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Degree of Master of Fine Arts

"An Interactive Kiosk on Amphibian Limb Deformities"

by

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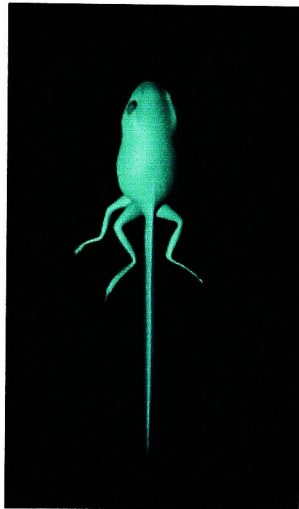
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PART ONE: THE SCIENCE

In the past three years, the alarming images of the recent incidences of deformed frogs in Minnesota have come to haunt the dreams of schoolchildren, political leaders, developmental biologists, and myself. Although limb deformities in amphibians have been recorded as far back as 1740, it appears that we are living in a time when the occurrence of this phenomenon is unprecedented. David Hoppe, professor of biology at the University of Minnesota, has said "I've observed literally thousands of Minnesota frogs over the last 20 years. Up until 1995 I has seen only 2 with macroscopic limb defects. In 1996, I saw over 200." (Schmidt, 324) What began in 1995 as field observations of some Minnesota schoolchildren has escalated into a sizable scientific, environmental, and political issue.

I have seen these frogs on television, in the newspapers, and in magazines. But as I looked at these images, I was compelled to wonder, not necessarily about what agents might cause this phenomenon, but rather, about how these limbs developed and came to be distorted. I wondered about the biological processes at work and how they had gone awry. Hence, the purpose of this thesis; to explain the processes of normal limb structure and development so as to understand, as much as possible, what has occurred to cause these frogs to develop their alarming and grotesques appendages.

NORMAL LIMB DEVELOPMENT

The duration of tadpole metamorphosis varies with species. All species, however, have in common their basic stages of development. This discussion is limited to the development of the limb, which occurs after the organism has existed for some time as a tadpole.

THE THREE GERM LAYERS

The body of the amphibian embryo, like that of other vertebrates, consists of three primary germ layers: ectoderm, endoderm, and mesoderm. The ectoderm is the outermost layer that will develop

into the epidermis and the nervous system. The endoderm is the innermost layer that will develop into the organs of the gastrointestinal tract. The mesoderm is the middle layer that will develop into the muscle, the skeletal system, and the circulatory system. During development, it is normal for some cells from these germ layers to separate themselves and migrate throughout the body as populations of single cells called mesenchyme (Wanek, e-mail). Mesenchyme cells are important in the development of many organs and structures, including the vertebrate limb.

THE LIMB BUD

Limb development begins with the proliferation and migration of mesenchyme cells from the somatic layer of the lateral plate mesoderm, to locations below the epidermis, in the areas of the prospective limbs (Oppenheimer and Lefevre, 230). The epidermal layer (ectoderm) above these mesoderm cells thickens, forming a structure called the *apical ectodermal ridge*. Hence, the limb bud consists of two tissues: a core of mesoderm with an ectoderm covering. While I will focus the discussion on the structure and development of the limb bud, I feel that a brief summary of the rest of limb development is necessary. Following the appearance of the limb bud, there is a rapid proliferation of mesoderm cells, which causes the bud to elongate. The distal end becomes flattened into a paddle shape. Bones and muscle develop as a result of mass cell movements. Finally, the details of the "hands" and "feet" develop as a result of programmed cell death (Hopper and Hart, 314).

The cells of the limb bud plus those cells in the surrounding area are referred to as the *limb field*. The limb field is an example of an *embryonic field*; other examples of which include the eyes, the ears, and the heart. An embryonic field is defined as "a group of cells whose position and fate are specified with respect to the same set of boundaries" (Gilbert, 609). Embryonic fields satisfy four specific principles. These principles are summarized as the following:

1. If the entire field is removed, the structure will cease to develop.

2. If the entire embryonic field is transplanted to a new location on the organism, the normal structure will develop in this new location.

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3. If part of the field is removed, the cells of the field will regulate and the normal structure will develop.

4. If tissue is added to the field, the cells of the field will regulate and the normal structure will develop (Oppenheimer and Lefevre, 230) (Wanek, e-mail).

The limb field is composed of the *limb disk* and an outer ring of cells. The limb disk contains the lateral plate mesoderm that will give rise to the limb itself, and an area of peripheral tissue that will form the peribrachial tissue and the shoulder girdle. If some of these cells are removed, a normal limb will develop, as the remaining cells compensate for the removed tissue. If all of this tissue is removed, the normal limb will still develop, from a ring of cells which surrounds the limb disk. If this outer ring of cells is also removed, however, the limb will not develop. The limb disk plus this outer ring of cells represents all of the tissue capable of forming a limb, the limb field (Gilbert, 609). The limb field represents the "whole prospective limb bud potential" (Hopper and Hart, 314). It appears that the cells within the limb bud have the ability to recognize both their position and their fate, and have the ability to change their positions and fates in response to disruption. This characteristic is called pluripotency. Pluripotent cells can develop into various parts of an organ. This capacity is limited by time, as it diminishes or ceases with the progression of development (Hopper and Hart, 315).

THE LIMB AXES

The next logical question is, how are the cells organized within the limb bud, so as to have this ability to regulate limb development? Pattern formation is a term often used when speaking about limb development. It refers to the way that "embryonic cells form ordered spatial arrangements of differentiated tissues" (Gilbert, 607). Developmental biologists think of the limb bud in terms of a three dimensional coordinate system, a complex system of patterns. The three axes are as follows, and develop in the following order:

1. *Anterior-posterior*, an imaginary line from the "thumb" and "pinkie"

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2. *Dorsal-ventral*, an imaginary line from the top of the "hand" to the "palm"

3. *Proximal-distal*, an imaginary line from the "shoulder" to the "fingertip"

(Gilbert, 611). The presence of the anterior-posterior axis, the pattern from anterior to posterior, is the first change that occurs in the vertebrate limb from its pluripotent state. Scientists have determined, from grafting experiments, that anterior-posterior information exists before the limb bud is even visible. A gradient model is one way to explain how information about this kind of polarity is determined. It is suggested that there is a gradient of some kind of substance, perhaps a morphogen, from the posterior end of the limb bud to the anterior end. There is a block of mesoderm at the point where the posterior portion of the limb bud joins the body wall, called the *Zone of Polarizing Activity,* or ZPA. The concentration of the morphogen is highest here, at the posterior pole, and decreases toward the anterior. Theory suggests that the concentration serves an orientating function. The level of concentration is speculated to specify the different digits of the limb. At its highest concentration (posterior), digit four is specified, and at its weakest (anterior), digit one is specified. All those digits in between have their own threshold concentrations as well, as they occur along this concentration gradient. (Gilbert, 614 - 615).

Dorsal-ventral information also exists early in development, and may also result from a some kind of concentration gradient.

The proximal-distal axis is defined after induction of the *Apical Ectodermal Ridge*, the AER, by the mesoderm below. The AER is a thickening of the ectoderm of the limb bud that occurs early in development. This structure is believed to be both induced by and maintained by the mesoderm below (Hopper and Hart, 316 - 318). It is believed that the mesodermal cells of the limb bud are organized in a proximal-distal sequence, according to their distance from the AER. Proximity to the AER thought to be what determines the structures that these cells will become. The limb elongates as the mesoderm proliferates by mitosis, and the cells develop into limb structures, again, as specified by their distance from the AER. Thus, the AER is responsible for the elongation (distal outgrowth) of the limb. (Hopper and Hart, 317). There is a

region on the limb bud where this mesodermal cell division takes place, called the *Progress Zone* (Gilbert, 611). As the cells leave the zone, they have their proximal-distal values assigned. The first cells to leave the zone develop into the most proximal structures of the limb, i.e.. the shoulder girdle. Continuing in this manner, the limb develops in a proximal to distal sequence. While polarity and position information have been found to be inherent within limb mesoderm, a limb cannot develop without the AER (Hopper and Hart, 317). Experimentation has shown that removal of the AER results in the cessation of cell differentiation in the progress zone, and the absence of the more distal structures of a normal limb (Gilbert, 611). The extent of these "deficiencies" depends upon the time at which the AER was removed (Hopper and Hart, 317). Thus, both the mesodermal component and the ectodermal component are necessary for limb development. The mesoderm provides the intrinsic pattern and positional information and the AER "maintain[s] the apical mesoderm cells in a state of developmental plasticity and...elicit[s] the proper sequencing of level-specific patterns in the limb mesoderm" (Hopper and Hart, 318).

To summarize, the limb bud consists of two types of cells, ectoderm and mesoderm. The mesoderm cells are organized within the limb bud along three axes. These three axes are important to the normal development of the limb. The cells have positional values along these axes, which correspond to the fates of those cells, i.e. what parts of the limb the cells will become. The regulative ability of the limb results from the abilities of the cells of the larger area, the limb field, to change their fates in response to a disruption.

LIMB DEFORMITIES

According to the website for NARCAM, the North American Reporting Center for Amphibian Malformations, there are four categories of deformed limbs: missing limbs, extra limbs, split limbs, and abnormal limbs. They are defined as the following:

1. *Abnormal limbs* - The correct number of limbs and digits is present, but one or more is deformed in shape or size.

2. *Extra limbs* - An extra limb is one that starts from the body and is not attached to any of the four normally occurring limbs. These can include extra hind limbs, extra fore limbs (sic), and extra limb buds.

3. *Split limbs* - A split limb is one that starts from one point on the body, but branches into two (or more) limbs.

4. *Missing limbs* - Missing limbs occur when a limb never develops. (NARCAM, online)

Abnormal limbs, because it is an ambiguous category, defies much explanation. Some of these kinds of deformities can be attributed to injury or predation, as well as to developmental problems. This category is still relatively unclear. Because much research has been done regarding amphibian limb regeneration, it appears that scientists can, at least partially, make speculations about the other three categories of deformity. Given the information about the regulatory ability of the limb bud, it is likely that limb deformities result from a two-fold process. First, there is an *external* force, which disturbs the organization of the cells of the limb, the axes of polarity that I have just discussed.   Second, there is an *internal* response from the limb bud. Recall that the limb bud has the ability to regulate its development.   It is most likely the anterior-posterior axis that has been affected when we see split limbs, extra or supernumerary limbs, and missing limbs.   Although changes in the proximal-distal axis can result in missing or incomplete limbs, it is on the anterior-posterior axis that I will focus my attention.

REGULATORY RESPONSES IN LIMBS

While we know the four basic regulatory principles that apply to an embryonic field, researchers have experimented with limbs specifically and have generated a list of principles specific to limbs. According the Bryant and Gardiner, all vertebrate limbs exhibit similar regulatory responses. These responses are:

1. The development of supernumerary limbs when cells with opposite polarities are confronted.

2. The ability to create, by intercalation, any missing structures along the pattern.

3. The ability to regenerate parts of the pattern that have been removed.

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(Bryant and Gardiner, 1). These principles can be used to understand one theory as to how the missing, supernumerary, or split limbs occur. The gradient from anterior to posterior represents a pattern. Each cell along this pattern has a positional value, its polarity. Further, each cell can recognize its polarity and its position within the larger pattern. When cells with oppositional polarities are confronted, it represents a disruption in the pattern. Cell division occurs in response to this change in the pattern. It is a typical regulatory response for cells to divide by mitosis to reestablish the pattern. This growth is called *intercalation*. Growth by intercalation occurs when cells with oppositional positional values suddenly find themselves next to each other. Of course, in limbs this is occurring in three dimensions, but as a simple model, it will be visualized in two dimensions. As an example, suppose that the values "1,2,3,4,5" represent the pattern along a limb bud in the anterior-posterior axis. Each cell is "aware" of its values and "aware" of the values of the cells surrounding it. Suppose that the cells are rearranged so that the values are 5,2,3,4,5. Cell 5 and cell 2 are now located next to each other, when there should be some values between them. Their regulatory response is to divide by mitosis to fill in the values between them. The result of their intercalary growth is: 5,4,3,2,1,2,3,4,5, where the larger numbers represent the confronted cells

that we began with. Since 5,4,3,2,1 represents one limb, here we have enough for the development of almost two complete limbs. In addition, these limbs would be mirror images of each other. It appears that it makes no difference whether or not intercalation creates an overall normal pattern, only that the process of intercalation serves to maintain the correct sequence of positional values. (Sessions, online). Thus, intercalary growth is that internal response which creates supernumerary or split limbs, depending upon where the disturbance occurs in the limb bud, and when it occurs in development. To reiterate, a simple model for how supernumerary and split limbs develop is as follows: There is an *external* force, which rearranges the positions of cells within the limb bud. The *internal* response of the cells is growth (mitosis) by intercalation. Intercalation serves to reestablish the correct pattern, no matter how abnormal the structure which results is. It is concluded, then, that "positional differences drive growth and pattern formation" (Bryant and Gardiner, 4) In contrast, "in the absence of positional differences, growth and pattern formation cease" (Bryant and Gardiner, 4). A limb will not develop when there is a lack of differences in the values

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along an axis. For example, using the same two-dimensional model, imagine a limb bud with values of 5,5,5,5,5 from anterior to posterior. Since there is no gradient of polarity, or no difference in values, there is no limb development.

CAUSES OF LIMB DEFORMITY

Because it is an area where scientists disagree greatly, I have intentionally chosen not to focus on what the suspected agents of deformity are. However, now that we have an understanding of the processes, it is possible to consider how the agents are causing the change, regardless of what the cause(s) turn(s) out to be. (Just a note, though, the suspects include: UV radiation. parasitic trematodes, chemicals, particularly a group called *retinoids*, and tadpoles or tadpole predators.)

The two agents at the forefront of the debate are retinoids and parasites, but this is an areas of much controversy in biological academia and research. Both have the ability to serve as that external force spoken of earlier. Recall that it is the external force which rearranges the order of the cells, and causes the intercalary response.

Trematodes are parasitic worms that have been found to burrow into the developing limb buds of amphibians and to create cysts. The presence of these cysts, as well as the action of the burrowing trematodes, is suspected to have the ability to rearrange the cells in the limb bud. This *physical* disruption has the potential to put cells with oppositional values in contact (Sessions, 45). Through experimentation and manipulation, Stanley Sessions has proven that a physical disruption is enough to cause the intercalary response and the development of supernumerary limbs. He has also found a direct relationship between limb deformities and the presence of trematode cysts (Sessions, online).

Retinoids have been shown to have the ability to *chemically* change the positional values of cells in the limb bud to values that are more posterior-ventral-proximal (Bryant and Gardiner, 5). Global exposure to retinoids can unify all the positional values in a limb bud, creating a situation of no growth. Recall the limb bud with values of 5,5,5,5,5. Local exposure of retinoids can "reprogram" the cells adjacent to the retinoid source to values that are posterior-ventral-proximal. This change causes a confrontation between cells of

different positional values (Bryant and Gardiner, 9). This confrontation causes the intercalary response and results in the development of supernumerary or split limbs.

To summarize, supernumerary and split limbs are likely to result from a disruption in the limb bud's anterior-posterior pattern. Whether it is a chemical or parasite causing the disruption, the internal response is the same. The cells grow by a process called intercalation, to reestablish the correct pattern. This growth produces more limb tissue, enough of which can mean an additional part of or complete limb.

PART TWO: THE ART

It was my intention, from a creative standpoint, that the thesis project showcase the variety of skills that I had acquired while in graduate school: illustration, design, animation, and interactive media. I also wanted to choose a topic that hadn't been explored in this format before, something that was interesting, and a topic that meant something to me. I wanted it to be both medical and scientific, since I have interests in both of these areas. I chose to do my thesis on the recent incidences of limb deformities in amphibians because it was a hot issue in the media and among science professionals. There had been numerous television news broadcasts, newspaper articles, radio broadcasts, websites, and magazine articles devoted to this topic. None of them, however, really told what was happening in terms of biology. Their discussions focused primarily on the individual frogs that were found and the suspected agents of deformity. I wanted to know more, and I thought that if I could learn the biological information that was behind this phenomenon and illustrate/explain it, the result would be an exciting and challenging thesis project.

I began the process by outlining, in my head, what kinds of imagery I wanted to use to tell this story. I decided on schematic ink illustrations and 3D animations. I am fond of the medium of pen and ink and I felt that my line work was fairly strong. I chose 3D animation because it was a medium that I wanted to learn. It's true, I hadn't done much 3D work at the time of my thesis proposal.

ILLUSTRATION

It was my intention for the ink drawings to be simple, but elegant. I tried to think about how the drawings would look both on the screen and hanging in the gallery. I am quite fond of the way that both sketches and line drawings look when scanned into the computer. There is something about ink drawings on the screen that is very attractive to me. I hoped that I could come up with a creative way to hang them in the gallery.

To begin the research for the drawings, I wrote e.mail to several people in Minnesota with regard to what the most common frog species seen with deformities were. The response: Northern Leopard Frog, Wood Frog, Mink Frog, and Green Frog. I began sketching these species from field guides at the library. This approach turned out to be unsuccessful for several reasons. I was quite unhappy with the sketches and initial ink drawings. They were schematic, but in a cartoon sense, not in an elegant sense. I also felt that they were static, that they lacked movement, and that they had little indication of texture and tissue. I sketched often, never achieving the results that I was after. Later, when I learned that the deformity problem wasn't species specific, I gave up on the field guide approach. The species names that were given to me by the people in Minnesota were those most commonly seen with deformities simply because they are the most common species in that area. So I abandoned that approach, and focused my efforts onto one species, the Northern Leopard Frog, *Rana pipiens*. I tried to buy one from a pet store. The idea was that I would have a live, 3 dimensional subject to observe and to sketch from. I soon learned, however, that pet stores do not sell this frog because it is too common. They sell Brazilian Frogs and Exotic toads and the like. So I found myself out of luck. Finally, with some faculty help, I ordered a frog skeleton and a preserved frog specimen from Carolina Biological Supply. Luckily, the Northern Leopard Frog is the species used in high school biology classes for dissection, so this is the species it is supplied to schools. I was much happier with the sketches and ink drawings from these subjects, as I was able to stay simple and still convey a sense of realistic form and texture. Acquiring these specimens made a significant difference in the quality of the line work.

The drawings were done with a Hunt 100 crowquill pen on Herculean film. I used a Mars eraser in the dispenser with a good amount of saliva to clean up mistakes. I liked this particular film because it was quite easy to work with, and it had a level of translucency that I knew would work well in the gallery when mounted with a textured paper. I made five drawings: a detailed drawing of a normal Northern Leopard Frog, an outline drawing of a frog with a missing limb, an outline drawing of a frog with split limbs, an outline drawing of a frog with supernumerary limbs, and a drawing of a normal frog skeleton. I also made some

sketches of the internal bony structures for each deformed frog illustration: missing, split, and supernumerary.

THE OUTLINE DRAWINGS

After inking, the drawings were scanned into the computer. In Photoshop, I executed the following steps: First, I used the magic wand tool to select the area outside of the drawing. Second, I selected the inverse, so that I had the entire areas within the figure's outline selected. Third, I used the eyedropper tool to grab the green color from a render of the 3D frog (which will be discussed later) and I used a *60 % opacity, darken fill* on each frog drawing. I used the "Darken" fill because it fills everything that is lighter than the color chosen. In this way, I kept the black ink lines pure black, rather than a mix of black and green. I also added a few black arrows, as needed, to indicate the area of the frog's body that showed the particular deformity. Next, I added a new layer, filled it entirely with a cream background color and moved it behind the layers with the frog and arrows. The last step was to save this file and then save it again with another name with all of the layers flattened. Before I take a Photoshop file into Director (yes, I imported them as Photoshop files, not PICT), I am sure to merge all of the layers so as to make it as small as possible. I do keep a version with all of the layers intact so that I can go back, if needed, and make changes. I did the same with the skeleton outline drawing, except for dropping the green color. This illustration, however, never made it to the final interactive piece.

THE BONES SKETCHES

I also scanned the bones sketches, though these were never inked, into Photoshop. I cleaned them up, turned up the levels, and dropped each, as a separate layer, into a file with the corresponding green frog image. I saved each as a separate file. At this point, I had two versions of each frog illustration: one with the green frog image and the bones, and one with just the green frog. These would be later used as rollover states in the Director movie.

THE NORMAL FROG DRAWING

This drawing was also scanned into Photoshop. In addition, I scanned a piece of canvas and a newspaper article about the deformed frogs. I moved the layer with the canvas so that it was the layer furthest back. Next, I cleaned up the newspaper scan, cut and pasted a little to give a collage effect, and put this as a layer on top of the canvas. I added another layer on top of these two with the same cream colored fill and reduced the layer's opacity so as to soften the textures of the canvas and the newspaper. Then I pasted the ink drawing on top of these layers. This top layer consisted of the detailed ink drawing with on its white background. I wanted the frog to be on the canvas/newspaper background, so I had to remove this white area surrounding the drawing. I used the same techniques as before, where I selected, with the magic wand, the area outside the drawing, and then selected the inverse. This selection was a little tight, so I modified the selection to expand 2 pixels. Then I deleted this selected area so that I had just the frog on this layer, but it still had some white area inside of its body. I selected these areas with the magic wand, modified the selection to contract one pixel, and then deleted these areas. The result: a frog drawing on top of a muted background, which showed some of the background through. The ink lines of the frog drawing had a thin white halo, the result of expanding / contracting by one pixel. I felt that this had a nice look to it.

THE ALIAS MODELING AND ANIMATION

This was the most difficult and challenging aspect of the whole project. I decided that as long as I had access to the SGI Lab and the Alias software, I should learn as much as I could. I took *3D Modeling and Animation 1* with Marla Schweppe in the computer animation program during the fall quarter of my last year, and I took *Character Animation* in the winter quarter. Neither of these classes directly helped with my thesis, but they did serve to make me more comfortable using the software's tools and I learned a great deal about animation. When I wasn't working on the class projects and tutorials, I was experimenting with what I needed to learn to accomplish my thesis.

MORPHING VS. SHAPESHIFTER

Because I would be making something change its shape, I did the *Morphing* and *Shapeshifter* tutorials many times. Making something change shape in a complex manner, ie. to grow, turned out to be a difficult task in Alias. Alias was designed primarily for character animation. I was determined, however, to make the software work for me. I began experimenting with simple shapes that mimicked the actions that I wanted to happen. I used spheres for the body and cylinders for the limbs. Both the Shapeshifter and Morphing tools involve using two objects: the beginning object and the ending object. Both of these objects have to be identical in terms of geometry, ie. they have to have equal numbers of CVs and isoparms. Ultimately, I chose to use Shapeshifter over Morphing because it provides more control. With Shapeshifter, there are slider bars that control how much the beginning object changes into the ending object. In contrast, with Morphing, there is one continuous movement from the beginning to the ending object. So I set up the geometry so that I would have a total of five sets of sliders: one set to control the body, and one set for each of the four limbs. An important thing to be careful about with Shapeshifter is where the pivot point of the object is located. The pivot point is the location from which the change in shape occurs. In the Shapeshifter settings box, I checked "Scale Pivot" meaning that I could set the pivot to any location, and that would be the location of the movement. Had I checked "Centroid", the change in shape would occur from the center of the object, resulting in the limbs growing out from their center and moving away from the body. Setting the pivot to the proximal tip of the limb object made the limb grow out from the body, which is what I wanted. It took a great deal of experimentation and consultation with other animation students and with Marla before I understood this and executed it successfully. Throughout this whole process, I was constantly thinking about how this was going to work, jotting notes and making sketches of the ideas that I would try out in the lab. Once I had a simplified model that worked the way that I wanted it to, I put that file aside and created a new file where I experimented with modeling what would become the final objects.

MODELING THE FINAL OBJECTS

I began with the body as a modified sphere and the tail as a modified cone, with the two attached via a *blend*. Conceptually, the limbs were especially challenging to model because the beginning object (the limb bud) had to have the same geometry as the ending object (the mature limb with webbing details). In addition, they each had to be one object. This greatly limited the amount of complexity that the shapes could have. After trying at least a million model scenarios during several months, I finally figured out that I could make each of these objects by creating a series of curves and *skinning* them with the *skin tool*. This process is similar to stretching a tent over a series of tent poles. I began modeling the final limb, the most complicated structure. I created a series of curves of different sizes using the *circle tool*. These curves defined the frame of the object, like the tent poles. I was careful to keep track of both the number of curves and the number of CVs on each curve. Within a skinned object, each curve has to have the same number of CVs for them to skin properly. The result was a cylindrical object, sculpted to look like a limb. The last curve was scaled to zero in the Y direction, so that it would close the object. This last curve was much wider than the others, with the CVs pulled outward so as to create many waves in the skin. The result was that the skin flared both outward and had convolutions, creating the webbing. Given that I knew the number of curves and the number of CVs on each curve on this final limb shape, I was able to create both a limb bud object and an intermediate limb object, so that all three objects would have identical geometry. Both the limb bud object and the intermediate limb object also had to have this last curve scaled to zero in the Y direction, to close the object, but without causing the webbing "flare." For the limb bud, this final curve was rounded to follow the contour of the bud shape, and for the intermediate limb, this final curve was slightly rounded and only slightly wavy. I chose to make three limb shapes because the animation from just the bud to the final limb was too abrupt and unrealistic. The intermediate shape afforded some real appearance of growth. It was important that the direction of the CVs on each curve of each object were the same and that the pivot points of each limb object were in the same places. I had to rebuild these objects many times to ensure that they would work properly in Shapeshifter. I pulled a couple of CVs on the final limb shape to make it less

mechanical and to suggest the presence of bone underneath. In the end, I had three limb shapes for both hind limbs: a bud, an intermediate shape, and a final limb shape. I set the pivot of each of these to the Y-axis, and scaled each to -1 in the X direction, so I would have a mirror image copy of each one for the other side of the body. Then I reset their pivots to all be at the proximal tip of the object, closest to where the body would soon be located.

After making the limb shapes out of skinned curves, I began to rethink the way that I had built the body shapes. I was dissatisfied with the modeling in general, and with the blend between the cone-tail and the sphere-body. As the tadpole "grew," this blend was stretching in inappropriate ways and losing construction history. I decided that I needed to rebuild the body objects, with the skinning method I had used to create the limbs. I used children's science books as reference at this point, because some had large, close-up photographs in them, which helped a great deal in the final modeling of the frog shapes. As I had done with the limbs, I made three body shapes: a tadpole, an intermediate, and an adult frog. Each of these shapes consisted of a series of curves (circles) with the same number of CVs. I pushed and pulled to created the eye bulges and dorsal ridges. The first curve was left open so that there would be a mouth, and the last curve was scaled almost to zero (but not quite zero, as this caused Shapeshifter problems). The result was one long object that consisted of a body and a tail. A real tadpole's tail has a transparent portion and a thinner inside structure. To get this effect, I created another skinned shape that would go over the tail portion of the previous object, and this shape was blended to the body. This shape was given a transparent shader, as was the blend. In this way, the thinner tail portion showed through the transparent object and it really did look like a tadpole. I was quite pleased with the new model and proceeded to create the intermediate and the final body shapes. The final frog shape had to be different in structure from the others because it would not have a tail, but still had to have the same number of curves. I could easily eliminate the second skinned object of the tail, the transparent one, but not the curves that created the inner tail, as these curves were part of the singular body object. For the final frog, these curves did exist, but were arranged in very close proximity with each other at the end of the frog's body, a structure called the "vent." This is the area of the frog body that was formerly the tail, and in the adult, it is a slight protrusion. By

cramming all these leftover curves into this area with close proximity with each other, I was able to maintain the correct number of curves for the shape, so that Shapeshifter would work with the geometry. An added bonus was that I also created a correct anatomical structure.

Finally, I created the forelimbs. Frog forelimbs do not have webbing, so I could not simply duplicate the structures that I had already created. I did, however, build these using the same method; skinning a series of curves. I created each forelimb object and each of its four digits this way. Using the *project tool*, I projected four curves into the "hand" portion of the skinned forelimb object, and trimmed them out. Next, I blended each digit (also a series of skinned curves) to the limb using the *blend tool*. While it is not necessary to trim out the surface after projecting a curve, it usually makes for a smoother, more accurate blend. I grouped the digits and forelimb (but not the blends) into one object and duplicated it to a mirror image as described earlier. Had I included the blends in the group, they would lose construction history, which compromises the integrity of the blend. In life, the forelimbs develop entirely within the frog's body and literally break out of the body wall. Thus, the developmental stages are not seen from the outside. For my animation, I made three versions corresponding to three positions of the forelimbs: one folded up to go inside the body, one partially unfolded, and one extended normally. In this way, Shapeshifter will "unfold" the limb and it will break out of the body wall.

SHAPESHIFTER ANIMATION

Having created three body shapes, three hind limb shapes, and the three forelimb variations, I was ready to put it all together. Shapeshifter was originally designed to create different facial expressions in character animation. The method is quite simple. There is a *base object* and a *target object*, both with identical geometry, i.e. the same number of CVs and isoparms. After selecting the base and target objects, Shapeshifter calculates and builds the slider geometry. To make the shapes change, one simply needs to pick the slider object and move it along its slider bar. As the slider moves, the object changes in shape from the starting shape to the ending shape. It can be moved to any point along the bar, and even beyond it if so desired.

In my scenario, I have three objects for each of the three structures: body, hind limb, and forelimb. Therefore, I created two sliders for the body development, two for each hind limb, and two for each forelimb. To clarify, there is a slider to control the change from tadpole body to intermediate body, one to control the change from intermediate body to the adult frog body. There is a slider to control the change from limb bud to intermediate limb, and one to control the change from intermediate limb to adult limb. Finally, there is a slider to control the change from the fully folded forelimb to the partially folded forelimb, and one to control the change from partially folded forelimb to the fully unfolded forelimb. I followed the same generalized steps for each, summarized in the following:

1. Clicked on the Shapeshifter icon.

2. In the dialogue box, checked "Scale Pivot."

3. Clicked "Go".

4. Alias prompts the user to select the base object and then the target object. In each case, the base object is the one that represents the earliest stage in development. For the first body slider (recall that there will be two). the base object is the tadpole body. For the first hind limb slider, it is the limb bud, and for the first forelimb sliders, it is the limb folded inside the body. The target objects for each are the intermediate shapes: the intermediate body, the intermediate hind limb, and the partially folded forelimb.

5. Selected each slider (the triangle thing) and set keyframes for their location at zero.

6. Moved each slider to a location somewhere along its slider bar, so that the shape of the base object changes.

7. Set keyframes for this location.

8. Clicked on the Shapeshifter icon again.

9. In the dialogue box, checked "Scale Pivot."

10. Clicked "Go".

11. Alias again prompts the user to select the base object and then the target object. In this case, the base object is still the same as when I created the sliders before; I used the very same object,

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only this time, its shape is a little different because I already moved the sliders to a location along their slider bars. The target objects for each slider in this second round are the final objects: the frog body, the adult hind limb, and the unfolded forelimb.

This summarizes, but also simplifies, the process I used to create the animation. I cannot stress enough that there were months of experimentation, consultation with animation students, and much heartache. These are, however, the results of my trials.

THE INTERACTIVE PIECE

The illustrations and animations were brought into Director 6.0 to create the final interactive piece. This was by far the easiest part of the whole project. Instead of elaborating in detail on every step taken with the Director piece, I will explain some of the important hints that I learned while assembling the piece.

The images were imported as Photoshop files, although I now have learned that using PICT files is much better in terms of file size. I was under the impression that Photoshop files were better for importing the individual palettes for each image. It was my intention to work first with the images as 32-bit Photoshop files and get the piece looking the way I wanted. Then I would go back to Photoshop and create a custom palette for my images and make them all 8-bit, mapped to this new palette. The result would be 8-bit images of a much higher quality than if they were mapped to the system palette. I did, however, run out of time. Instead, I changed the 32-bit castmembers to 16 bit within Director. In the cast window, I selected them all, and from the Image menu, I scrolled down to Transform Bitmap. In the dialogue box, I selected 16-bit, System Palette. Obviously, 8-bit would have been best, but this worked and the movie ran smoothly. Even though I did not create the custom palette, I think that it is important to elaborate on the steps for this procedure. A custom palette is created using the following steps:

1. Place all images for the movie within one Photoshop file, as in a collage.
2. Save the file, something like "Master Palette.".
3. In Photoshop, select "Image/Mode/Indexed Color" from the title bar.
4. In the dialogue box that appears, select the following: "8-bit, Adaptive and No dither".

5. Click "OK."

6. Go back to "Image/Mode" and this time scroll down to "Color Table." This brings up the palette that was created for your images in steps 4 and 5. It represents the 256 colors that will best represent what is seen in those images.

7. Click "Save" and name the palette .

8. Go back to the original image files, those that were used to create the collage for the palette, but not that file itself.

9. With each file, in Photoshop, select "Image/Mode/Indexed Color" from the title bar.

10. In the dialogue box, under "Palette", click "Custom." In the next dialogue box, click Load. Photoshop prompts you to select a palette. Choose the "Master Palette" that you created in the above steps.

11. Save each file as an indexed version. This process will change the images slightly, as it remaps their colors to those in your master palette.

In Director, the animations were imported as quicktime movies from Adobe Premiere. These movies were assembled into quicktime movies by a two step process: First, I assembled all of my frames into a quicktime movie using Debabelizer. When an animation from Alias Wavefront is rendered, it exists as a series of frames, or stills. There is an option to create a quicktime movie as you render from Alias, but some animation students informed me that the quality is poor. Debabelizer does a much better job of making quicktime movies. So after rendering, I brought in all of the frames contained within a folder into Debabelizer. In DeBabelizer, I specified "Quicktime" as the kind of output that I wanted. For compression, I chose "Animation compression," "Millions of colors", and High quality." Depending on quality and compression, making the quicktime can take quite a while. Unfortunately, at the time, there was only one machine at RIT with Debabelizer on it, one of Macintoshes in the Grad Animation Lab in Building 7B.

Once the quicktime movies were made, I brought them into Adobe Premiere to add the titles. I decided to have titles rather than narration for two main reasons: time constraints, and because I knew that the gallery would be very crowded and loud on opening night. Ideally, a multimedia program should

have both text and narration, to accommodate both hearing and non-hearing individuals. Titles area added in Premiere from the Filters menu, by selecting Alpha Channels, and then typing whatever one wants to appear on the screen of the quicktime. Dragging the box, which contains the type to the desired location, can change the location of these titles. In this way, the titles can scroll up the screen, like the credits of a film.

In Premiere, I did a great deal of experimentation regarding further compression. After selecting "Make movie" from the title bar, I clicked on "Options" to select my compression rate for the final quicktime movie with titles. In Premiere, the combination of animation compression, thousands of colors, and low quality gave me the best result with quite a low file size. This is an area where one needs to do a series of tests to find out what will work best for the particular piece. After importing the movies into Director, I highlighted the quicktime movie cast member in the cast window, I selected "Modify/Castmember Properties", from the title bar, and clicked "Enable preload" in the dialogue box. Doing this makes the movies play smoother, because they are completely loaded into Director before they are played. I also wrote the following movie script:

On startMovie,

        preload 1,306

end

This preloads all of the cast members, 306 of them, before the movie begins. This, as well as "Enable preload" in the quicktime movie castmember properties dialogue box, causes a small delay when initially starting the movie. The pause is well worth it when the Director movie finally plays. The rest of what I did was fairly simple. I added ambient sound to the sound channels, made rollovers for the navigation and some of the illustrations, and created a slider for an animation of how an extra limb develops.

THE FINAL PRESENTATION

The final interactive piece was run on a Power Macintosh 7100PC in the Bevier Gallery. I put the computer and monitor into a kiosk structure, which was built by a student in Industrial Design. I did some adjustments to the kiosk structure, including installing new canvas and painting some of the wood. On the wall behind the kiosk hung the 5 large ink illustrations: the detailed drawing of a normal frog, the three outline drawings of frog deformities, and the drawing of normal frog skeleton. These drawings were framed in simple black metal frames, mounted to pieces of canvas to match the kiosk.

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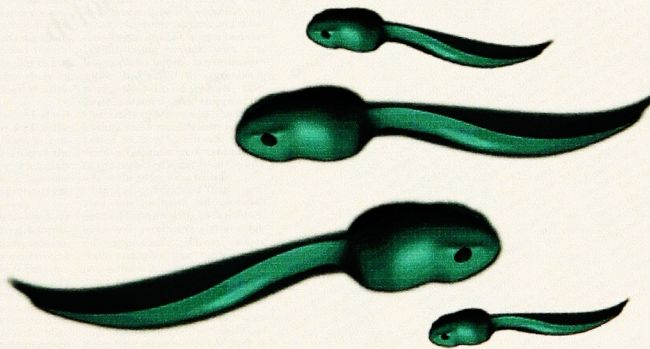
AMPHIBIAN LIMBS:

DEVELOPMENT AND DEFORMITY
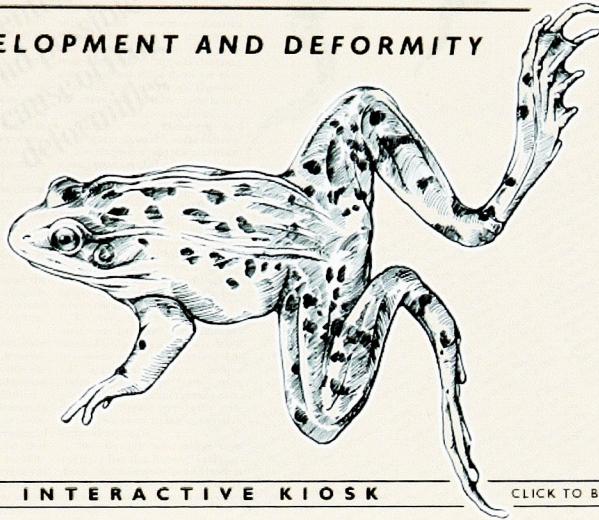
AN INTERACTIVE KIOSK

CLICK TO BEGIN

| BACKGROUND | DEFORMITIES | METAMORPHOSIS | ABNORMAL DEVELOPMENT | HELP | CREDITS | QUIT |

MAIN MENU

Frogs begin life in the water as tadpoles, and later gain the ability to live both as aquatic and a terrestrial creatures. Metamorphosis is the process that prepares the amphibian for it's terrestrial life, and depending on the species, can take from a few weeks to a few years to complete.

The important changes that occur during metamorphosis include, but are not limited to, the development of limbs.

◄ MAIN MENU ►
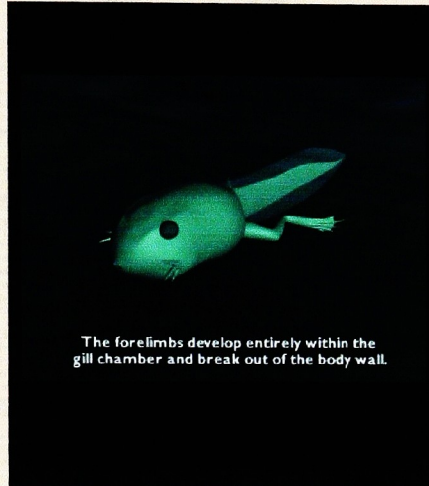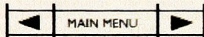
The forelimbs develop entirely within the gill chamber and break out of the body wall.

SPLIT LIMB

In this case, there is a second, incomplete limb which branches off of an otherwise normal limb. The extraneous limb structure is often a mirror image of that which it branches off from, and because it is probably poorly innervated, it is likely to affect the animal's normal locomotion, and thus, its means of escape from predators.
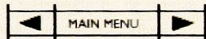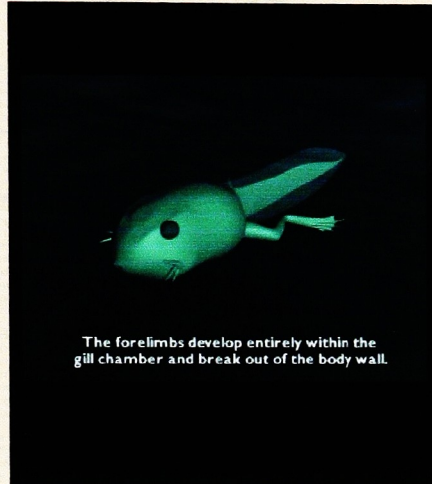
◄ MAIN MENU ►
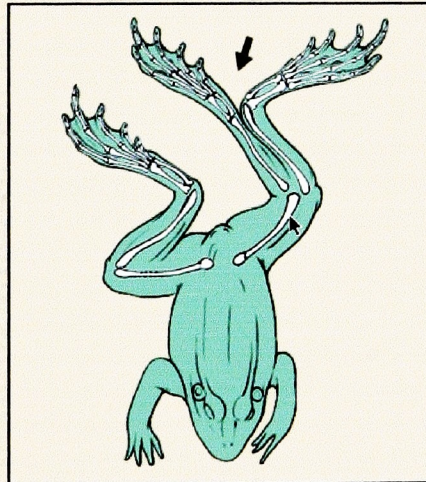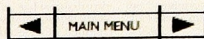
Place the cursor over the image to see the skeletal structures.

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◄ MAIN MENU ►

The forelimbs develop entirely within the gill chamber and break out of the body wall.

THE LIMB BUD

The precursor of the amphibian limb is a structure called the **limb bud**. The limb bud is an aggregation of mesoderm cells which migrated to the site of the future limb. These cells are arranged within the bud in a three dimensional coordinate system. The organization of the limb bud is understood by developmental biologists to consist of three axes of polarity.

Place the cursor over the terms below to see them illustrated to the right.

anterior - posterior

dorsal - ventral

proximal - distal

◄ MAIN MENU ►