Ultimate goals

1. Determine the genetic basis of 'complex' traits.
2. Improve genetic and phenotypic prediction.

using high throughput genomic technologies

BROAD ADVICES

1. DONT BE SCARED, BUT BE RESPECTFUL: **QUALITY CONTROL IS A MUST.**
2. LEARN LINUX, AWK & R as much as you can
3. LEARN A SCRIPTING LANGUAGE: PYTHON SUGGESTED.

Scenario

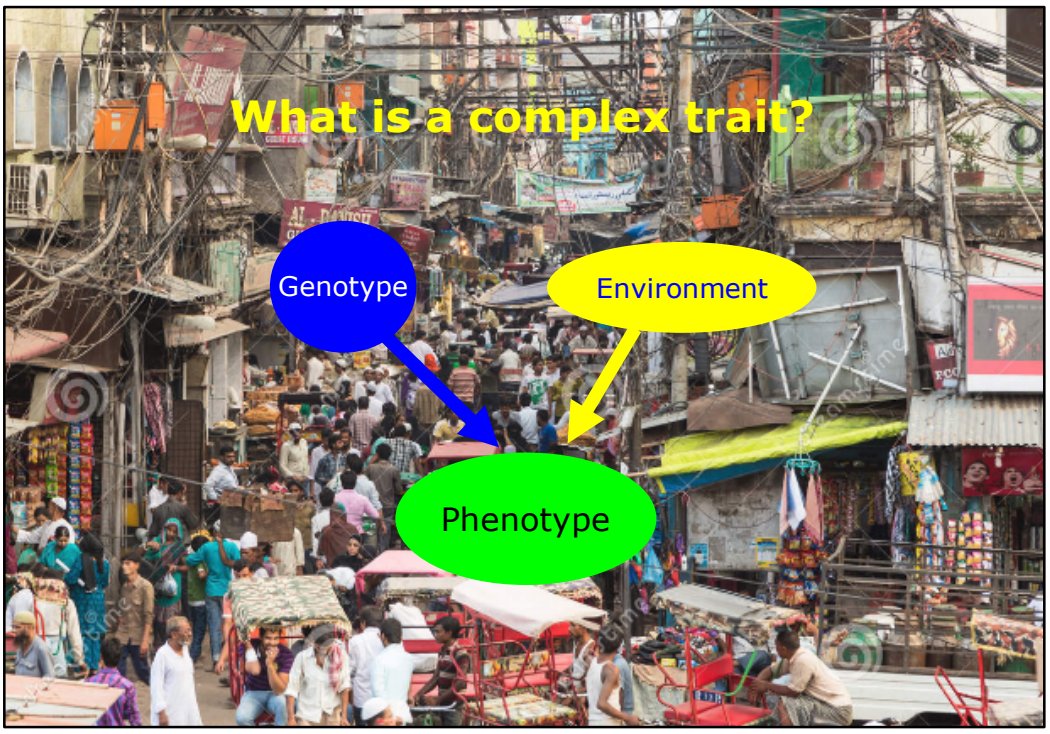
- ✓ Biology has become a data rich science, where the limiting step is the data analysis, rather than obtaining the data.
- ✓ Nevertheless, large data sets with reliable phenotypic measurements and genomic data are still rare.
- ✓ As a result, a combination of computer and experimental approaches is key to success.
- ✓ Although there has been a large gap between human and the rest of species, next generation sequencers have filled these gaps; and the same technologies can be applied to virtually any species in a reasonable time.

What is a simple trait?

A ⟷ ▲

T ⟷ ◆

1 Genotype ⟷ 1 Phenotype




AL - DANISH
GUEST HOUSE
In general, we simplify complexity by taking the linear (additive) part only


AL - DANISH
GUEST HOUSE
Our approach to complexity is indeed VERY simple!

Why traits are 'complex'?

- The primary reason for complexity to arise is a continuous dialogue between the individual's genotype and the environment. This dialogue is fundamental for adaptation and survival, it is builtin and we cannot escape from it (sorry).
- A secondary reason is that the genome itself is complex, it is full of motifs (structural, regulatory,...) and only a tiny part is identified and characterized.
- A third reason is that the genome is highly unstable and resilient, it bears a large number of different kinds of polymorphisms.

Main consequences of complexity

1. There is no unique relationship between phenotype and genotype. The relationship is measured instead in probabilistic terms.
2. As a result, we cannot escape from Statistics and Mathematics (sorry again).
3. It is going to be (very) difficult to prove causality.
4. Many unreplicated results. Many undiscovered causal mutations <=> High type I and type II error rates <=> High similarity between alternative models.
5. Dominance, epistasis, GxE interaction …

Main genomic data types

- ✓ DNA Sequence (e.g., GenBank)
- ✓ DNA polymorphism (marker, e.g., dbSNP)
- ✓ RNAseq (functional genomics, e.g., GEO), epigenomics…
- ✓ Annotation data, including interactions, pathways,…

http://www.ncbi.nlm.nih.gov/sites/entrez

Outline of this course

1. Background
2. Genome Wide Association Analysis
3. Genomic Selection
4. Sequence Data Analysis
5. Big Data and Machine Learning

1 - Background

1. Population Genetics Concepts
2. Statistics Concepts
3. Statistics vs. Machine Learning

2 - Genome Wide Association Studies (GWAS)

1. Why disequilibrium?
2. What could go wrong?*
3. Testing: False Discovery Rate (FDR)
4. Population Structure
5. Mixed Models

* almost everything…

3 - Genomic Selection (GS)

1. Fit vs. Prediction in modern genomics
2. The large p, small n paradigm
3. Variable selection vs. Ridge regression methods
4. Merging data: Single Step
5. GS using sequence data

4 - Sequence Data Analysis

1. It is all about quality
2. Main data formats
3. Genomic pipeline
4. RNAseq pipeline
5. Metagenomics pipeline

5 - Big Data and Machine Learning

1. Big is Beautiful
2. Big Datasets in Genomics
3. Machine Learning vs. Statistics
4. Ensemble Methods
5. Deep Learning

BACKGROUND:
Population Genetics Concepts

Genetic Variability

- Factors that affect variability
  - Mutation: the ultimate source of all variability
  - Recombination
  - Genetic drift
  - Selection
  - Migration

- Measures of variability
  - Number of pairwise differences per length DNA sequenced (Tajima's)
  - Number of SNPs per length DNA sequenced (Watterson's)

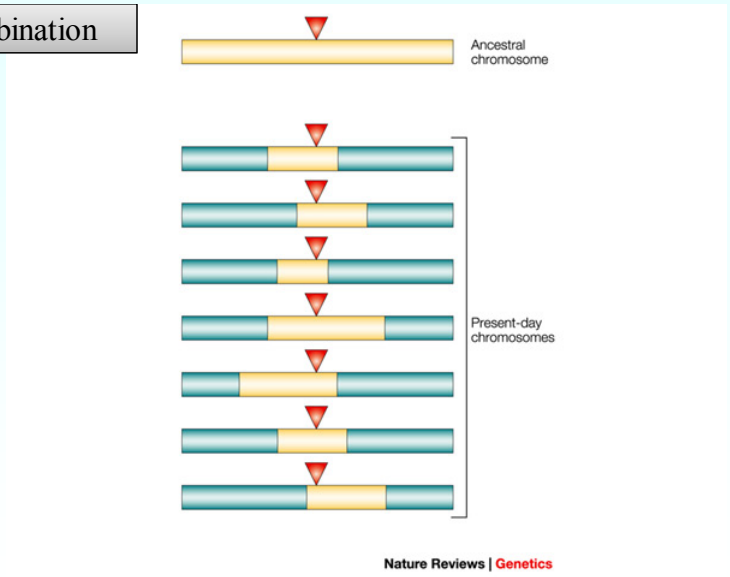
Sources of variability: Mutation

- It is caused by errors in DNA replication.
- DNA variability would soon become exhausted if mutation did not exist.
- Although mutation occurs in all mitotic and meiotic cell divisions, the only mutations that matter for genetic variability are those that occur in the germ line and are transmitted to offspring.
- Mutations cause different kinds of polymorphisms: SNPs, microsatellites, copy number variations ...
- Mutation rate varies along the genome, between sexes and across evolutive lineages.

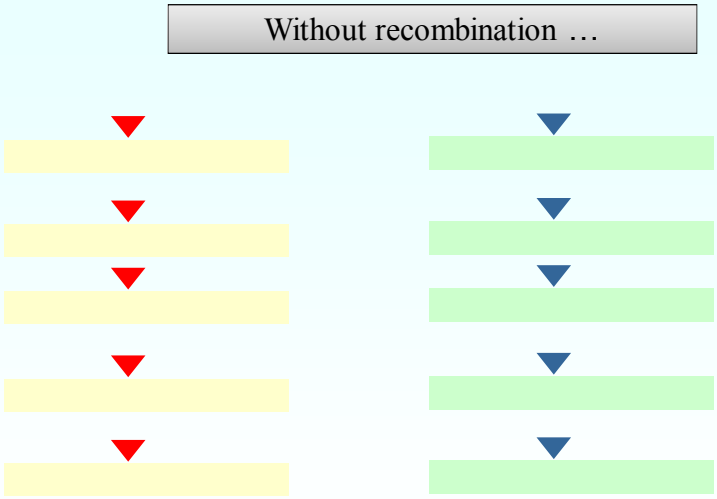
Sources of variability: Recombination

- Its frequency is not constant along the genome and differs between sexes.
- It is also affected by the environment and is partially under genetic control. Recombination rate is a trait that can be selected for.
- It breaks down the genealogical history shared between two linked loci, making it possible association mapping, i.e., it establishes a link between physical distance and linkage disequilibrium.

recombination



Ardlie et al. 2002



NO GWAS!!

Sources of variability: Drift

- Genetic drift it refers to the changes in allele frequencies caused by sampling. It is an unavoidable consequence of the finite size of populations.
- Given a population of size N and an allelic frequency p, the probability that stays the same in the next generation is given by the binomial distribution:

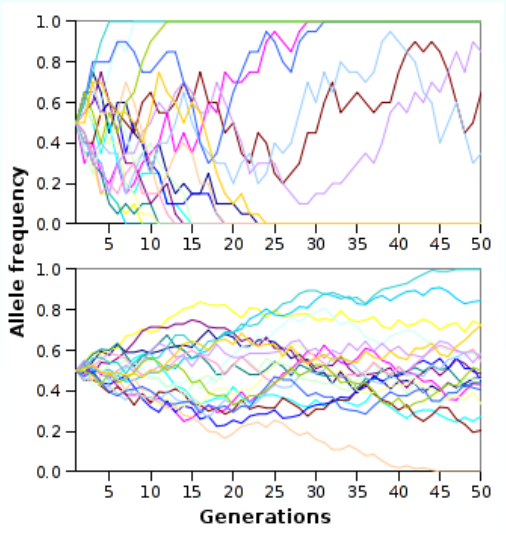
$$P(p_1 = p_0 \mid N, p_0) = \binom{N}{Np_0} p_0^{Np_0} (1 - p_0)^{N(1-p_0)}$$

Sources of variability: Drift

$$P(p_1 = p_0 \mid N, p_0) = \binom{N}{Np_0} p_0^{Np_0} (1 - p_0)^{N(1-p_0)}$$

- Mean: Np_0
- Variance: $N\ p_0\ (1\text{-}p_0)$, maximal at $p = 0.5$
- If nothing else happens, eventually either allele 1 or 2 will become fixed.
- The probability of fixation, under the neutral model (Kimura), is simply its present allelic frequence.
- Note that this process is a Markov chain process: what happens in generation t depends ONLY on the situation at generation t-1, and not on earlier generations.

Genetic drift



N=10

N = 100

wikipedia

Exercise: drift.R script

http://scit.us/redlynx/

```
#----- simulates drift
drift <- function(N,f) {
   t=0
   while(f>0 & f<1) {
      genotypes <- rbinom(N,1,f)
      f<-mean(genotypes)
      t=t+1
   }
   c(t,f)
}
```

Sources of variability: Selection

- It is usually the main force we are interested in, because selection targets the loci for traits of relevance: fitness (natural selection) or phenotypes of economic interest (artificial selection).
- It affects only a small subset of loci, whereas drift influences the whole genome.
- Several kinds of selection: directional, balancing, purifying, artificial …
- Normally, we are only interested in directional selection.

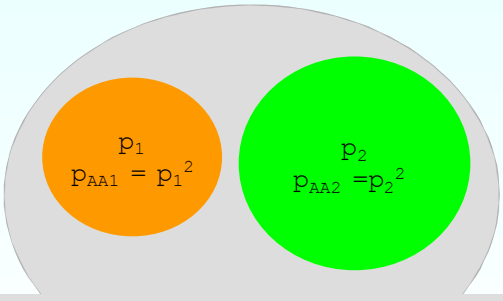
Sources of variability: Selection

- Directional selection: an allele is selected for because it confers an adaptive advantage. It results in a selective sweep and in a decrease in variability in the surrounding genome.
- Balancing selection: Selection favors the presence of more than one allele. There are different non mutually exclusive causes: heterozygote advantage (overdominance), frequency dependent selection, environmental heterogeneity. It results in an increased variability.
- Background selection: selection against deleterious alleles maintained by recurrent mutation. Difficult to distinguish from the neutral hypothesis.

Sources of variability: Admixture

- Finite population size causes genetic drift.
- Neutral alleles will evolve differently in each population (because it is a Markovian process), causing a differentiation between populations.
- Admixture or migration between individuals of different populations cause a departure from expectation of the null model.
- A common phenomenon is analyzing a pool of populations ignoring opulation structure (Wahlund's effect).

Sources of variability: Admixture



$$p_T = (p_1N_1+p_2N_2) / (N_1+N_2)$$

$$p_{AAT} = (p_{AA1}N_1+p_{AA2}N_2) / (N_1+N_2) > p_T^2$$

Wahlund's principle: apparent excess of homozygotes (deficit of heterozygotes)

Measures of admixture: F statistics

Population structure (subdivision) results in an increased inbreeding – or decreased heterozygosity – relative to a single population of equal sum of sizes.

Three levels can be distinguished:

- H_I = Individual heterozygosity in a population, observed heterozygosity averaged across subpopulations.
- H_S = Expected individual heterozygosity in an equivalent random mating subpopulation.
- H_T = Expected individual heterozygosity in an equivalent random mating total population.

Measures of admixture: F statistics

Observed heterozygosity over m populations:

$$H_I = \sum_{i=1}^{m} H_i / m$$

Expected HW heterozygosity in subpopulation s, a alleles:

$$H_S = 1 - \sum_{j=1}^{a} p_{j,S}^2 ; \quad \overline{H}_S = \sum_{s=1}^{m} H_s / m$$

Exp. heterozygosity in an equivalent total population

$$H_T = 1 - \sum_{j=1}^{a} \overline{p}_j^2$$

Reduction in Hi within its subpopulation:

$$F_{IS} = \frac{\overline{H}_S - H_I}{\overline{H}_S}$$

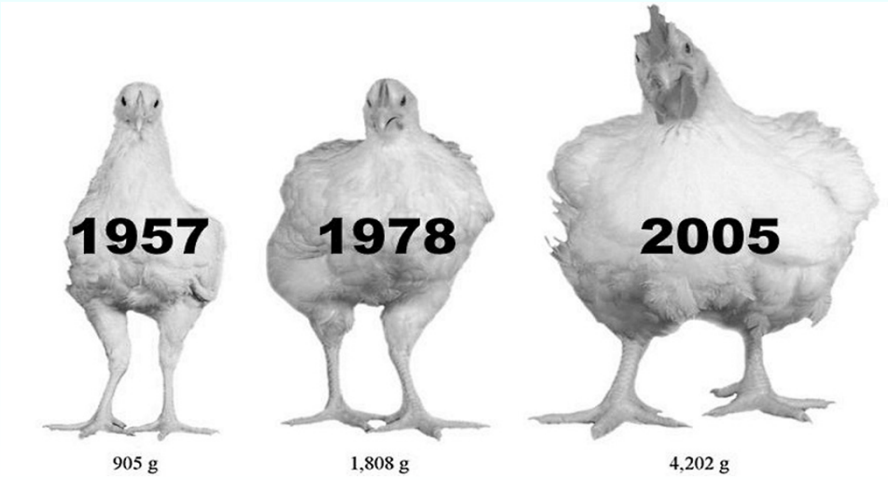
Reduction in heterozygosity because of population subdivision

$$F_{ST} = \frac{H_T - \overline{H}_S}{H_T}$$

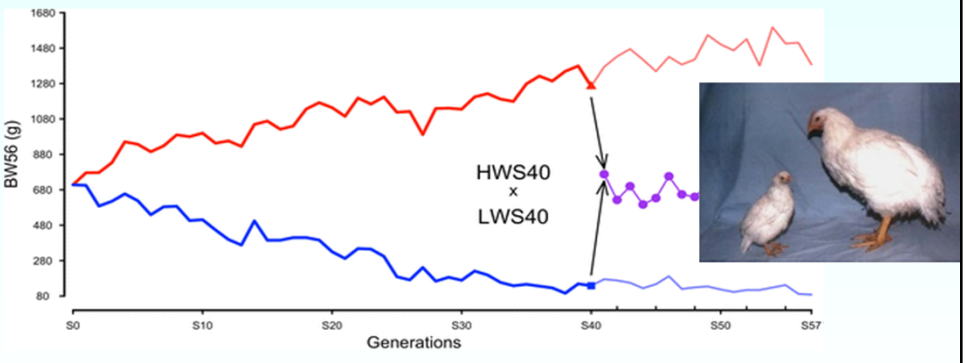
Sources of variability: Selection

- Artificial selection: differential reproduction according to some criteria set by humans.
- It is extremely efficient.
- Involves strong bottlenecks.

Increase in average performance: chicken



Divergence selection in animals

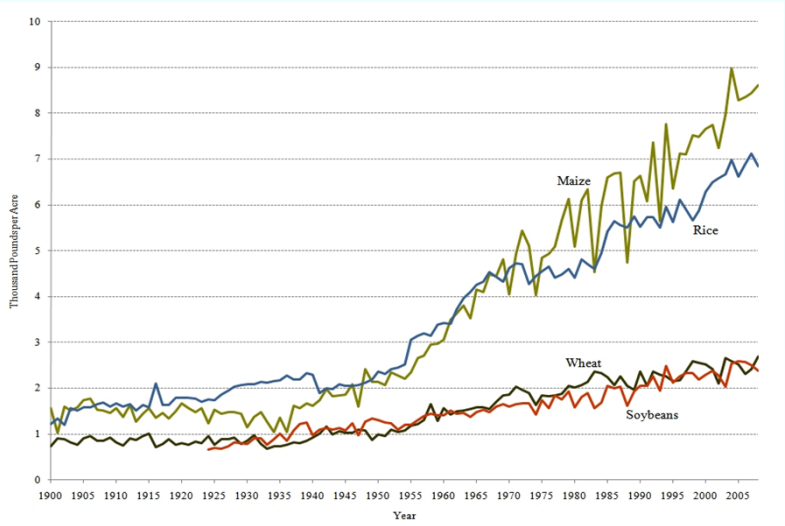


Long-Term Divergent Selection for Eight-Week Body Weight in White Plymouth Rock Chickens

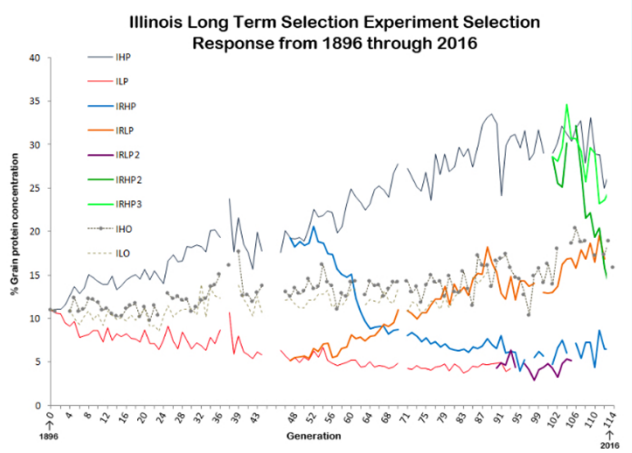
E. A. DUNNINGTON and P. B. SIEGEL

Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0306

Increase in average performance: cereals



Illinois long term selection experiment http://mooselab.cropsci.illinois.edu/longterm.html



In 1896, University of Illinois scientists initiated an experiment that is now the longest running continuous genetics experiment in higher plants. Beginning with an open pollinated variety of maize, Burr's White, the founders selected for ears with the highest or lowest concentrations of grain protein or oil. Over a century has elapsed, and one-hundred fifteen cycles of recurrent selection has created twelve populations that vary significantly in their grain protein and oil composition.

Perhaps the most surprising result of modern genomics is how dramatic phenotypic changes are accompanied by barely undetectable changes in DNA sequence

This is called 'The infinitesimal model'

It presupposes that quantitative traits are explained by a large number of genes, each acting individually and of small effect. In addition, quantitative traits are also modified by the environment.

XV.—**The Correlation between Relatives on the Supposition of Mendelian Inheritance.** By **R. A. Fisher**, B.A. *Communicated by* Professor J. Arthur Thomson. (With Four Figures in Text.)

(MS. received June 15, 1918. Read July 8, 1918. Issued separately October 1, 1918.)

Practicals: chapter 1

BACKGROUND:
Statistics Concepts

In God we trust, all others bring data*

* Found in Tibshirani et al., attributed to both Deming and Heyden

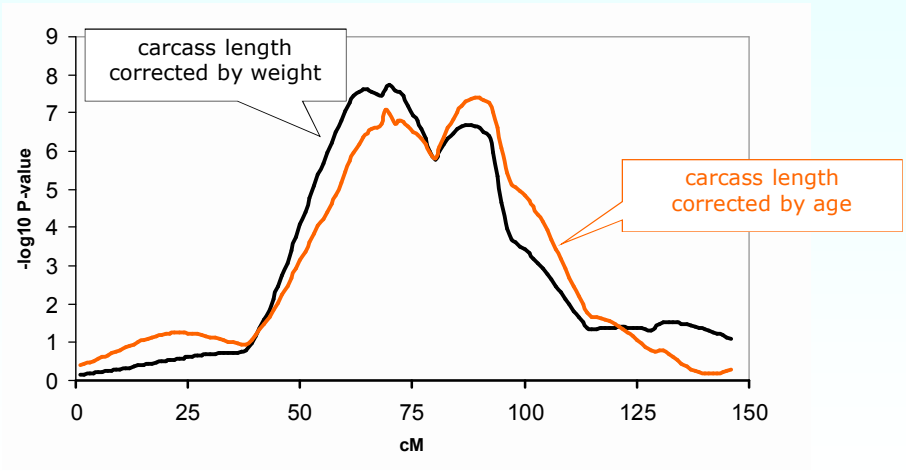
Statistics

- The term 'Statistics' and 'State' share the same etimology, as Statistics was initially (end 18th century) a means for the State to have censuses. This is now called **descriptive** statistics.
- But the real importance of Statistics in science lies in its role as **quantifying uncertainty**.
- The concepts of **inference** and **model** are central to this goal.
- Statistics is not a coherent and unified framework, there coexist many different schools (frequentist, Bayesian, non parametric) where many angry disputes have been witnessed.

Concept of Model

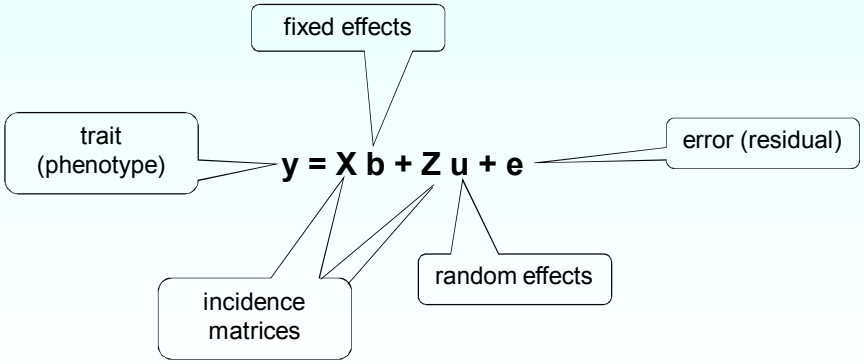
- A model is an abstraction of reality, it never exists a *true* model.
- 'The value of a model is that it often suggests a simple summary of data in terms of the major systematic effects together with a summary of the nature and amount of unexplained variation' (McCullagh and Nelder, 1983).
- Two desirable characteristics: **parsimony** and **goodness of fit**.
- In Statistical Genetics, a model is also a definition of the trait.

A model is a definition of a trait



Iberian x Landrace pig cross, SSC4

Usual models: Mixed Linear Models



Usual models: Mixed Linear Models

$$\mathbf{y = X\,b + Z\,u + e}$$

$$\begin{pmatrix} y \\ u \\ e \end{pmatrix} \sim N\left[\begin{pmatrix} Xb \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} ZGZ'\sigma_u^2 + I\sigma_e^2 & ZG\sigma_u^2 & I\sigma_e^2 \\ GZ'\sigma_u^2 & G\sigma_u^2 & 0 \\ I\sigma_e^2 & 0 & I\sigma_e^2 \end{pmatrix} \right]$$

Normality and additivity are two tightly linked phenomena

Inference methods: General

- Statistical inference means quantifying the values of the parameters in the model, e.g., b in previous formulas, or θ in general terms.
- It is about prediction rather than description.
- There are several inference frameworks (frequentist, Bayesian...) and several criteria within each framework (maximum likelihood, minimum least squares, maximum a posteriori, ...).

Inference methods: Maximum Likelihood

$$\mathbf{y = X\,b + Z\,u + e}$$

$$V = ZGZ'\sigma_u^2 + I\sigma_e^2$$

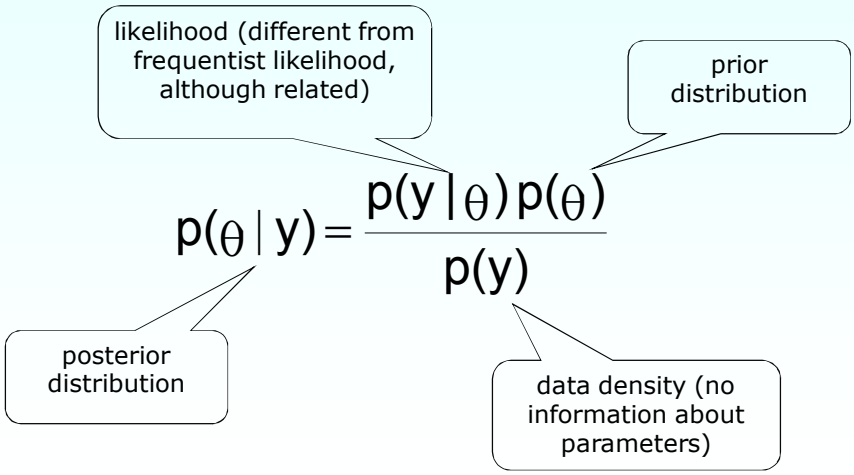
$$L(y) = k\,|V|^{-1/2}\,\exp\{-0.5(y-Xb)'\,V^{-1}\,(y-Xb)\}$$

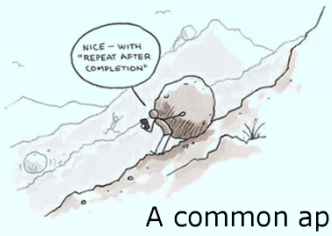
- A maximum likelihood estimate is the the set of values b and variances (σ^2) that maximizes the (log) likelihood.
- Nice properties but difficult to compute in most cases.

Inference methods: Bayesian methods

- In a frequentist framework (like the maximum likelihood estimate) parameters are evaluated in terms of how good is its performance under conceptual repeated sampling.
- In the Bayesian approach, inference is conditioned on the actual data, and parameters are evaluated in terms of decision theory.
- While the parameters are fixed unknown quantities that we want to know with as least error as possible in the frequentist paradigm, for Bayesian theory the parameters follow a distribution that reflect the uncertainty that we have about them.
- Bayesian methods are generally intensive computationally, and depends on fine tuning that requires a certain expertise.

Inference methods: Bayesian methods





Inference methods: Bayesian methods

A common approach for inference (obtaining the marginal posterior) for each parameter is Gibbs sampling.

Sample $p(\theta_1|\theta_2,\theta_3,..\theta_n,y)$
Sample $p(\theta_2|\theta_1,\theta_3,..\theta_n,y)$
…
Sample $p(\theta_n|\theta_2,\theta_3,..\theta_{n-1},y)$

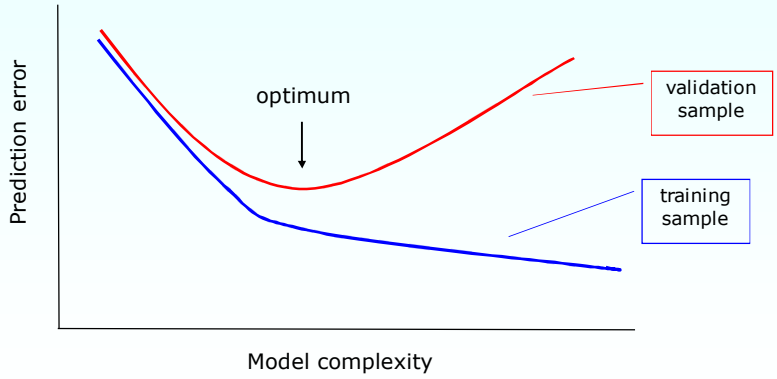
→

$p(\theta_1|y)$
$p(\theta_2|y)$
…
$p(\theta_n|y)$

conditional posterior

marginal posterior

Model comparison: A modeling tradeoff



Hastie et al. Elements of Statistical Learning

Model comparison

- Goodness of fit (distance between prediction and observation) is a good thing.
- But an increase in number of parameters should be penalized. This means that parsimony is also a desirable property.
- Model comparison is basically about choosing a compromise between these two concepts. There are many criteria to choose the best model. The ranking of models will depend on the criterion chosen.

Model comparison

- Likelihood ratio test: for two hierarchical models that differ in p parameters, $-2\ln (L_1/L_0) \sim \chi^2$ with p d.f. under the null hypothesis that L_0 is the true likelihood.
- Akaike's Information Criterion: AIC = $-2\ln L + 2k$. The lower the better.
- Bayesian Information Criterion (BIC) or Schwartz criterion: BIC = $-2\ln L + k \ln(n)$, where k is number of parameters and n is number of observations. It is more conservative than AIC. The best model is that with lowest BIC, as in AIC.
- Crossvalidation: The most sensible and pragmatic approach. The method of choice in machine learning.

Model comparison: Bayesian criteria

- An usual approach is to compare **Bayes factors** between competing models M_1 and M_2.

$$BF_{12} = \frac{p(y \mid M_1)}{p(y \mid M_2)} = \frac{\int p(y \mid \theta_1, M_1)\, p(\theta_1 \mid M_1)\, \partial\theta_1}{\int p(y \mid \theta_2, M_2)\, p(\theta_2 \mid M_2)\, \partial\theta_2}$$

- Bayes factor is often interpreted as the odds in favor of one or other model given by the data (although BF depend in general on prior choice).
- Similar to likelihood ratio test except that uncertainty about parameters is averaged out and that non hierarchical models can be compared.

Multiple comparisons: Bonferroni correction

- Recall the definition of type I error, α, it is the number of times the null hypothesis is rejected and is true. At the usual α=0.05 the number of times H_0 is rejected can be very high if the number of tests say SNPs is very high.
- A traditional approach has been to control the total number of false positives by raising the significance threshold.
- Bonferroni correction is to set the threshold to α/n where n is the total number of tests. Bonferroni is highly conservative as it assumes independence between tests.

Multiple comparisons: Permutation tests

- Under H_0 the relationship between the data and the parameter is circumstancial.
- Thus, one can generate the distribution of a given statistic by randomly shuffling the data and the explanatory variable(s).
- Advantages: robust, it does not assume any null distribution, easy to compute.
- Inconveniences: difficult to apply with hierarchical tests (e.g., 2 QTL vs. 1), when there is an additional relation between individuals (e.g., relationship matrix).

Multiple comparisons: False discovery rate (FDR)

- FDR is the number of false significant tests divided by the total (false + true) number of significant tests.
- Suppose a number n of P-values ranked from lowest to highest $P_1 < P_2 < \ldots. P_n$, then a β FDR is attained by selecting P-values below the threshold (find the largest k s.t.):

$$P_k \leq \frac{k}{n}\beta$$

Multiple comparisons: Example

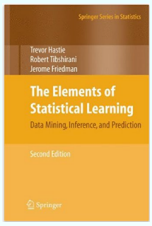
α=0.05/10

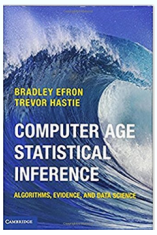
i	P-value	i/n	i/n 0.05	FDR=0.05	i/n 0.5	FDR=0.5	Bonferroni
1	0,002	0,1	0,005	-0,003	0,05	-0,048	0,002
2	0,001	0,2	0,01	-0,009	0,1	-0,099	0,001
3	0,003	0,3	0,015	-0,012	0,15	-0,147	0,003
4	0,006	0,4	0,02	-0,014	0,2	-0,194	0,006
5	0,091	0,5	0,025	0,066	0,25	-0,159	0,091
6	0,3	0,6	0,03	0,27	0,3	0	0,3
7	0,34	0,7	0,035	0,305	0,35	-0,01	0,34
8	0,42	0,8	0,04	0,38	0,4	0,02	0,42
9	0,54	0,9	0,045	0,495	0,45	0,09	0,54
10	0,73	1	0,05	0,68	0,5	0,23	0,73

Multiple comparisons: Important remark

Unfortunately, one of the consequences of complexity is that raising the significance threshold over a given (unknown) optimum does not reduce the rate of false positives but does decrease power.

BACKGROUND:
Statistics vs. Machine Learning





- Machine Learning is a wide field related to developing algorithms that can automatically identify patterns in data (and use them for prediction of future records).
- Statistics and ML can be highly interrelated with many shared concepts and procedures (albeit often with distinct vocabulary).
- Historically, ML has been developed by Computer Scientists whereas Statistics has been linked to Mathematical Faculties. This is no longer true, especially with intensive use of computers by modern Statistics.

Statistics	Machine Learning
➢ Focused on inference	➢ The target is prediction
➢ Based on Models	➢ Model free
➢ Theoretically founded	➢ Pragmatic
➢ Problem constrained	➢ Data heterogeneity is no problem
➢ Clear interpretation	➢ Often cannot be interpreted
➢ General solutions	➢ Specific solutions