Lab 2
Genes and Behavior
Fall Quarter 2011

Introduction

The goal of this lab is to provide you with "hands on" experience with issues concerning the relationship between development, behavior, and genetics. To do this lab you will be using four software packages (the first three of which you have had some experience with from the last lab):

Extend. A powerful computer simulation system.
Microsoft Excel. A spread sheet program that allows you to rearrange, manipulate and graph data (it also has limited statistical analysis capabilities).
Microsoft Word. A word processing program that will allow you to combine text with tables and graphics.
Statview. A statistical analysis package, which allows you to do very sophisticated statistical analyses with your data.

There are five main parts to this lab:
A short background section on the lab
The 3P’s of Science and Science Education: Problem Posing, Problem Solving, and Persuasion
Problem posing
Problem solving
Persuasion
The Lab: Parental handling in Two Strains of Mice Reared by Foster Parents
Introduction and Methods
Modern psychology is a multidisciplinary science and no field of psychology illustrates this more clearly than developmental psychobiology. Fundamental discoveries in psychology have been made, for example, by integrating genetics and endocrinology into the study of behavioral phenomena. From the integration of genetics into psychology the field of behavioral genetics emerged. But the integration of different fields of science is by no means a simple task. Different fields tend to focus on different levels of organization.

The problem of integration is not merely one of simple methodological reductionism (i.e. the view that entities--for example genes--at one level causally explain all the phenomena at upper levels, the development of individual behavior) Successful integration must account for all the major (minor conditions are those with little effect with respect to the time scale in question) conditions that explain the behavior in question. In particular it must account for downward and upward causation and the interactions between these directions of causation. This type of integrative approach can be termed wholistic reductionism, by which we mean that upper level phenomena (in this case behavioral development) is explained by including lower level causes, but not at the expense of ignoring the downward effects of organization and function on the composition of a system and the interactions of its parts.

Behavioral geneticists are primarily interested in characterizing the relationship between genetic differences among individuals in a population and their behavior. The developmentalist focuses on
understanding *conditions* of ontogeny that are necessary for the occurrence of some behavior, change in behavior, or physiological process that plays a role in behavior. Thus, behavioral geneticists and developmentalists do not perform the same types of analyses of behavior, and so it is not appropriate to merely substitute behavioral genetic analyses for developmental analyses. Later in the course we will discuss in some detail the relationship between genetics and development. In this lab, however, we will begin to focus on the complexities that distinguish these two approaches.

A typical way of showing the genetic influences on behavior involves investigating the behavior of inbred strains of animals in nearly identical environments. (In the laboratory no two animals can be raised in *exactly* the same environments. There will always be some differences. For example, different locations in a room--though they are housed in exactly the same type of housing units, small differences in the time animals are handled by their human caretakers. In a laboratory colony all of these potential differences are kept to a minimum. Lighting is kept as uniform and regular as possible. Temperature and humidity are held as constant as possible. Animals are housed in the same type of cages, fed the same type of food, and their bedding is changed on a regular schedule and at about the same time. But, these "external" conditions are not the only components of a developing animal’s environment as we will see later.)

In the simulated experiments that you will be performing, we will suppose that we are working with inbred strains of mice raised in virtually identical laboratory environments. Inbred strains, as mentioned in the previous lab are produced by many generations of brother-sister matings. Any behavioral differences such as fighting, maternal care, visual exploration, between strains under these conditions (i.e. many generations of brother-sister matings and virtually identical laboratory environments) are *presumed* to result solely from genetic differences.

The simulated experiments that you will be performing are based on a
series of experiments performed by Robert H. Ressler (Behavior Genetics Laboratory, Department of Psychology, Western Reserve University, Cleveland, Ohio) in the early 1960s. You will be using two strains of "virtual" inbred mice: BALB and C/57. As you discovered from last week’s lab, the two strains differed in their average litter size. In this and subsequent labs, we will determine whether there are other behavioral differences among these two strains and what the possible causal sources of these differences might be.

The labs will be challenging in that you will use simulated experiments in Extend to generate complicated raw data. With this raw data you will perform sophisticated statistical analyses, interpret the results, and write them up in reports. **We do not, however, suppose that you have a strong background in statistics.** Some of the basic statistical ideas that you will need to know will be described later and a statistical analysis software system (Statview) will be used to perform the analyses, but how to perform the analyses and interpret their results will be up to you. This includes figuring out how to format the data and enter it into Extend. **When you get to this stage and do not know how to proceed, talk among the members of your group, talk to other groups (you are free to communicate with each other as much as you want, but each group must produce their own reports), talk to us. This is all part of the problem-solving process in science that you will read about in the next section.**

Before continuing, we would like you to read some excerpts from "Problem-Posing, Problem-Solving, and Persuasion in Biological Investigations," a paper written by Jim Stewart University of Wisconsin-Madison and John R. Jungck Beloit College. These excerpts were written for BioQuest, a set of simulated experiments for various areas of biology, but their discussion applies equally here. These excerpts describe three basic phases of scientific research that you will find in any science. Problem posing and problem solving may seem obvious phases of science, but without the ability to persuade your audience of your discoveries, they will typically go unnoticed!
Problem-Posing

Of the 3Ps, problem-posing often receives the least attention in introductory courses--most of the problems students are asked to solve have been chosen by others. When that happens it is easy to lose sight of the significance of posing problems. But as Ann Sayre has written in her biography, *Rosalind Franklin and DNA* (1975):

The choice of both the problem and the approach to solving it is of vast consequence to a scientist. A wrong choice in either direction can be disastrous, a waste of productive years in unproductive labor, to an end which is at best negative?in other words, to the conclusion that it doesn’t work. Inevitably the particular choice made is less dispassionately followed than appears on the surface, or?in fact?than scientists are usually willing to admit. (p. 131-132)

While posing one’s own problems may be uncommon in undergraduate education, it is at the heart of biology. A biologist could stand in her laboratory or in the field forever, and no textbook-stated problems would come to her. Problems cannot spring from nothing; a particular problem has a history in a particular discipline. And the way that a discipline or individual scientists look at the world has everything to do with which
problems are felt to be worth investigating. Only by being encouraged to pose problems is it possible to begin to appreciate how problem-posing effects the direction that science will take. Sir Peter Medawar (1984) calls this agenda-setting and problem-posing "the art of the soluble." Donald Schon (1987) has described the role of "on the job" experience in professional education in architectural design, music, and clinical psychology. Students in these areas are provided with realistic practice with the tools and problems of the profession: designing buildings, playing music, psychoanalyzing patients. In other words, what is at the heart of professional education in these fields is experience with the posing and solving of realistic problems. There is no reason for this not to be the case in biology education as well. Students should have the opportunity to experience the excitement and satisfaction of doing something that is intellectually engaging. Problem-solving in biology is **realistic** when it captures the open-ended essence of science as it is practiced; problems must be posed and solved by the problem solver.

When using BioQUEST computer simulations, students are required to pose problems and to ask questions, both critical features of a genuine scientific education. When students are engaged in posing problems, it becomes obvious that problem-posing and problem-solving are inseparable, and that drawing warranted inferences depends upon knowing the assumptions that were made at the time the problem was initially posed.

The posing of problems makes apparent many of the blatant biases of culture based on race, gender, ethnicity, class, and religion. However, other biases, such as reductionism, teleology, anthropomorphism, anthropocentrism, speciesism, and confirmation bias also often creep into the formulation of problems, as well as into the strategies used to test hypotheses and persuade peers. If these assumptions are ignored during problem-posing, they become more difficult to detect, much less eradicate, at the problem-solving and persuasion stages of scientific practice. Not including problem-posing in biology courses makes it easy for students to overlook those factors, both in science and in the larger
cultural context, that influence the problems that scientists pose.

Some of these points about problem-posing are illustrated in the following composite story taken from the history of the development of knowledge about a single biological and social phenomenon.

As Europeans began to settle on the African continent, they had to cope with many hardships associated with their decisions to leave the familiarity, comforts, families, and friends of their native lands. One hardship was the number of debilitating diseases that had not been found, or at least only rarely, in Europe. One of the more common of these diseases affected them so that afflicted individuals would "...alternately shiver in paroxysms of chills as though submerged in a trough of ice and then be beset by fevers elevated to the point that could cause dangerous side reactions." The disease, called Uhlonzane (in Zulu), was by no means limited to Europeans; native Africans had been plagued by it for centuries. However, it was well know that not all Africans were equally susceptible to Uhlonzane; many individuals suffered from milder cases than others, while some seemed never to be afflicted at all.

However, not all of the diseases that were common in Africans were debilitating for the European settlers. For example, the Europeans seemed to be immune to one of the African diseases. Lakuregbee (Yoruba) left those affected severely anemic, often with debilitating joint pain due to blockages of the circulatory system; it ultimately led to a general deterioration of many body organs. Biochemist Stuart Edelstein (1986) has described a belief common to many African societies, that of the ogbange child: a "wicked soul [who] had come repeatedly to a family to be born as a baby, only to leave soon after birth" (p. 18) and to reappear in children born later to the same family. This belief, which Edelstein thinks is associated with Lakuregbee disease, is movingly expressed by Chinua Achebe in Things Fall Apart (1969):

As she buried one child after another her sorrow gave way to despair and
then to grim resignation. The birth of her children, which should be a woman’s crowning glory, became for Ekwefi mere physical agony devoid of promise. The naming ceremony after several market weeks became an empty ritual. Her deepening despair found expression in the names she gave her children. One of them was a pathetic cry, Onwumbiko--"Death, I implore you." But death took no notice; Onwumbiko died in his fifteenth month. The next child was a girl Ozoemena--"May it not happen again." She died in her eleventh month.

With the introduction of western European medicine and scientific technology to the African continent, it was discovered that those unfortunate enough to suffer with Lakuregbee had crescent-shaped red blood cells as compared to the round cells of unaffected individuals. Eventually it was realized that those who had the crescent-shaped red blood cells were not afflicted with Uhlonzane: people who suffered from Lakuregbee seemed to be spared the ordeal of Uhlonzane. By now it is probably obvious to you that the English names of the diseases are malaria (Uhlonzane) and sickle-cell anemia (Lakuregbee).

Considering for a moment that these observations had just been made, it is possible to imagine that today’s biologists could pose a set of diverse problems, from a variety of disciplinary perspectives. A classical geneticist might ask if there is a genetic basis for either of the diseases, and want to know the modes of inheritance. An epidemiologist or ecologist might ask about causal mechanisms of the diseases, ways in which the tropical environment might contribute to their transmission or whether or not they are found in other populations. A molecular biologist might ask if there is a protein structure basis for the unusually shaped red blood cells. An evolutionary biologist might ask if there is an evolutionary explanation for the relationship between the diseases, if they are of common origin, or did either emerge more than once in the evolution of humans. Not all problems posed need to be biological: others might want to know about the social and economic effects on individuals who have been diagnosed and labeled as having sickle-cell anemia.

There are many problems that can be posed about any given phenomena
or data set. Which questions are posed will, in large part, be a function of the background, interests, and even funding sources of particular researchers. In the African disease case, a complete picture of the two diseases will not emerge until questions are asked from a variety of perspectives. Questions from sociological, economical, and political directions may also be necessary to develop a complete understanding of the two diseases as parts of a system.

The following vignette on the history of the development of eugenics, like the picture developed with sickle-cell anemia, illustrates some of the issues related to problem-posing, including how cultural knowledge influences both the posing of problems and the interpretation of data that are gathered to seek solutions to those problems.

**A Vignette on Eugenics**

The hereditary theories of T.D. Lysenko are commonly used as an example of how science, Mendelian genetics in this case, can be subverted by a cultural/ideological agenda, in this case Leninism. Lysenko was a Russian geneticist/agriculturalist who resurrected neo-Lamarckian interpretations of inheritance, presumably because they provided a comfortable fit with the "environmentalist" soviet ideology of the 1930’s. The "Lysenko affair" has the trappings of a modern-day melodrama, with the villain being soviet ideology, and the moral the thinly veiled claim that such cultural influence on science is rare, particularly in the West. That the Lysenko story is not so straightforward has been described by the biologists Richard Levins and Richard Lewontin (1985) in their book *The Dialectical Biologist*. For us, though, the significant feature of the Lysenko tale is that it draws attention to the ever-present interconnectedness of science and culture. An example of this interconnectedness can be found in the rise of the science of eugenics in the early part of this century, particularly in the United States and Great Britain. In the words of the coiner of the term, Darwin’s
cousin Francis Galton, eugenics is:

...the science of improving stock, which is by no means confined to questions of judicious mating, but which, especially in the case of man, takes cognizance of all influences that tend in however remote a degree to give to the more suitable races or strains of blood a better chance of prevailing speedily over the less suitable than they otherwise would have had. (Galton, 1883)

The concept of eugenics spread to the popular press, and became a popular idea outside the scientific community. For instance, in Kansas, medals were given to Grade A individuals in the "Governor’s Fitter Family Contest." Vice President Calvin Coolidge asserted in 1924 that "America must be kept American. Biological laws show...that Nordics deteriorate when mixed with other races." The American psychology establishment, who through their involvement with IQ testing contributed to immigration restrictions for certain nationalities, was sympathetic to such pronouncements as that made in 1923 by C. C. Bridgham: "The decline of American intelligence will be more rapid than [that] of European national groups, owing to the presence here of the negro. These are the plain, if somewhat ugly, facts that our study shows. The deterioration of American intelligence is not inevitable, however, if public action can be aroused to prevent it." (reported in Gould, 1981, p. 230)

An indication of the extent to which eugenics permeated the American consciousness was found in Supreme Court Justice Oliver Wendell Holmes’ 1927 opinion on compulsory sterilization, as expressed in Buck vs Bell:

We have seen more than once that the public welfare may call upon the best citizens for their lives. It would be strange if it could not call upon those who already sap the strength of the State for these lesser sacrifices...in order to prevent our being swamped with incompetence....the principle that sustains compulsory vaccination is
broad enough to cover cutting the Fallopian tubes. (Quoted in Gould, 1981, Epilogue)
Flushed with the success of plant and animal breeders, eugenicists sought to apply these new-found tenets to the development of a science of human husbandry. Galton envisioned two ways to improve the "english Stock." Positive eugenics would encourage those from upper class lineages to produce more children, with appropriate incentives from the state if necessary. Negative eugenics was the active discouragement of inferior stock (the "feeble minded") from procreating. Borrowing from his cousin’s evolutionary views, Galton wrote that "what nature does blindly, slowly, and ruthlessly, man may do providently, quickly, and kindly" (quoted in Kevles, p.13).

It is one thing to argue that it would be possible for non-geneticists, including racists, to use the work of geneticists for eugenic purposes. After all, can scientists be held responsible if their intellectual efforts are misused? However, it is quite another thing to claim that societal views, including classist, racist, and sexist ones, could condition the scientific activity of scientists. Yet this is apparently what happened during the early days of eugenics.

In England, leading scientists such as Francis Galton and Karl Pearson not only endorsed eugenic programs, they developed the science that underlay them. In the United States, geneticist Charles Davenport was its champion, his Eugenics Records Office being housed at the prestigious biological research facility--Cold Spring Harbor. The picture painted of Galton, Pearson, Davenport, and other eugenicists in two recent books (Daniel Kevles, In the Name of Eugenics, 1985; and Hamilton Cravens, The Triumph of Evolution, 1988) is not as clean as the progression from objective data to scientific truths to societal implications that is so often claimed to characterize science/society interactions. Rather than a unidirectional channel of influence, a two-directional one prevailed in this case. In the example of eugenics, previously held views of non-white "races," non-British cultures, and the naturalness of class status led at least in part to the development of
hereditary views associated with eugenics. These were the goggles through which data were sought, interpreted, and verified.

Stephen Jay Gould, writing in *The Mismeasure of Man* (1981), makes the point about this two-way influence particularly forcefully:

...I criticize the myth that science itself is an objective enterprise, done properly only when scientists can shuck the constraints of their culture and view the world as it really is....My message is not that biological determinists were bad scientists or even that they were always wrong. Rather, I believe that science must be understood as a social phenomenon, a gutsy, human enterprise, not the work of robots programmed to collect pure information. (p. 21)

Historian of science Will Provine (1973) traced the history of the involvement of geneticists in eugenics. He argued that between the 1920’s and 1950’s, even though no substantive new data became available on "race crossing" in humans, the public positions of geneticists underwent dramatic changes. During that time, advances in basic genetics led to the realization that inheritance wasn’t quite as straightforward as Mendel’s simple dominance relationships. New phenomena, including linkage, multiple alleles, and the increasing awareness of the important effect of the environment on phenotype, made the earlier, more simple views untenable. Whereas the turn-of-the-century view could be characterized by one gene producing one trait, by the end of this period it had become necessary to acknowledge that one gene could influence many traits, and that many genes could influence one trait. It was also beginning to be understood that environmental factors such as temperature could effect the manner in which a gene or genes mapped to a trait or traits. Provine concluded with a provocative question: why had so many geneticists, in light of a relative constancy of data, progressed from being proponents of eugenics, to agnostics, to opponents? He answers his own question:

Few geneticists wanted to argue, as had the Nazis, that biology showed
race crossing was harmful. Instead, having witnessed the horrible toll, geneticists naturally wanted to argue that biology showed race crossing was at worst harmless. No racist nation could misuse that conclusion. And geneticists did revise their biology to fit their feelings of revulsion....It is necessary and natural that changing social attitudes will influence areas of biology where little is known and the conclusions are possibly socially explosive. (p. 796)

So now the story has come full circle. The science of eugenics, which at least partly grew out of 19th century views on social class and race held by prominent scientists such as Galton, Pearson, and Davenport, had become undermined by the public values of post-World War II society.

**Problem-Solving**

We have all solved many problems, both in and out of biology classes. However, there is often a significant difference in what is involved in solving in-class as compared to out-of-class problems. One such difference is that most in-class problems are posed for students. Another difference is that in-class problems can often be solved by using algorithms (rules that, if used correctly for the class of problems that they were designed to solve, will guarantee a "correct answer"), even if a problem solver doesn’t know why an answer is correct.

We have all used algorithms for adding multiple-digit numbers with carrying. It is possible to write such an algorithm in a programming language so that a computer can do the multiple-digit adding. Yet no one would claim that just because the computer can execute the algorithm and produce a correct answer, it understands adding with carrying. Similarly, in genetics, it is common to use an algorithm to generate gamete types from given parental genotypes (simulating meiosis) in preparation for using a Punnett square algorithm to determine offspring genotypes and phenotypes. Successful use of algorithms, however, does
not guarantee that a problem solver has any understanding of the many underlying genetics concepts. So, for example, a solver may have little understanding of chromosomes, genes, alleles, meiosis, or even that a Punnett square represents fertilization. Performance is not always a reliable indicator of understanding, although a major objective of biology instruction should be performance with understanding.

For most problems that we solve outside of class (or that biologists solve) algorithms will not suffice. Everyday problems are too open-ended, too unstructured to be solvable using algorithms. When faced with everyday problems, from finding a hotel in a new city to figuring out a way to explain to parents how the fender of the family car got crumpled, we all resort to a variety of heuristics, or rules of thumb, that allow us to make progress toward a solution, but do not guarantee a correct answer. Heuristics, such as breaking a complex problem into subproblems, working backwards, or thinking of a similar, previously solved problem, may be useful in a wide variety of situations, although there is no guarantee that they will produce the best solution, or even that they will produce any solution.

**Persuasion**

Research is not part of science until colleagues in a research community have been persuaded that the solution to the particular problem is adequate—that is, has both internal logical consistency and consistency with appropriate, accepted knowledge in the discipline within which the solution to the problem is being sought. In the end, experimentation or data analysis is important only in the context of a theory; yet often, student labs stop at the data-collection phase. This is the central point of physicist John Ziman’s (1968) book, *Public Knowledge: The Social Dimension of Science*. In it he asserts:

...a really **good** experiment, a really novel and exciting one, is connected,
in the mind of the experimenter, with the proof of some novel and exciting hypothesis. His communication of the experiment to his colleagues is not merely an exposition of the peculiar events that occurred when he put a piece of litmus paper in the solution; it is an attempt to show that the world behaves as he has conceived it. After the private moment of illumination, there must come the public demonstration, the deliberate process of persuasion. That is why I say that a good experiment is a powerful piece of rhetoric; it has the ability to persuade the most obdurate and skeptical mind to accept a new idea; it makes a positive contribution to public knowledge. (p. 36)

This persuading of peers is important for students, too, if for no other reason than that by doing so they will better understand the importance of persuasion to science. Angelo Collins (1986) talks about "solutions as hypotheses" that can be used to convince peers. They result from the drawing of warranted inferences from well-collected data. No matter how many experiments have been done, how many data have been collected, or how many puzzles have been solved, science hasn’t been done until reports of results have been used to convince peers that an hypothesis is reasonable.

The write-up is the traditional persuasive activity in science laboratories. Unfortunately, practice in scientific writing is not part of many student laboratories. Because of the speed with which reports can be written and revised with computers, this situation could be changed One lesson that could be learned is that writing and persuasion are much more social than simply tabulations of data in a lab notebook.

The social dimension of writing occurs in two contexts. One is multiple authorship: a small team of students will bring more resources to the writing process than will a single person. By taking a team approach to writing, students may be more likely to become aware of the teleology, anthropomorphism, circular reasoning, speciesism, sexism, and racism that can be a part of biological writing. The second context is a larger one, connecting a single report, through citations to other research, to the
..science [is a] continuous activity of criticism, reassessment, and re-evaluation. A scientific paper is seldom the report of an isolated inquiry; it is deeply embedded among all the other papers on the subject. Its content does not become a scientific truth until it has passed through the furnace of critical appraisal and has been adopted by all (or nearly all) the other workers in the field. (p. 28)

The Lab: Parental handling in Two Strains of Mice Reared by Foster Parents

In this lab we will be following the 3P’s of science. The problem posing phase has been initially setup for you, so the main focus of the lab will be on the problem solving and persuasion phases. But in the persuasion phase (the writing up of the lab report, you will be asked to describe problems posed by these results and how you might go about experimentally investigating them).

Introduction and Methods

In the early 1960s experiments in behavioral genetics, with different highly inbred strains of animals, demonstrated behavioral differences among strains. Under the assumption discussed above that environmental conditions were held relatively constant, the results of those early studies have been interpreted as demonstrating direct effects of genetics on behavior (i.e. upward causation). However, as Robert Ressler argued, not all aspects of a mouse pup’s developmental environment can be held constant in the laboratory. Both the prenatal and postnatal environments of each mother may provide a different environment for each litter of pups. Thus, the prenatal and postnatal
environments of the pups are confounded with genotype (confounded in this context means that in previous experiments with inbred strains, the experimental designs did not consider the possibility that either (or both) of the prenatal and postnatal environments of pups may influence their subsequent behavioral development.) Failure to examine the effects of these environments, leaves the question of direct genetic influence indeterminate.

The simulated experiment you will run and analyze is designed to provide some information about the possible differences between the two inbred stains of mice in the parental handling of pups (this would be a component of the pup’s postnatal environment). Handling is an important variable to examine since it is well known that variations in the handling an animal receives either from its natural parents or by human lab technicians can have profound influences on a number of behavioral characteristics in adulthood. Thus considerations of the effects of handling in the postnatal environment are particularly important in assessing whether there are purely direct genetic effects on behavior.

In order to adequately evaluate the differences in handling due to the strain of mice, a simulated cross fostering scheme will be used. To do this, 10 litters of C57 mice will be reared with foster parents of their own strain and 10 litters will be raised by the BALB strain. Similarly, 10 litters of the BALB strain will be raised by foster parents of their own strain, and 10 litters by the C57 strain. This experimental scheme is illustrated below.

The day after litters are switched, parental handling will be measured for 10 successive days. On each test day, you will suppose that the foster parents are removed from their home cage and placed in an empty cage. The pups will be taken from the nest in their home cage and placed at the other end of the cage. The foster parents are then returned to their home cage. The simulation will then record the total number of second of handling of pups by the foster parents (This data is stored in the data
The handling data for all four litters is in the right most data unit. Handling is defined as carrying, dragging or oral manipulation of a pup by either of their foster parents. The total amount of handling recorded for a litter on each day will be divided by the number of pups in the litter to produce a measure of the average amount of handling per pup each day.

**Figure 1.** The structure of the cross fostering experiment. The handling data are in the right most data unit. The other data units contain the handling data plus the litter size data (see Model-2).
Procedure

Open Model-2. Each time you run a simulation, you will get a result that looks like Figure 2. There is a plot of the average handling times per pup per litter over 10 days (i.e. trials). Each simulation run is of one litter in each of the cross fostering conditions. Thus, in order to get 10 litters from each condition, you will have to copy the data from each run into an Excel worksheet. Once the data are properly assembled in an Excel worksheet, the worksheet can be opened in Statview for statistical analysis. 
Figure 2. Data from four litters in each of the cross-fostering conditions. You will have to run 10 simulations to get the 40 litters required for this experiment. In each column (e.g. BALB/BALB) the first entry is the foster parent strain and the second is the litter strain. Note: Delete trial 0 from all simulations, since it is not "real" data. (In order to run the simulations, trial 0 had to be included.)

Hypotheses:

If the prenatal and postnatal environments are truly the same for these two inbred strains of mice, then there should not be a difference in the mean time spent handling each pup in each of the cross fostering conditions.

The mean amount of handling each pup receives should decrease over time as the pups mature and become more and more independent of their parents.

Statistical Analysis

You will be using a two factor (parent strain and pup strain), repeated measures (handling each day) analysis of variance to analyze this data (this is a mouthful! But, you only need to know what the results mean and how to interpret them). The two most difficult aspects of this part of the lab will be figuring out how to get the data into the right form for Statview and interpreting the hypothesis testing analysis you will perform. (see the section below on hypothesis testing).

Discussion

When you finish this lab, you will need to put together a preliminary report of your results. You do not have to give this report to the lab instructor. I will become part of your midterm report for this set of simulated experiments with mice. It is, however, useful to put together an initial report since this will provide the basis of your final midterm report, which will include the results of all of your studies. To get
started, this report should contain the following elements:

1. An introduction to the problem based on what you have read here and what you have learned from class.

2. Describe the procedure that you simulated (do not describe how you performed the simulation). That is, you are to suppose that you actually performed the experiments, and these are the methods you used. They are briefly described above.

3. Present the results of your statistical analysis. Include an analysis of variance table describing what each of its elements are and what it means for the hypotheses you are testing. Visually illustrate your results with figures. Are there any other effects? If so, what do they mean?

4. Discuss your results. The discussion should not merely restate the results, it should interpret these results in light of their implications for direct genetic effects on behavior. Describe in a few sentences one or more experiments you would like to perform to test questions these results have raised for you.

**Hypothesis Testing**

We are assuming that you already understand some basic statistical concepts such as the *mean* and *variance* of a population. Moreover, it is assumed that you have the basic idea of a probability distribution such as a binomial distribution or a normal distribution. If you do not, you can discuss it among members of your group, other groups or us (This is one reason to break up into groups of 3, in your interactions you can share knowledge with each other and your lab reports can be truly emergent!).

In classical hypothesis testing, which you will be doing in this lab, a **statistical hypothesis** is typically a statement about one or more population distributions. It is important to remember that a statistical hypothesis is *always about the populations distributions and not*
about the sample you are using to test the hypothesis. Statistical hypotheses are also typically never exactly the same as "real-world" hypotheses, which are statements about phenomena or their causes. However, when a problem is posed in an appropriate way (i.e. by developing an appropriate research design), statistical hypotheses can be inferred from real-world hypotheses, with the limitation that we must be prepared to relate the structure (i.e. research design) we imposed on the problem to the real world. This is the important issue concerning how carefully designed research applies to real world contexts. This aspect of science belongs to the persuasion stage of scientific discovery.

For convenience exposition, we can represent statistical hypotheses by the letter H. A hypothesis that completely specifies the population may, for example, have the form:

\[ H: \] The population in question (e.g., BALB mice) are normally distributed with respect to mean = 25g weight with standard deviation = 5g.

It is often the case we have (1) less than completely specified hypotheses and (2) that our hypotheses are stated as comparisons between parameters (e.g., means of two populations). For example:

\[ H: \] The populations of BALB and C/57 mice are normally distributed and BALB have larger mean litter sizes than do C/57 mice (i.e. \( \mu(BALB) > \mu(C/57) \)).

To test hypotheses in classical hypotheses testing we set up alternative hypotheses from which to choose. One hypothesis stating the difference we expect to find and the other stating that there is no difference. It is conventional to label the hypothesis we are making as \( H_1 \) and the hypothesis that states there is no difference as \( H_0 \) (i.e. the Null hypothesis). Thus, for example, we would state the hypothesis above as:

\[ H_1: \] The populations of BALB and C/57 mice are normally distributed and have larger mean litter sizes than do C/57 mice (i.e. \( \mu(BALB) > \mu(C/57) \)).
μ(C/57)).

And the null hypothesis as:

**H0**: The populations of BALB and C/57 mice are normally distributed and have the same mean litter sizes as do C/57 mice (i.e. μ(BALB) = μ(C/57)).

We can almost never look at a data sample and from that alone determine which hypothesis is most likely correct. Population means for both populations may be essentially the same, but because our sample size was not the whole population of these mice, the sample means may not be equal (even though the actual populations means may be virtually the same).

What is required is a method for deciding whether a hypothesis is likely true or not. Typically, we have to make this probability calculation for the null hypothesis **H0** simply because we lack sufficient population distribution information to calculate **H1**. To calculate the probability of a null hypothesis in parametric statistics using the null hypothesis **H0**, we assume that the population distributions for both populations are the same and then given the actual sample data, we calculate the probability that the two populations of mice have the same litter sizes.

If the probability of **H0** is sufficiently small, then we should reject **H0** in favor of **H1** if the means are in the direction specified in the hypothesis. It could, of course, turn out that the data favor

**H2**: The populations of BALB and C/57 mice are normally distributed and have larger smaller litter sizes than do C/57 mice (i.e. μ(BALB) < μ(C/57)).

In either case a decision must be made about whether to accept or reject the null hypothesis in favor of one of our alternative hypotheses. It is important to realize that there are many possible decision rules for
rejecting $H_0$ in favor of say $H_1$. In many areas of the biological and social sciences, it is conventional to make this decision based on a threshold probability below which we believe that $H_0$ is too improbable to accept. This probability is the rejection threshold, \( \alpha \) for $H_0$. In classical hypothesis testing, errors can be made! The kinds of errors recognized by statisticians can be summarized in a 2 X 2 table comparing the truth of the statistical hypotheses we make and our decisions:

**True Situation**

<table>
<thead>
<tr>
<th>Decision</th>
<th>$H_0$</th>
<th>$H_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don't reject $H_0$ $p &gt; \alpha$</td>
<td>Correct</td>
<td>Error</td>
</tr>
<tr>
<td>Reject $H_0$ $p \leq \alpha$</td>
<td>Error</td>
<td>Correct</td>
</tr>
</tbody>
</table>

Statisticians distinguish between **Type I** errors (in which the null hypothesis is rejected when in fact its true) and **Type II** errors (in which the null hypothesis is not rejected when in fact it is false). The probabilities of making these two types of errors are $\alpha$ and $\beta$ receptively, and the corresponding correct decisions are $1 - \alpha$ and $1 - \beta$.

The two types of errors are important and not independent. Decreasing the likelihood of one error can increase the likelihood of the other. But in classical hypothesis testing, the Null hypothesis is given the "benefit of the doubt" as in our legal system (i.e. we assume a person is innocent-the null hypothesis--and want the error of conviction when actually innocent to be low) and we set the probability error, $\alpha$, relatively low, typically $\alpha = 0.05$ or $0.01$. In the simulated experiments you will be conducting, the $\alpha$ level you will use is 0.05.

The hypotheses you are testing, as mentioned above, involve two factors
(parent strain and pup strain) and 10 repeated measures (handling over days). Below is what a Statview data sheet would look like for a repeated measures analysis of variance for one factor only (parental strain) and 10 repeated measures. Note that time is a nested variable with 10 measures. Your Statview data sheet will be more complicated because you will have two factors: pup strain in addition to parental strain.

Note: This data sheet has only one categorical variable, strain. In your data you will have Foster Parents as one variable and the strain of the pups as the other. In the Extend data above, BALB/C57 means BALB foster parents and C57 pups. So make two columns instead of one.
Figure 3. Statview data sheet. Time is a compact variable. Note the "compact" button on the upper left corner. Select the columns, press the compact button and give the compact variable a name such as time or trials.

Below is an analysis of variance table. This will be the main output of your analysis. In the first column are the degrees of freedom, the second column the sum of squares, the third the mean square, the fourth is the F-Value, the fifth is the P-Value, and we will not worry about Lambda and the Power of the test. The P-Value is the probability that the Null hypothesis is true based on the data. The P-Value is determined by calculating the F-Value, which is the Mean Square of the condition divided by the subject mean square. (Note that the Mean Square is the Sum of the Squares divided by the degrees of freedom). The ratio of these values has what is called an F-probability distribution. Thus, for example, given the degrees of freedom for the Strain factor (i.e. BALB, C/57), which is 1 (i.e., conditions - 1) and the degrees of freedom for the Subject group, which is 18 (the sum of the two groups minus one for each group, 20 - 2), and the F value, which is their ratio. Statview calculates the probability that the mean handling time for the two strains is the same: F(1, 18) = 15.6, p = 0.009 (note, that by convention we report the F value of an analysis of variance together with the degrees of freedom of the numerator and denominator, F(numerator df, denominator df)). Our alpha criterion is 0.05 for rejecting the null hypothesis. Thus, we conclude that the two strains significantly differ in the amount of handling time they devote to their young.
**ANOVA Table for time**

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>1</td>
<td>205.307</td>
<td>205.307</td>
<td>15.622</td>
<td>.0009</td>
<td>15.622</td>
<td>.974</td>
</tr>
<tr>
<td>Subject (Group)</td>
<td>18</td>
<td>236.562</td>
<td>13.142</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Category for time</td>
<td>9</td>
<td>431.693</td>
<td>47.966</td>
<td>5.265</td>
<td>&lt;.0001</td>
<td>47.385</td>
<td>1.000</td>
</tr>
<tr>
<td>Category for time * Strain</td>
<td>9</td>
<td>185.665</td>
<td>20.629</td>
<td>2.264</td>
<td>.0205</td>
<td>20.380</td>
<td>.896</td>
</tr>
<tr>
<td>Category for time * Subject (Group)</td>
<td>162</td>
<td>1475.870</td>
<td>9.110</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>