

Our study focuses on an assay discovered by Reynolds and Fink[12]. They noticed that when Baker's yeast (Saccharomyces cerevisiae) grows on a low glucose medium they adhere to plastic substrates and form biofilms. The ability of the yeast to stick to plastic substrates was traced back to FLO11, a yeast gene encoding a cell surface glycoprotein that allows cells to adhere to agar and to each other. This gene can be turned off (producing the mutant Flo11Δ) or overexpressed (producing the strain Sfl1Δ), so that three independent strains of otherwise identical cells with different adhesion strengths exist. Reynolds and Fink found that when wild type (WT) yeast grows on low agar concentration (0.3%), it forms a complex structure with reproducible features. Since the cells are nonmotile, the structures that form are entirely the result of the forces that act upon them. The morphologies observed in the Reynolds-Fink experiments are determined by a large number of related effects, including adhesion, nutrient consumption, and water content.
2.2.2 Materials and Methods

Yeast Strains We use Baker's yeast *Saccharomyces cerevisiae* with different levels of expression of the adhesive protein FLO11 (obtained from the laboratory of Dr. G. Fink, Whitehead Institute). There are three strains Flo11Δ, wild type (WT), and Sfi1Δ that express low(zero), normal, and high levels of FLO11, respectively. The strains are characterized by the levels of adhesion as non-sticky, sticky, and super-sticky. The system has many advantages. First, these cells are spherical and nonmotile with an average cell division time of two hours. Cellular rearrangements are possible through the forces the cells exert on each other and on their environment. An aggregate of these cell types has an effective surface energy \( \gamma_{CC} \) due to adhesive interactions between individual cells. The magnitude of \( \gamma_{CC} \) is set by the concentration of this cell surface protein, which is genetically controlled.

Preparation of Agar Substrate and Yeast Colonies. The growth medium YPD is composed of water, 1% Difco yeast extract, 2% Bacto peptone, and 2% Mallinckrodt dextrose. A desired amount of Bacto agar is added to the growth medium. The mixture is then autoclaved for 20 min at 122°C to dissolve the agar and sterilize the medium. The substrate is prepared by pouring 30 ml of the sterile mixture into a sterile petri dish (Corning) and allowed to set for an hour. A sterilized glass plate is placed at the bottom of the petri dish before pouring in the mixture. This makes the transfer of the substrate between the petri dish and the microscope stage more stable and easier. When the plates are set, they are ready for inoculation. Colonies are inoculated by spreading 25\( \mu l \) of a dilute mixture of yeast cells and liquid YPD. The inoculation procedure ensures that for each plate the number of colonies is small (< 20) and spread out. The inoculated plates are placed in a humidified incubator at 28°C for a couple of days.

Imaging and Data Analysis Once the colonies are visible by eye the imaging process begins with a side-view microscope (Leica Monozoom 7) with an attached CCD camera. This allows the measurement of contact angles that a yeast colony makes with the agar substrate and the two dimensional shape of the colony as a
function of time. For imaging, the glass plate with the agar substrate is cut and re-
moved from the petri dish and then placed on the microscope stage. A dual cold light
source (Fiber Lite MI-150, Dolan Jenner, Inc.) is used to illuminate the colonies from
the sides. Time lapse images of the colonies on the same plate are taken every few
hours; between images the plates are placed back in a humidity controlled environ-
ment. Each time lapse step takes about 20 minutes to images all the colonies in the
plate. Even with the glass plate, the transferring of a substrate of agar concentration
below 1% is not stable. This limits the experiments to agar concentrations of 1% and
higher. We acquire and analyze the images using Metamorph software (Universal
Imaging Corporation).

2.3 Results

Contact Angle The first set of experiments is designed to measure the contact angle
of a yeast droplet for fixed agar concentration, and to determine if it remains con-
stant throughout the growth of the colony, as implied by equation (2.1). Time lapse
images of colonies of the same plate were taken every two hours. Our initial exper-
iments showed that although the shape of small yeast droplets remains spherical, the
contact angle actually increases with time, contradicting Young's law with constant
surface energies. We hypothesized that the increase in the angle might arise from the
evaporation of water from the colonies and the substrate during the imaging process.
We therefore conducted a set of experiments using a number of identically inoculated
plates to verify this hypothesis. After the colonies on a given plate are imaged, the
plate is discarded to avoid evaporation. Images at later times were taken from a fresh
plate from the incubator. These experiments demonstrate that the contact angle re-
mains constant during the entire growth of the colony (figure 2-1). The constancy of
the contact angle is obeyed even after the instability (to be discussed subsequently)
occurr.

Figure 2-2 shows images demonstrating that an increase in cell-cell adhesion in-
creases the contact angle. The supersticky Sf1Δ strain has the highest contact angle
at a given agar concentration, consistent with its higher surface tension. The sticky wild-type (WT) and nonsticky Flo11Δ strains have similar contact angles. Although one might expect the wild-type strain to have a larger contact angle than the mutant Flo11Δ strain owing to the expression of the adhesive protein, this neglects the effect of water on the surface energy of the colony. We hypothesize that the surface of the colony contains capillary bridges of water between the yeast cells which also act to pull cells together. When the adhesion between cells is sufficiently weak, one would expect the cell-cell adhesion energy $\gamma_{CC}$ to be dominated by the surface tension of water; although we have no direct way of measuring $\gamma_{CC}$, we believe this is a consistent interpretation of the data.

Figure 2-2 also shows images documenting the change in $\theta$ on varying the agar concentration from 1% to 3%. The contact angle increases with increasing agar con-
Figure 2-2: Dependence of contact angle on adhesion and agar concentration. (a) Side view of colonies from three strains on substrate of different agar concentrations. Increasing cell adhesion is horizontally across and increasing agar concentration is vertically down. (b) Plot of contact angle as a function of agar concentration for sfl1Δ (square), WT (left triangle), and Flo11Δ (right triangle). The scale bar denotes 100 μm.
centration. The mechanism through which the yeast colony adheres to the agar is unknown. If the yeast cells adhered to the agar directly, one would expect the contact angle to decrease with increasing agar concentration. This is because higher agar concentrations would give a higher concentration of binding sites between the yeast and agar, consequently changing $\gamma_{CS}$. On the other hand, increasing agar concentration also visibly dehydrates the yeast colony; this effect will also change the cell-cell surface energy $\gamma_{CC}$.

![Figure 2-3: Time lapse images. A time lapse images of a WT colony on 2.1% agar concentration. The series started from top left to bottom right. The scale bars on the top and bottom rows denote 100$\mu$m and 1mm, respectively.](image)

Although the molecular mechanisms controlling the contact angle are interesting, the most important conclusion for the present study is that the contact angle remains constant in time, and can be manipulated by changing either the agar concentration or the cell adhesion. A timelapse images of a WT colony is shown in figure 2-3.

**Colony Shape** When the colony is sufficiently small, the shape is always a spher-
Figure 2-4: Types of instabilities. Images of the different types of unstable morphologies from WT(b) and Sf1Δ(b,c) on 1.8%, 1.2%, and 2% agar concentrations, respectively. The three types of morphologies imaged are staircase(a), staircase with dimple(b), and dimple(c). The scale bar denotes 1mm.

Figure 2-5: Contour plot of timelapse images of a WT colony on 2.1% agar concentration. Two hours elapse between each two contours starting from the inner most curve.
After the instability, the resulting morphologies include staircase, staircase with centered dimple, and spherical cap with dimple (2-4). A contour plot of timelapse images of a WT colony (2-5) demonstrates the transition of the colony from spherical to nonspherical. Extensive observations indicate that the character of the instability is determined entirely by the contact angle $\theta$. For instance, a superadhesive Sfi1$\Delta$ colony on 1.5% agar concentration has a similar contact angle to a wild-type colony on 2.1% agar; although the surface tension $\gamma_{CC}$ of Sfi1$\Delta$ is higher than the wild-type, the Young's law equation (2.1) implies that this is compensated by the higher agar concentration. Despite the differing mechanisms leading to the contact angle, both types of colonies come to a staircase morphology. This suggests that morphology is controlled completely by the adhesion level and agar concentration, which together determine the contact angle of the colony.

This observation suggests that the critical volume where a non-spherical shape occurs can only depend on the contact angle, $\theta$, with no explicit dependence on the type of cells or the agar concentration. To test this hypothesis we measured a “phase diagram” (2-6) of colony morphologies as a function of colony volume and contact angle. The transition to non-spherical shapes indeed occurs above a contact angle dependent critical volume. In Figure 2-6, for high contact angles and low colony volumes the shapes are spherical; for low contact angles and high colony volume the shapes are non-spherical. The non-spherical regime is divided into three sub-regimes based on the contact angle: at low angles ($\theta < 40^0$), the shapes are staircases; at mid angles they are staircases with dimple; at highest angles ($\theta > 70^0$) they are dimples.

The experimental phase diagram is obtained with the WT and Flo11$\Delta$ strains on four different agar concentrations ranging from 1.8% to 3% and Sfi1$\Delta$ strain on 1% to 1.5%. For each agar concentration, we make eight identical plates. Images are taken from a different plate every 2 hours. At the end of the experiment we have about 150 images per agar concentration. The detected edge $y_{expt}(x_i), \{i = 1..N\}$ is analyzed to obtain contact angles, radii, and areas of the colonies. The edges are then fit to a
Figure 2-6: Theoretical (solid line) and experimental (points) phase diagram. The bifurcation curve demonstrates when the transition from spherical to non-spherical shape occurs. This line arises from solving the equation of the perturbed solution from the spherical cap solution with the ratio of the surface tension, $\gamma$ to the shear modulus $G$ equals to 0.1. It denotes the stability boundary. Above the theoretical curve is the spherical regime and below is the non-spherical regime. Experimental data come from WT colonies (left triangle) and flo11Δ (right triangle) on 1.8, 2.1, 2.4, 2.7, and 3 percent agar concentrations, as well as Sfl1Δ (square) on 1, 1.2, and 1.5 percent agar.

circular cap $y^{fut}(x)$ using a least squares method. We then calculate $\chi^2$, defined as

$$\chi^2 = \frac{1}{N} \sum_{i=1}^{N} \frac{(y^{fut}(x_i) - y^{exp}(x_i))^2}{\sigma^2},$$

where $\sigma$ is the measurement error per data point. When the colony transitions from a spherical to a nonspherical shape, $\chi^2$ rapidly increases. We are interested in detecting the early stage of the instability. We define the instability to occur when $\chi^2$ is in the range between 0.1 and 0.3. Applying this threshold to each set of conditions then yields the phase diagram (2-6).
The fact that the spherical colony shape destabilizes above a critical volume implies that there must be a force other than surface tension affecting the colony shape. The obvious candidates for this additional force are gravity, gradients in adhesivity caused by nonuniform nutrient consumption or waste production in the colony, and elastic stresses. Although gravitational forces should play a role when the colony is sufficiently large, we ruled out gravity by performing experiments on colonies grown while inverted. The colony becomes unstable at exactly the same volume independent of its orientation relative to gravity. We tested for the importance of nutrient consumption or waste production by carrying out experiments where the glucose level in the substrate is varied. Since the expression of adhesive protein is directly controlled by the level of glucose [12], varying the glucose level simulates the effect of nutrient consumption. The experiments showed that the instability occurs at the same critical volume for different levels of glucose, ruling out the possibility of nutrient depletion on causing the instability.

The only remaining candidate is the possibility of developing elastic stresses in the colony. Elastic stresses might be generated by cell growth; however, the very slow cell division timescale (typically a couple of hours) makes this unlikely. Assuming the colony material is similar to particulate gel, we can compare the growth rate to the stress relaxation rate. Yeast cells divide on average every 90 minutes; hence, the growth rate is 1/5400 sec. The shear modulus of a particulate gel of 0.5 volume fraction is approximately 3x10^3 dyn/cm^2 and the maximum dynamical viscosity is about 6^2 (dyn)(sec)/cm^2 [13]; hence, the stress relaxation rate is approximately 6.5x10^5 which is much larger than the growth rate. Therefore, the elastic stresses induce by cell growth is negligible. Elastic stresses might also arise due to a direct instability of the spherical cap, in which the elastic energy to support a non-spherical shape is less costly than the surface energy for the shape to remain spherical. To explore this possibility, we developed a mathematical model of an elastic droplet on a solid surface. The model demonstrates that the spherical cap solution is unstable at a contact angle dependent critical volume. Both the phase diagram for this transition and the resulting non-spherical shapes agree with the experiments.
2.4 Mathematical Model

To assess the possibility of an elastic instability of the yeast colony, we study the stability of a spherical cap with fixed contact angle to non-spherical perturbations, assuming that the total energy is the sum of surface and elastic energies. The strategy of our stability analysis is as follows: First, we assume that the shape of the colony with fixed volume and fixed contact angle is $h(x)$, not necessarily a spherical cap. Here $h(x)$ denotes the thickness of the colony a horizontal distance $x$ from its center. We then compute the elastic stresses that are necessary for this shape to be in equilibrium, and finally the total energy, summing surface and elastic energies. Minimizing this energy subject to constant volume and constant contact angle constraints gives the preferred shape of the colony.

2.4.1 Elastic Lubrication Theory

We begin by calculating the elastic strain that must exist in the colony for a non-spherical shape to remain in equilibrium. We consider a two dimensional colony with height $z = h(x)$. The strain field in the colony is $\nabla u(x, z)$ where $u(x, z)$ is the displacement. For small deviations from a spherical cap (for which there are no elastic strains) we assume the displacement field is measured relative to the spherical cap with identical volume. The yeast colony is incompressible ($\nabla \cdot u = 0$), owing to the water in the yeast droplet. Displacements in the colony then follows from the equilibrium equations of an elastic droplet,

\[
G \nabla^2 u - \nabla p = 0 \tag{2.2}
\]

\[
\nabla \cdot u = 0 \tag{2.3}
\]

where $G$ is the shear modulus of the material [16]. We remark that the magnitude of $G$ is not the same as the elastic modulus of a single yeast cell [14], since the elastic deformations of a yeast colony result in deformation of the network of cells in the colony, instead of the individual cells themselves [13]. The bulk elasticity $G$
is therefore much smaller than that of the cells themselves. A typical value of $G$ for particulate gel is $\sim 3 \times 10^3$ dyn/cm$^2$ for a volume fraction of 0.5 [13].

To compute the strain predicted by equations (2.2.2.3), we assume that the characteristic length scale of the colony in the horizontal (x) direction, $L$, is much larger than that in the vertical (z) direction, h. Such a "lubrication approximation" is common in analyzing thin film flows in fluid mechanics[15]. Denoting the components of the displacements $u$ in the x and z directions as $u_x$ and $u_z$, respectively, the equilibrium equations are

$$G\nabla^2 u \approx G \frac{\partial^2 u}{\partial z^2} = \nabla p$$

where we have used the fact that the horizontal scale is much larger than the vertical scale to approximate $\partial^2_x \ll \partial^2_z$. Similarly, the incompressibility condition $\partial_x u_x + \partial_z u_z = 0$ implies $u_x = -z \frac{\partial u_z}{\partial x} + \ldots$, so that $[u_z] \sim \frac{h}{L}[u_x]$. Hence when $h/L \ll 1$ we have $u_z \ll u_x$, and vertical displacements are unimportant. Similarly, equation (2.4) implies that $\partial_x p \ll \partial_z p$, so that we can assume the pressure primarily depends on the horizontal coordinate $p = p(x)$.

With these simplifications the equilibrium equations reduce to a single equation for $u_x$. Henceforth we drop the subscript x and denote the elastic displacement by u.

The boundary conditions are that the displacement vanishes on the agar substrate $u(z = 0) = 0$, and the shear stress at the yeast-air interface vanishes $\partial_z u(z = h) = 0$; finally, the pressure at the yeast-air interface is given by the Gibb's condition[17] $p(z = h) = -\gamma h''$. This last condition enforces the balance of the pressure at the interface with the surface tension force.

Applying the boundary conditions and solving equation (2.4) give the displacement in terms of $h(x)$,

$$u = \frac{\gamma h''}{G} \left[ \frac{z^2}{2} - zh \right].$$

A straightforward calculation then gives the total energy of a yeast colony with arbitrary shape $h(x)$,

$$E[h] = \frac{2\gamma}{3G} \int h''^2 h^3 dx + \gamma \int \sqrt{1 + h'^2} dx + p_0 \int h dx,$$

(2.6)
where the first term is the elastic energy, the second term the surface energy, and \( p_0 \) is a Lagrange multiplier ("pressure") that enforces the constant volume constraint. Note that if the shape is exactly a spherical cap (so that \( h(x) - h_0(x) \) has constant curvature), the elastic energy vanishes identically, so the spherical cap solution is a stationary solution to equation (2.6).

### 2.4.2 Instability of Circle Cap

Our goal now is to demonstrate that there are colony shapes with a fixed volume \( v_0 \) and equilibrium contact angle \( \theta \) which can lower their energy by deviating from a spherical cap. We first give a qualitative argument exposing how this instability can arise, and then proceed with a detailed calculation.

**Scaling Argument**

Consider a colony with volume \( v_0 \) and contact angle \( \theta \). If \( h \) is the characteristic thickness of the colony and \( R \) is its radius, then \( v_0 \sim hR \sim R^2 \theta \). From equation (2.6), the elastic energy of such a colony is of order \( \frac{\gamma^2}{G} \left( \frac{h}{R} \right)^2 h^3 R \sim \frac{\gamma^2}{G} \theta^5 \) and the surface energy is of order \( \gamma \left( \frac{h}{R} \right)^2 R \sim \gamma \theta^2 R \). At large enough radius, the surface energy contribution dominates the elastic energy, and thus the colony will deform. These two energies are the same order of magnitude when \( \theta^* \sim (G/\gamma R_*)^{1/3} \) or \( \theta^* \sim ((G/\gamma)^2 v_0^*)^{1/7} \), where \( \gamma/G \) is the characteristic length scale representing the competition between surface tension and elasticity. For a typical yeast colony, \( \gamma \sim 10 \text{ dyn/cm} \) [8] and \( G \sim 3 \times 10^3 \) dyn/cm\(^2 \) [13] so the characteristic scale of the instability is \( 10^{-2} \) cm. For volumes \( v_0 > v_0^* \) an instability to a non-spherical solution will occur.

**Quantitative Argument**

The scaling argument can be made quantitative by studying the first variation of equation (2.6). Assuming that \( h' \ll 1 \), we have

\[
\frac{2\gamma^2}{3G} [- (h'' h^3)''] + 3h''^2 h^2] - \gamma h'' - p_0. \tag{2.7}
\]
We are interested in solutions to equation (2.7) that are close to a spherical cap. Denote \( h_0(x) = \frac{2x}{3R}(1 - x^2/R^2) \) as a spherical cap with radius \( R \) and volume \( v_0 \). The radius is related to the contact angle through \( tan(\theta) = \frac{4v_0}{3R^2} \), and the pressure enforcing the volume constraint is then \( p_0 = \frac{4\gamma}{3R} \). Taking \( h = h_0 + c\rho \) and expanding equation (2.7) to leading order in \( \rho \) and integrating twice we obtain,

\[
(h_0^3 \rho''')' + \alpha \rho = 0. \tag{2.8}
\]

where \( \alpha = 3G/2\gamma \). A non-spherical solution exists if there exist nonzero solutions to equation (2.8), satisfying the boundary conditions. The boundary conditions are that the solution is symmetric around the origin \( \rho'(0) = \rho'''(0) = 0 \); at the radius \( R \) the profile vanishes \( \rho(x = R) = 0 \) and the slope obeys the contact angle condition \( \frac{4v_0}{3R^2} + \rho'(x = R) = -tan\theta \). (For the spherical cap solution, \( \rho = 0 \), so the radius satisfies \( R = \sqrt{4v_0/(3tan(\theta))} \).) Finally, since we are considering perturbations to the shape at constant volume, if we fix the volume of the solution to be \( v_0 \), then the volume associated with \( \rho \) must vanish (\( \int_{x=0}^{R} \rho dx = 0 \)). The boundary conditions correspond to five conditions on the solution; equation (2.8) is fourth order, and in addition we have the unknown critical volume \( v_0^* \). Hence these conditions are sufficient to uniquely specify the instability.

The most convenient way to find additional solutions is to rescale the horizontal coordinate \( y = x/R \), and introduce \( \nu' = \rho \). The volume constraint on \( \rho \) then implies that \( \nu(y = 0) = \nu(y = 1) = 0 \). The equation for \( \nu \) is

\[
[(1 - y^2)^3\nu''''']' + \Gamma \nu' = 0 \tag{2.9}
\]

with boundary conditions \( \nu''(0) = \nu'''(0) = \nu'(0) = \nu(1) = \nu'(1) = 0 \). Now we can view \( \Gamma = \alpha 2R^4/3v_0 \) as an eigenvalue. We numerically computed the smallest eigenvalue for which nonzero solutions to this equation exist: \( \Gamma = \Gamma^* = 65.12 \). Hence, we have an explicit formula for the bifurcation curve \( tan(\theta^*) = 0.72 \ast ((\gamma/G)^2 v_0^*)^{1/7} \). Notice that \( \gamma/G \) is a characteristic length scale. Normalizing the volume by letting
\[ V = \frac{v_0^2}{(\gamma/G)^2} \] gives
\[ \tan \theta^* = 0.72V^{1/7} \quad (2.10) \]

For volume \( v_0 > V \) than those given in equation (2.10) the spherical cap solution is unstable. The solid line in Figure 2-6 shows the theoretical bifurcation curve. In this comparison we have assumed that \( \gamma = 73 \text{dyn/cm} \) (the surface tension of water) and \( G = 5 \times 10^3 \text{dyn/cm} \), as described above. The theoretical curve quantitatively captures the trends of the experiments.

![Graph](image)

Figure 2-7: Theoretical solution and corresponding energy. Predicted non-spherical shape solution vs. spherical cap solution for the same sample volume \( v_0 = 0.5 \), \( \theta = 27.4^\circ \) and \( dv_0 = v_0/10 \). Inset figure plots total energy as a function of \( dv_0 \), demonstrating that the non-spherical shape has lower energy.

Finally, we note that the shape of the colony close to the bifurcation point also
follows from this analysis. The shape of the colony is \( h(x) = h_0(x) + c\rho(x) \). A weakly nonlinear analysis around the bifurcation point demonstrates that if the volume of the colony increases from \( v_0 \to v_0 + \delta v \), the solution is \( c = \frac{3\delta v}{\rho'} \). To leading order in \( \delta v \), the radius of the colony is constant.

Figure 2-7 shows the two possible configurations for the colony near the bifurcation point, the spherical solution, and the nonspherical solution. The inset shows the energy of both solutions as a function of distance from the bifurcation point. As advertised, the nonspherical cap shape is energetically favorable to the spherical cap.

### 2.5 Conclusion

We have demonstrated that the shape of a growing yeast colony is governed entirely by surface energy minimization and surface adhesion when the colony is sufficiently small, but that above a critical volume elastic stresses play an equally important role in determining the colony shape. In the elastic regime, the colony shape is contact angle dependent. The role of elastic stresses in determining the contact angle dependent morphology is illustrated through a mathematical model, which demonstrates that above a critical (contact angle dependent) volume, the spherical-cap solution is unstable and elastic stresses are important. The contact angle dependence of the critical volume is quantitatively consistent with the experiments. Our mathematical analysis is limited to the neighborhood of the instability threshold. Beyond the threshold there are a zoo of contact angle dependent colony shapes (e.g. the “staircase” morphology occurs when \( \theta < 40^\circ \), and when \( \theta > 70^\circ \) the colony has a single dimple in the center).

The demonstration of elastic instability in this simple model of tissue growth points to the possibility of elastic effects in more complex situations. For example, the instability we have identified is the precursor to the complex morphologies discovered by Reynolds and Fink [12]. The discovered instability is analogous to the stress-driven morphological instability of prestressed solids [18, 19]. The surface morphology changes due to the development of stress within the solid. This planar instability is
found in the case of grown thin film on a substrate where the lattice constant of the film and of the substrate are different [20]. Another example is the elliptical inclusion in an isotropic matrix. The spherical inclusion lowers the strain energy by transforming into an elliptical one [21].

The precise role of elastic stresses in determining tissue morphologies under more general conditions remains to be seen. The present experience with yeast droplets demonstrates that at least two different materials with different adhesive energies are needed for an elastic instability. The general requirements for elastic stresses to play a role in determining tissue morphology remain to be worked out. It seems possible that the fundamental notion of selective adhesion as a driving force for tissue development needs to be supplemented with elastic effects. If so, there is the fascinating possibility of elastic stresses being regulated during development through, for example, cells modifying their individual stiffness.

There are problems remain to be worked out. We can extend the current model to calculate the colony nonlinear shapes and to study the subregimes of the nonphysical regime. We need to resolve the discrepancy between the theoretical instability boundary and the experimental one. The discrepancy might cause by the negligence of elastic stress exists in agar substrate.
Chapter 3

Parabolic Singularity in Thin Elastic Sheets

3.1 Introduction

There are many applications where deformation of a thin plate is important, ranging from self-organization and the formation of patterns in plants [34] to determination of forces involved in the motility of cells and micro-organisms [35]. For most boundary conditions, as the plate thickness, $h$, tends to zero there are no smooth solutions. Therefore, it is important to understand how stress singularities arrange themselves when stress is applied to a constrained plate.

The large deformation of elastic plates is determined by minimization of elastic energy. For a thin plate, the total free energy consists of two parts, bending energy and stretching energy. These energies are defined respectively as follows,

\[
E_{\text{bending}} = \int \Psi_1 df \tag{3.1}
\]
\[
\Psi_1 = \frac{D}{2} \left( (w_{zz} + w_{yy})^2 + 2(1 - \sigma)(w_{xy}^2 - w_{xx}w_{yy}) \right)
\]
\[
E_{\text{stretching}} = \int \Psi_2 df \tag{3.2}
\]
\[
\Psi_2 = \frac{1}{2} u_{\alpha\beta} \sigma_{\alpha\beta}
\]

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where \( w(x, y) \) is the out of plane deflection, \( D = \frac{Eh^3}{12(1-\sigma^2)} \) is the plate rigidity constant, \( E \) is the Young’s modulus, \( h \) is the plate thickness, and \( \sigma \) is the Poisson ratio. \( \Psi_2 \) is the stretching energy per unit volume and is defined in terms of \( \sigma_\alpha \beta \) and \( \sigma_\alpha \beta \) which are the strain and stress tensors, respectively [16].

Foppl and von Karman were the first to derived a set of coupled equations that characterize the deflection of a thin plate and the associate in-plane stress, by minimizing this energy. The Foppl von Karman plate equations [16, 23] are,

\[
\begin{align*}
D \nabla^4 w(x, y) &= [w, \phi] \\
\nabla^4 \phi(x, y) &= -\frac{E}{2} [w, w]
\end{align*}
\] (3.3)

where \( \phi \) is the in-plane stress function or Airy stress functions defined in terms of normal \( \sigma_x \) and \( \sigma_y \) and shear \( \sigma_{xy} \) stresses,

\[
\sigma_x = \phi_{yy}, \sigma_y = \phi_{xx}, \sigma_{xy} = -\phi_{xy}
\] (3.4)

and the differential operator \([a, b]\) is defined as,

\[
[a, b] = w_{xx} \phi_{yy} + w_{yy} \phi_{xx} - 2w_{xy} \phi_{xy}
\] (3.5)

Notice that \( \frac{1}{2}[w, w] \) in equation (3.3) is the gaussian curvature of the surface of the plate. Therefore, if the gaussian curvature term is equal to zero then no in-plane stress is necessary. The necessity of singular structure in elastic plates arise from the fact that surfaces of constant gaussian curvature do not satisfy general boundary conditions. Typically, in the limit where the plate thickness tends to zero, the stress becomes localized in space, creating stress singularities. In the context of crumpled paper and the deformation of shell, there are three known types of singularities:

- Circle [24]
- Line (ridge) [26]
- Point (Developable cones) [30]
The complex interaction between bending and stretching of a stress singularity in each of these cases has been well studied and the associated energy determined.

Motivated by controlled experiments on the crumpling of thin elastic plates, we introduce and study the parabolic singularity. Our experiments show a cascade of parabolic singularities when deforming a mylar sheet by a compressive load along its length with the boundary conditions of clamped edges along the sides length of the plate, free edge at one end, and the remaining edge constrains under foam pieces which acts like an elastic foundation. To our knowledge, this is the first observation of parabolic singularity in the literature. The circle singularity is studied by Pogorelov in the case of deforming a spherical shell. He derives the boundary layer energy for this case and generalizes the theory for any convex surfaces [25]. The line singularity is first studied by Witten et.al. They derive the scaling law for for the radius of curvature of a ridge as a function of its length and the plate thickness. A boundary layer analysis of the ridge singularity is developed by Lobkovsky [28]. Pomeau focused on the buckling of thin plates in the strongly nonlinear limit and constructed a network of ridges using the results from Witten and Lobkovsky [32]. The point singularity is investigated by Mahadevan et. al. They noted that for the plate thicknesses that are normally of interest, developable cones (d-cone) and not ridges actually possessed the lowest energy [30, 31]. Boudaoud studies deformation of an elastic plate using an elastic model based on point singularities to predict the subsequent deformations [36]. Parabolic singularities have higher energy than line and point singularities. However, energy is not the only factor determining the type of singularity produced by the applied stress; geometrical constraints play an important role.

In order to understand the cascade of parabola singularities, we need to develop a theoretical and experimental understanding of a single parabola; this task is the goal of the present study. Experimentally a single parabola can be generated by deforming one end of a semicylinder by a normal point load. Theoretically, we compute the solution by intersecting surfaces of zero gaussian curvature with each other; away from the intersecting curve this solves because they are solutions to the von Karman equations with no stress. In the asymptotic limit of small plate thickness, it is possible
to construct a boundary solution between the different regimes. The total energy of
the solution is then given as sum of the energies of the individual solutions plus the
energy of the boundary. A parabola singularity can be generated by intersecting a
cone and a semi-cylinder, both have zero gaussian curvature.

We present the details of the experiments in section II. The detailed calculation
of the energy at the boundary layer of a parabola singularity is presented in section
III. We elaborate on the minimization of the total energy to get the relationships of
the length, the width, and the applied force to the maximum displacement of the
semicylinder edge. The chapter is ended with the discussion section.

3.2 Preliminary Experiments

3.2.1 Cascade

The following experiment demonstrates that parabolic singularities occur under cer-
tain boundary conditions. Two long parallel clamps are attached to a stage with a
knob. This setup allows the two clamps to be pushed together, which is equivalent to
applying a compressive load along the clamped edges. A thin mylar sheet is placed
with its longer edges held in place by the clamps, leaving the remaining edges free.
At one of the free edges, two identical pieces of foam with lengths equal to the width
of the sheet sandwich part of the sheet. A wooden frame with height equal to that
of the two foams secures the foams in place when the compressive load is applied
(fig.3-1).

Once the foam is in place, the plate is compressed by turning the mechanical
knob about 1/4 of a revolution each time. This cranking is maintained until the first
curved singularity appears, at which time the compressed distance and the length of
the singularity are measured. The radius of curvature of the singularity is observed to
be independent of the applied load. Its length obeys a power law of the compressed
distance. As the applied load increases, the parabolic region travels towards the
free end. Eventually the initial singularity escapes beyond the free edge and a new
Figure 3-1: A picture of the experimental apparatus demonstrates a formation of a cascade of parabolic singularities on a mylar sheet of dimensions 30.5cm x 45.7cm. The plate thickness is 0.127 mm.

A parabolic region appears from underneath the foams. This second parabola never escape from the plate. In respond to further compression, more parabolic regions appear after the the second parabola and the plate organizes itself into a cascade of parabolic like curved singularities. The clamped edges imposes zero deflections. Three generations of cascade could be seen on a plate of dimensions 30.5cm x 45.7cm and the plate thickness of 0.127 mm (fig.3-1). The wavelength of the deformation under the foam is set by the foam stiffness, k, and the rigidity constant,D, of the mylar plate. The wavelength is proportional to $(D/k)^{1/4}$.

The cascade shows high concentration of stress at the boundary layer of the singularities. The energy calculation for the whole cascade is complicated. The essential need is to understand the energy associated with an individual parabolic singularity, to which we now turn.
3.2.2 Single Parabolic Singularity

Figure 3-2: Picture of the experimental apparatus and a single parabolic singularity (courtesy of A. Kudrolli).

The accompanying figure 3-2 shows an experiment in which a normal point load, $F$, is applied to one of the free edges of a semicylinder of radius $R$. The semicylinder is formed by clamping a rectangular piece of mylar along its length, leaving the remaining edges free, then compress the clamped edges until the desired $R$ is reached. A point force, $F$, is applied at the middle of one end of the cylinder causes it to deform inward. The boundary curve of the deformed region has the shape of a parabola. Increase the applied force cause the width of the parabola becomes wider but not its length.
3.3 Mathematical Model

To analyze the deformation of a curved singularity we intersect a cone and a cylinder and derive the expression of the associated energies. The advantage of intersecting these two surfaces is that they both have zero gaussian curvature. As mentioned before, surfaces of zero gaussian curvature have no stress and thus solve of the large deformation elastic equations. The intersection of two solutions also has no strain except at the boundary. Therefore, in addition to the bending energy of the intersecting surfaces, the bending and stretching at the boundary layer also contributes to the total energy. The cone and cylinder energies are proportional to the surface areas of the intersecting regions. To compute this, we first use geometric constraints to derive the expression for the intersecting surface area between the cone and the cylinder. We then tackle the complex interaction between stretching and bending to derive the energy of the boundary layer any curved boundary, following the classic work of Pogorelov [25]. We minimize to the total energy to obtain the functional forms of the apply load $F$, the length and the width of a curved singularity as a function of the depth of the deformation $\epsilon$.

3.3.1 Preliminaries: Geometrical Considerations

Figure 3-3 illustrates the geometry of a cone intersecting a cylinder. We assume that the axis of the cone is parallel to the axis of the cylinder (an assumption that can be easily relaxed). The cone has three parameters: the location of the apex, $(y_0, z_0)$, and the opening angle $\theta$. The goal of this section is to understand the relationships between these parameters when one end of the cylinder is displace by a distance $\epsilon$ below its initial position.

The equation of the cone according to the prescribed coordinate system is defined as,

$$\tan^2(\theta)(z - z_0)^2 = x^2 + (y - y_0)^2$$  \hspace{1cm} (3.6)
Figure 3-3: A side view and a top view of a cone intersects a cylinder where $R$ is the radius of the semicylinder, $\theta$ is the opening angle of the cone, $(y_0, z_0)$ is the apex of the cone, and $\epsilon$ is the displacement of the cylinder at $z = 0$. The top view picture shows the intersecting curve and the deformed region of width $w$ and length $l$.

and the equation of the upper half semicylinder is,

$$y = \sqrt{R^2 - x^2}$$  \hspace{1cm} (3.7)

We are interested in the limit where the area of intersection between the cylinder and the cone is small. First, the cone is placed so that the bottom half of the cone touches the cylinder at a point then $y_0 = z_0\tan(\theta) + R$. Second, leaving the parameters of the cone fixed according to equation (3.6), then we can make the cylinder intersect the cone by increasing the radius of the cylinder by a small amount $\epsilon$. Physically, this procedure is equivalent to applying a point load to the end of the cylinder, since any deformation of the cylinder from its initial shape requires an external force.

Taking $R \rightarrow R + \epsilon$ in equation (3.7) we calculate the intersecting curve to linear order in $\epsilon$,

$$z^*(x) = \cot^2\theta \left[ \frac{(R + z_0\tan\theta)x^2}{2z_0R} - \epsilon\tan\theta \right] = A - Bx^2$$  \hspace{1cm} (3.8)
where

\[ B = \frac{R + z_0 \tan \theta}{2z_0 R \tan^2 \theta} \quad (3.9) \]
\[ A = \frac{\epsilon}{\tan \theta} \quad (3.10) \]

The equation of the intersecting curve derived above is a parabola with length \( l = A \) and width \( w = 2 \sqrt{A/B} \).

The intersecting surface area between the cone and the cylinder must be equal by area conservation. The area of the surface of the cylinder bounded by the intersecting curve is,

\[ A_{cyl}(\theta, z_0, \epsilon) = 2 \int_0^{z_0} \int_0^{z_0'} \sqrt{1 + \frac{x^2}{R^2 - x^2}} \, dx \, dz \quad (3.11) \]

The area of the cone within the intersecting region is,

\[ A_{cone}(\theta, z_0, \epsilon) = 2 \int_0^{z_0} \int_0^{z_0'} \frac{\tan \theta \sec \theta(z - z_0)}{\sqrt{\tan(\theta)^2(z - z_0)^2 - x^2}} \, dx \, dz \quad (3.12) \]

The area constraint implies \( A_{cyl} \) is equal to \( A_{cone} \). We use condition to determine \( \tan \theta \) in terms of \( z_0 \). Then \( z_0 \) is the only parameter left in the problem which will be determined by energy minimization. The integral for \( A_{cone} \) must be evaluated numerically. Before proceeding to evaluate the areas, we nondimensionalize \( \epsilon \) and \( z_0 \) by the radius of the cylinder \( R \) and denote the dimensionless quantities \( \tau \) and \( z_0^* \). For a fixed \( \tau \) and a given \( z_0^* \), there is a single value of \( \theta \) for which the areas are equal. The functional form of \( \tan \theta \) as a function of \( z_0^* \) is determined by stepping through different values of \( z_0 \). Figure 3-4 shows the numerical result of \( \tan \theta \) as a function of the parameter \( z_0 \),

\[ \tan \theta = 1.04z_0^{-1} \quad (3.13) \]

This relationship is independent of \( \tau \) and the plate thickness. This result reduces the problem to determine one parameter \( z_0^* \). This analysis also implies an upper bound for \( \epsilon \). From the equation (3.12) if \( \epsilon > z_0^* \tan \theta(1 + z_0^* \tan \theta)/2 \) then the area is complex. Using the result from equation (3.13), the upper bound for \( \epsilon \) is found to be 1.08.
Figure 3-4: Plot of \( \tan \theta \) as a function of \( \frac{z}{R} \) computed from equating \( A_{cyl} = A_{cone} \). The plot shows that \( \tan \theta = 1.04 \frac{z}{R}^{-1} \).

However, we expect our analysis to break down well before this.

### 3.3.2 The Energy

The previous section constructed a family of solutions in terms of \( \varepsilon \) and \( \mathcal{Z}_0 \), using the geometrical constraints. For a given displacement \( \varepsilon \), the length of the cone \( z_0 \) is determined by energy minimization. When the cylinder is displaced by a fixed distance \( \varepsilon \), we want to look for a \( z_0 \) that minimize the total energy of the system. In order to hold \( \varepsilon \) in a fixed place we need to specify an applied force \( F \) which we will also calculate. The total energy of the system is comprised of three parts: (1) the energy of the part of the cone that intersects the cylinder, \( U_{cone} \); (2) the energy of the (undeformed) cylinder, \( U_{cyl} \); and (3) the energy of the boundary, \( U_{bound} \), i.e. the outline of the parabola.
The Cone

The first part of the energy involves the energy of the cone. Since the cone tip does not intersect the cylinder, the energy contribution from the cone part consists of purely bending energy and can be calculated as follows,

\[
U_{cone} = 2D \int_{0}^{\phi_{max}} \int_{R_0}^{R_{cone}} \frac{1}{r^2} r dr d\theta = 2D \frac{\sqrt{A/B}}{z_0 \tan \theta} \ln \left( \frac{z_0}{z_0 - l} \right)
\]  

(3.14)

where \( \phi_{max} = \frac{\sqrt{A/B}}{z_0 \tan \theta} \).

The Cylinder

For the cylinder, there is no stretching, so the energy of the cylinder is proportional to the product of the area of the cylinder (3.11) and square of its curvature,

\[
U_{cyl} = D \frac{A_0 - A_{cyl}}{R^2}
\]

(3.15)

where \( A_0 \) is the total area of the plate.

The Boundary

Finally we must compute the energy of the boundary layer between the cone and the cylinder. We are going to derive the energy for any boundary layer with curved boundary. The conditions for this to work generally are as long as radius of curvature of curve is smaller than the radius of curvature of the ridge calculated by Witten et al., which scales like \( h^{1/3} X^{2/3} \), where \( h \) is the thickness of the sheet and \( X \) is the length of the ridge and larger than the plate thickness. The approach is to use the equation the von Karman equations. We use scaling and similarity solutions to reduce to the von Karman equations to a boundary value problem [28]. Then using these solutions we will write the total energy for the boundary layer. The Von Karman equations in polar coordinates are defined as follows,

\[
D \nabla^4 w(r, \theta) = [w, \phi]
\]
\[
\n\nabla^4 \phi(r, \theta) = \frac{-E}{2} [w, w] \tag{3.16}
\]

and the differential operator \([a, b]\) is defined as,

\[
[a, b] = a_{rr} \left( \frac{1}{r} b_r + \frac{1}{r^2} b_{r\theta} \right) + \left( \frac{1}{r} a_r + \frac{1}{r^2} a_{r\theta} \right) b_{rr} - 2 \left( \frac{1}{r} b_\theta \right)_r \left( \frac{1}{r} a_\theta \right)_r \tag{3.17}
\]

To proceed we define a local coordinates \((x(s), z(s))\) along the intersecting curve, hence every point on this curve has a corresponding local normal \(\hat{n}\) and tangent \(\hat{t}\). In normal direction, the surface changes rapidly. There are three different curvatures that need to be analyzed. These curvatures are \(w_{nn}, w_{tt}, \text{and, } \kappa(s) = \frac{1}{\rho(s)}\), the normal curvature, tangential curvature and curvature of the boundary itself, respectively. Our experiment indicates that \(w_{tt} \ll \frac{1}{\rho(s)} \ll w_{nn}\). The second inequality is intuitive since the width of the boundary layer is much smaller compared to that of the boundary curve and the height of the boundary layer is of order \(\epsilon\). Taking this into account and use the new local coordinates (3.16) reduces to,

\[
Dw_{mnn} \approx \frac{1}{\rho(s)} (w_{nn} \phi_n + w_n \phi_{nn})
\]

\[
\phi_{nmmn} \approx -\frac{E}{\rho(s)} w_{nn} w_n \tag{3.18}
\]

Let \(\delta\) be the width of the boundary layer which is to be determined later. Scaling the equations (3.16) using (3.18) with respect to \(\delta\) we obtain,

\[
\frac{Dw_{mnn}}{\delta^4} = \frac{1}{\rho} w_{nn} \frac{\phi_r}{\delta} + \frac{1}{\rho} w_n \frac{\phi_{nn}}{\delta^2}
\]

\[
\frac{\phi_{nmmn}}{\delta^4} = -\frac{E}{\rho} w_{nn} w_n \frac{w_n}{\delta^2} \tag{3.19}
\]

Since bending and stretching energies are comparable, by balancing these we can get a scaling for \(w\) and \(\phi, [\phi] \sim \frac{Eh^2}{\delta}, [w] \sim \frac{w}{\delta}\) hence reduces (3.19) to,

\[
w_{mnn} = \frac{\delta}{\rho} \frac{1}{Eh^2} (\phi_n w_{nn} + w_n \phi_{nn})
\]

\[
\phi_{nmmn} = \frac{\delta}{\rho} Ew_n w_{nn} \tag{3.20}
\]

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To find the solution to the reduced and rescaled equations above, we seek similarity solutions of the form,

\[ w = h \left( \frac{L}{\delta} \right)^p F(\xi) \]
\[ \phi = E h^2 \left( \frac{L}{\delta} \right)^{\sigma} G(\xi) \]  

(3.21)

where \( \xi = \frac{z}{L} \). Substitute the similarity solutions in (3.20) and by requiring the bending and stretching terms to be equal, induced \( p = q = 1 \). Then equations (3.20) become a system of nonlinear fourth order dimensionless differential equations.

\[ F''' = G' F'' + F' G'' \]
\[ G''' = F' F'' \]  

(3.22)

Define new functions \( S = F' \) and \( T = G' \) to reduce (3.22) to a system of third order differential equation as follow,

\[ S''' = S'T + ST' \]
\[ T''' = SS' \]  

(3.23)

In order to solve the system above we need six boundary conditions. Since the solutions is symmetric through the z-axis we can just solve for solution in the domain \([0, \infty)\).

Before analyzing the boundary conditions, we are going to introduce the notion of matched asymptotic expansions. In solving a boundary value problem (BVP) on an interval that broken into two overlapping subintervals, we first find an asymptotic approximation of the solution which only valid in each of the subintervals. Then the uniform asymptotic solution of the BVP is obtained by requiring the functional form of the asymptotic approximations must be the same in the overlap region. This is useful in the context of boundary layer analysis. In the case of a cone intersect a cylinder, the region where stress concentrates is called the boundary layer or inner
region and every where else is called the outer region. The solution exists in the inner region is called inner solution and vice versa. In the asymptotic limit, matched asymptotic expansion requires the outer limit of the inner solution must be equal to the inner limit of the outer solution.

We use this method of asymptotic expansion to determine the boundary layer thickness. Note that in the case of a cone intersecting a cylinder, the inner limit of the outer solution clearly corresponds to the limit of approaching the intersection of the cylinder and the cone. In general, the slope of the boundary layer must match the slope of the initial configuration $\zeta$. Hence, in the limit $\xi \to \infty$, $S(\xi) \to \frac{\xi}{R}$ since $S = F'$ this is equivalent to matching slope condition. Since the solution is symmetric across the y-axis, the slope must equal to zero when $\xi = 0$ or $S'(0) = 0$. The last boundary condition for $S$ is to consider the curvature as $\xi \to \infty$ is equal to zero. The boundary conditions for stress function $G$ are stress equals to zero for $\xi = 0$ and $\infty$ and its derivative equals to 0 at $\infty$. In summary, here are the boundary conditions for $S$ and $T$ are

$$S(0) = 0, S(\infty) = \frac{\rho}{R}, S'(\infty) = 0$$

**(3.24)**

$$T(\infty) = 0, T'(0) = 0, T'(\infty) = 0$$

**(3.25)**

We solve the above system with the associated boundary conditions numerically using MATLAB. Figure 3-5 shows the converged solutions for the curvature and the stress.

The energy of the boundary has two parts, the bending and stretching energy. Neglecting the smaller terms to obtain,

$$U_{\text{bend}} = Eh^2 \int_{-\infty}^{\infty} (\nabla^2 w)^2 \, dA = \int_{-\infty}^{\infty} \frac{2\pi r \, dr}{\delta^4} \delta w''(\xi)$$

$$U_{\text{stretch}} = \frac{\pi}{E} \int_{-\infty}^{\infty} \frac{\phi''(\xi)}{\delta^4} \, r \, dr$$

**(3.26)**

Using the similarity solutions equation (3.21), $r = \rho + \delta \xi$ and $dr = \delta d\xi$, since $\rho \gg \delta$, 

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Figure 3-5: A plot the numerical solutions of the similarity equations (3.23). It shows the curvature (top) of the boundary layer and the corresponding stress (bottom).

The energies simplified to,

\[ U_{\text{bend}} = \frac{2\pi E h^2}{\delta^3} \left( \frac{h \rho}{\delta} \right)^2 \int_{-\infty}^{\infty} \rho F''(\xi) d\xi \]
\[ U_{\text{stret}} = \frac{\pi E h^2}{\delta^3} \left( \frac{h \rho}{\delta} \right)^2 \int_{-\infty}^{\infty} \rho G''(\xi) d\xi \]

(3.27)

Hence, the total energy of the boundary layer is equal to,

\[ U_{\text{bound}} = \frac{E h^4 \rho^3}{\delta^5} \left[ 2\pi \int_{-\infty}^{\infty} F'' d\xi + \pi \int_{-\infty}^{\infty} G'' d\xi \right] \]

(3.28)

We calculate the prefactor (the terms inside the bracket) of the boundary energy and found it to be 1.17 \(^1\).

The energy of the boundary layer derived above holds for any two intersecting surfaces of zero gaussian curvature. In the case of a cone intersecting a cylinder,

\(^1\)Similar to the 1.2 prefactor calculated by Pogorelov.
the inner limit of the outer solution is the average of the slopes of the cone and the
cylinder denoted by \( \zeta \). The outer limit of the inner solution is \( w \rightarrow \frac{h_0}{\delta} x \). By matched
asymptotic expansion, the width of the boundary layer is,

\[
\delta = \sqrt{\frac{h_0}{\zeta}} \tag{3.29}
\]

From equation 3.28 the total energy of the boundary layer is,

\[
U_{\text{bound}} = 1.17 D \int \frac{z^{5/2}}{\sqrt{\rho h}} ds \tag{3.30}
\]

Recall the equation (3.8) for the boundary curve is \( z = A - Bx^2 \). Thus, the curvature
is \( z'' = \frac{1}{\rho} = -2B \). \( \zeta \) is the average slope of the semicylinder and the cone which is
equal to,

\[
\zeta = -\left( \frac{x}{R} + \frac{x}{R_c} \right) \tag{3.31}
\]

The arclength element \( ds \) in terms of \( dx \) is \( ds = \sqrt{1 + 4Bx^2} dx \). Replacing \( \zeta, \rho, \) and
d\( ds \) as above into equation (3.30) and integrate the resulting equation with respect to
\( x \) to obtain the actual total boundary energy in terms of the parameters as follow,

\[
U_{\text{bound}} = 1.17 \frac{D}{2^{5/2}h^{1/2}} \left( \frac{1}{R} + \frac{1}{R_c} \right)^{5/2} B^{-5/4} \left( \frac{2}{7} A^{7/4} + \frac{4}{11} BA^{11/4} \right) \tag{3.31}
\]

### 3.3.3 The total energy and the force

The total energy of the deformed plate is \( U_{\text{total}} = U_{\text{cone}} + U_{\text{cyl}} + U_{\text{bound}} \). Minimization
of the total energy with respect to \( \bar{z}_0 \) gives the functional form in terms of \( \bar{\varepsilon} \). The
relationship between and \( \bar{\varepsilon} \) is obtained. For each \( \bar{\varepsilon} \), there is a \( \bar{z}_0 \) where the energy is
minimum. Stepping through a range of \( \bar{\varepsilon} \) to obtain the functional form of \( \bar{z}_0 \) as a
function of \( \bar{\varepsilon} \). Figure 3-6 shows that

\[
\bar{z}_0 \sim \bar{\varepsilon}^{-1} \tag{3.32}
\]

and its functional form as a function of \( \bar{\varepsilon} \) is plate thickness dependent. Figure 3-7
Figure 3-6: $z_0$ vs. $\bar{\epsilon}$. Plot of $z_0$ the length of the cone as a function of $\bar{\epsilon}$ the depth of the deformation of the plate, for different plate thicknesses where $z_0$ and $\bar{\epsilon}$ denote the length of the cone and deflection of the plate that normalized by the radius of the cylinder, respectively.

shows the numerically computed dimensions of the parabolic region as a function of $h$ and $\epsilon$. The length $l$ of the cone has a very weak epsilon dependence, and a stronger dependence on plate thickness. The width $d$ of the cone grows with increasing deformation; however it shows no dependence on the plate thickness. Figure 3-8 plots the total energy as a function of $\bar{\epsilon}$. The applied force, $F$, can be obtained by differentiating the total energy with respect to $\bar{\epsilon}$.

These numerical results give the characteristics of the parabolic singularity and demonstrate that the energy minimization problem that we have posed gives a reasonable solution that is qualitatively in accord with our experiments. More work must be done: in particular for the single parabola, we need to extract the asymptotic laws in the limit of vanishing plate thickness, and also test these results experimentally.
Figure 3-7: Plot of (a) parabola length $l$ and (b) parabola width $d$ as a function of $\bar{\epsilon}$ for different plate thicknesses where $\bar{\epsilon}$ is $\epsilon$ normalized by the radius of the cylinder.

### 3.4 Conclusion

We demonstrate that there are over constrained boundary conditions that cause the formation of higher energy singularities experimentally. Specifically, we observed a well organized parabolic singularities with different generations in a bifurcate fashion. We derive the energy of the boundary layer for any curved boundary by reducing the von Karman equations to a boundary valued problem using scaling and similar solutions. Our finding of the energy of the boundary layer recover the result of Pogorelov [25]. Our theoretical study of a single parabolic singularity utilize the
idea of intersecting surfaces of zero gaussian curvature. We show that a parabolic singularity can be generated by intersecting a cone and a cylinder. This study gives another example of this idea beside the case study by Pogorelov [24]. Furthermore, the preliminary functional relationship between the applied load, the length and the width of the deformed region to the depth of the deformation is derived from simple geometrical considerations and energy minimization. Our theoretical results gives an initial understanding and development of the parabolic singularity. These results need to be studied further to explain deformation in the asymptotic limit where the plate thickness h tends to zero. The quantitative comparison between experiment and theory is to be carry out in the near future. Once the results are validated with an experiment we can extend them to analyze the cascade of parabolic singularities. Another approach to study the cascade is to solve the von Karman equations subject to the described boundary condition numerically.
Bibliography


