Random Effects, and what to do when there is no existing method.

Biology 683

Heath Blackmon

Other kinds of regression

Logistic regression allows us to fit a binary response variable (absent/present; alive/dead) with one or more categorical or continuous predictor variables.

Poisson regression allows us to fit a response variable that is Poisson distributed (number of extinctions in a unit of time, number of colonies per plate, (number of occurrences for rare events)) with one or more categorical or continuous predictor variables.

```
fit.logi <- glm(obs ~ pred2 , family="binomial")

fit.pois <- glm(obs ~ pred2, family="poisson")
```

GLM and LM function in R

- The GLM and LM function in R takes equations that can be described with the following operators

+	+X include this variable
:	X:Z include the interaction between these variables
*	X*Y include these variables and the interactions between them
^	(X + Z + W)^3 include these variables and all interactions up to three way

R versus the math implied

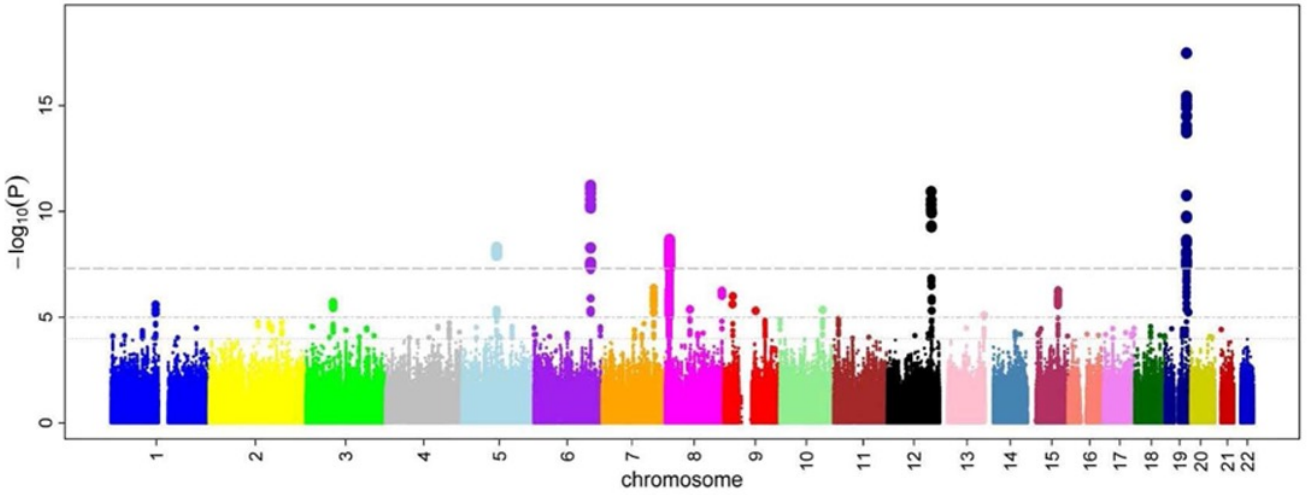
```
glm(y ~ X + W)
```

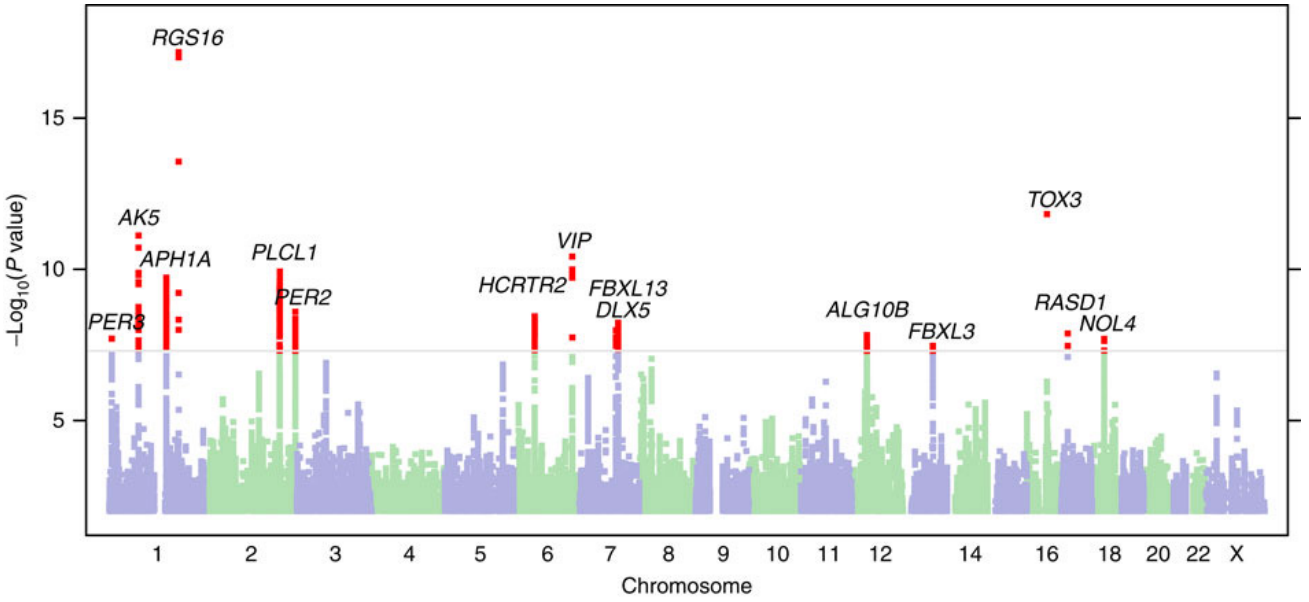
$$y_i = \beta_0 + \beta_1 X_i + \beta_2 W_i + \epsilon_i$$

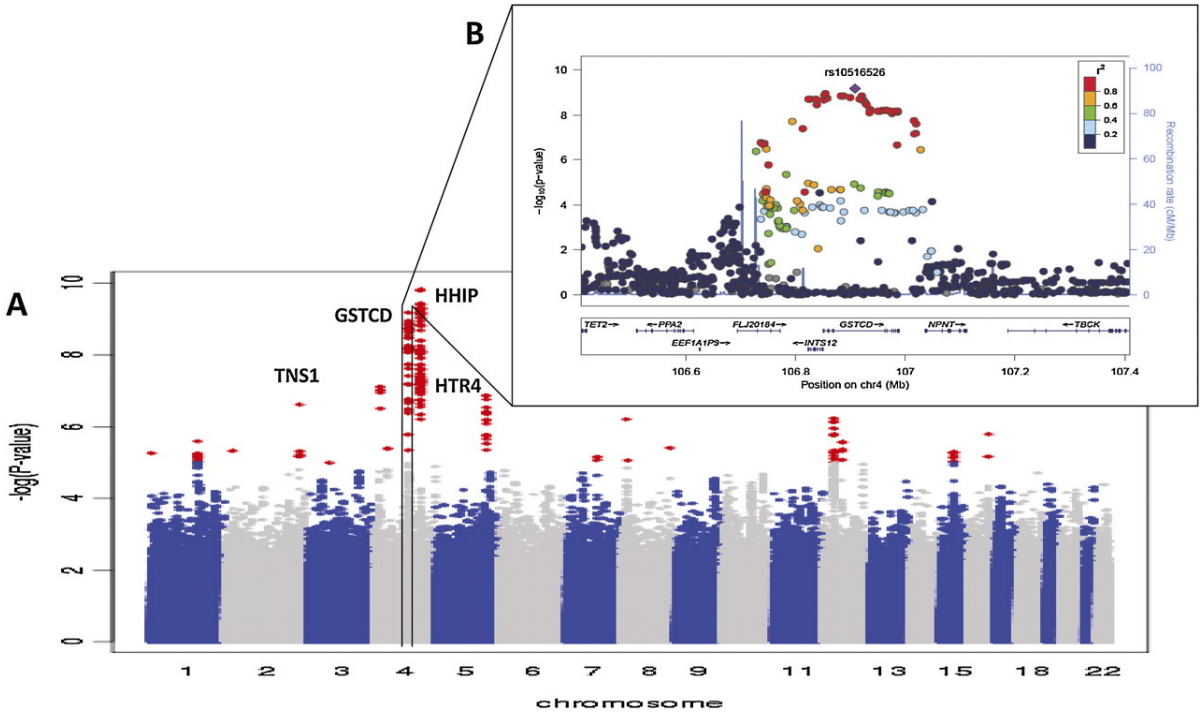
```
glm(y ~ X * W)
```

$$y_i = \beta_0 + \beta_1 X_i + \beta_2 W_i + \beta_3 X_i W_i + \epsilon_i$$

GWAS


−log10(P)
chromosome


RGS16
AK5
APH1A
PER3
PLCL1
PER2
HCRTR2
VIP
FBXL13
DLX5
ALG10B
FBXL3
TOX3
RASD1
NOL4
−Log10(P value)
Chromosome


A
B
rs10516526
TNS1
GSTCD
HHIP
HTR4
-log(P-value)
chromosome
Position on chr4 (Mb)

GWAS

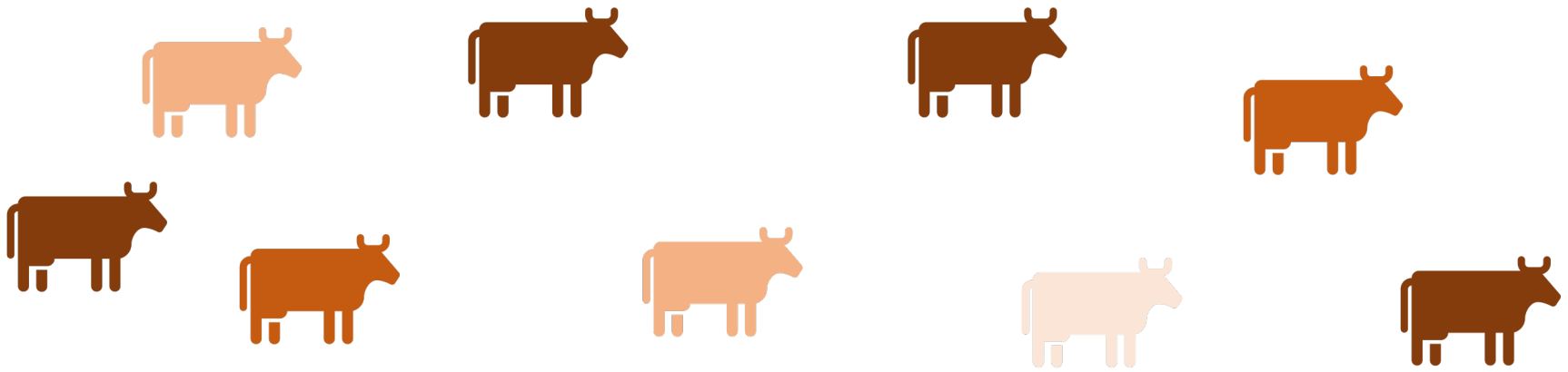
GWAS: Genome wide association study.

The goal of GWAS is to determine what genes have alleles that are responsible for a trait of interest. The trait can be any measurable trait in any organism that you wish to study. For instance, a disease in humans, an economically important trait of a crop or domestic animal, an adaptation like a certain color pattern in birds, etc.

GWAS – Discrete condition (often disease)


```
>case 1
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>case 2
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
>case 3
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>control 1
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
>control 2
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>control 3
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
```

GWAS – Continuous trait



```
>sample 1
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>sample 2
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
>sample 3
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>sample 4
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
>sample 5
CATACTACTACTGAACGTTTGCTCCTGCtactatctctctctctctctttctctctctctctctCATGC
>sample 6
AGTTGACTACTGCATACTCGTGCTAGCTGACTGTCGTACGTACGTAGCTAGTGATCGATCGATGCTAGCTA
```

GWAS – Continuous trait

AGTTGACTACTGCATACTCGT
AGTTGACTACTGCATACTCGT

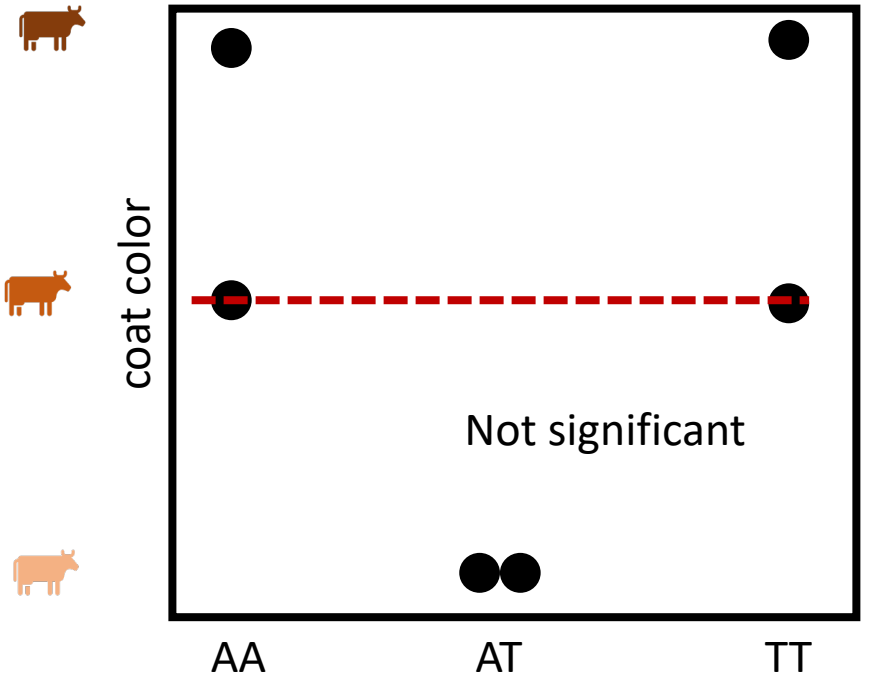
AGTTGACTACTGCATACTCGT
AGTTGACTACTGCATACTCGT

TGTTGACTACTGCATACTCGT
AGTTGACTACTGCATACTCGT

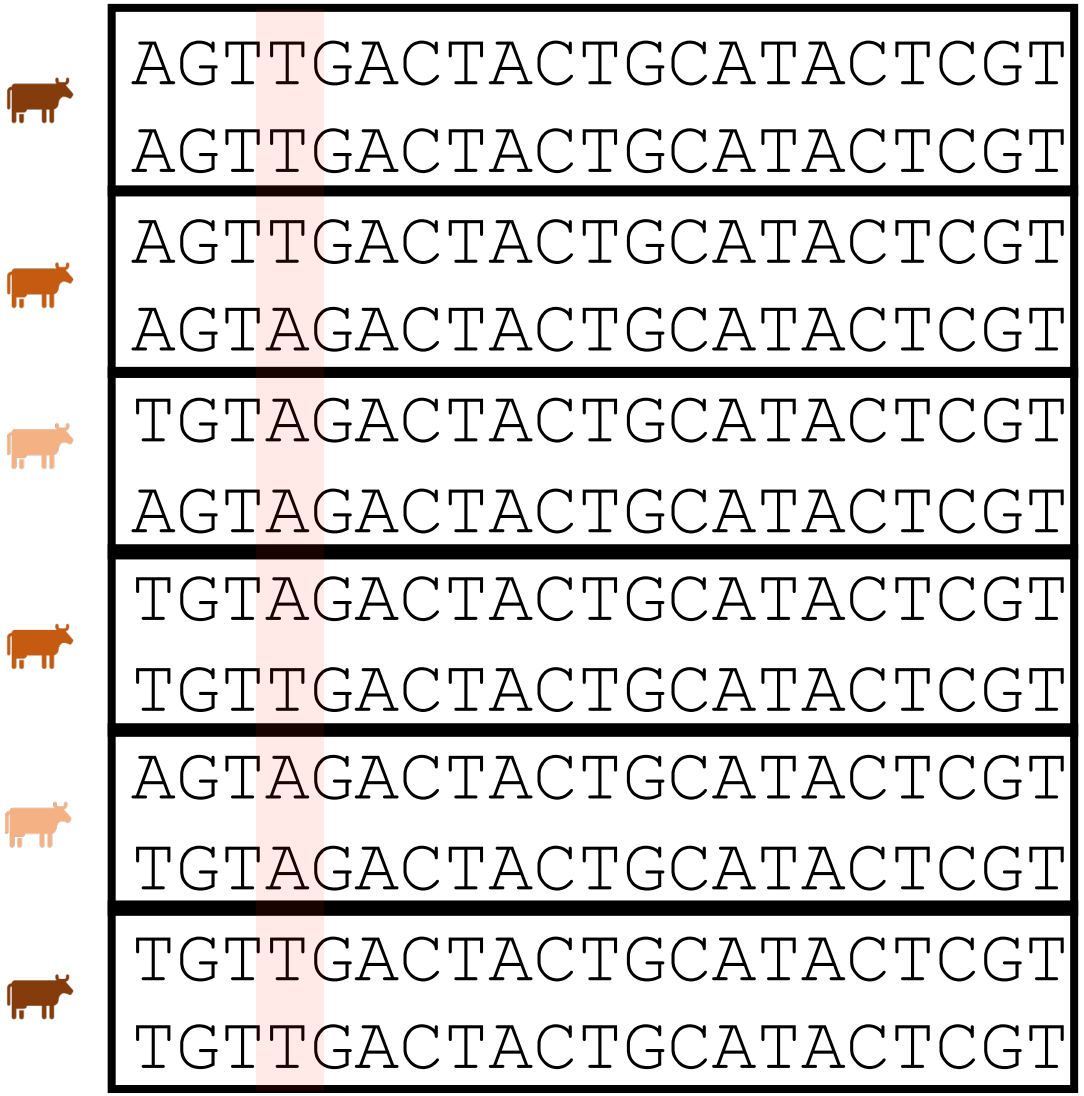
TGTTGACTACTGCATACTCGT
TGTTGACTACTGCATACTCGT

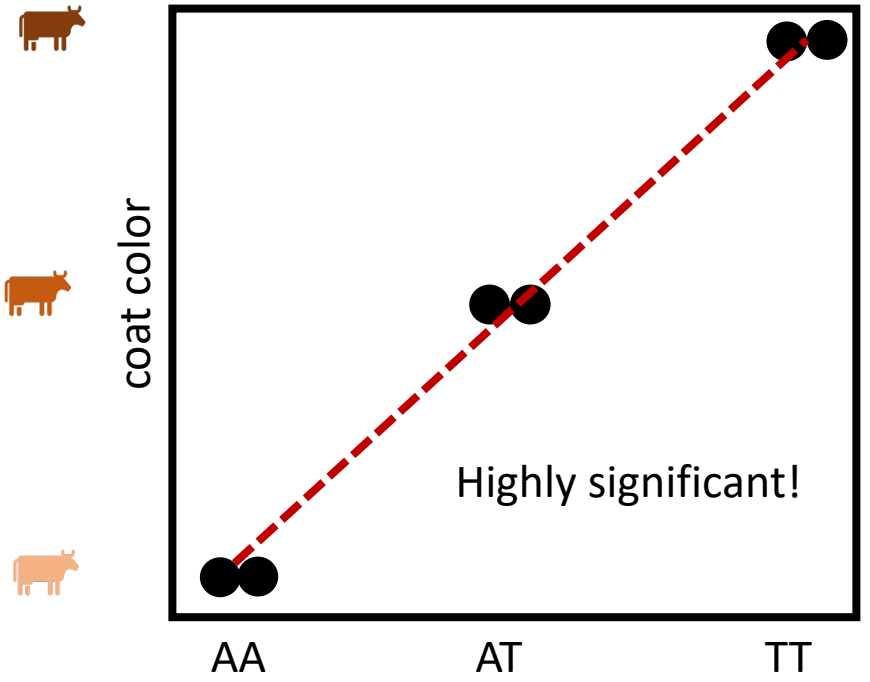
AGTTGACTACTGCATACTCGT
TGTTGACTACTGCATACTCGT

TGTTGACTACTGCATACTCGT
TGTTGACTACTGCATACTCGT



GWAS – Continuous trait





GWAS - Problems

What is the problem with doing this across the whole genome?

Multiple tests lead to more false positives!

1) require a higher level of significance $5x10^{-8}$
2) only look at the very most significant
3) lots of more complicated approaches too!

What is one of the most basic requirements of almost all statistical tests?

Tests normally assume independence of the data points!

1) Samples from a population will be related to each other due to ancestry (trees!)
2) DNA sequencing is not done equally in all groups of people (western samples are usually over represented

GWAS - Problems

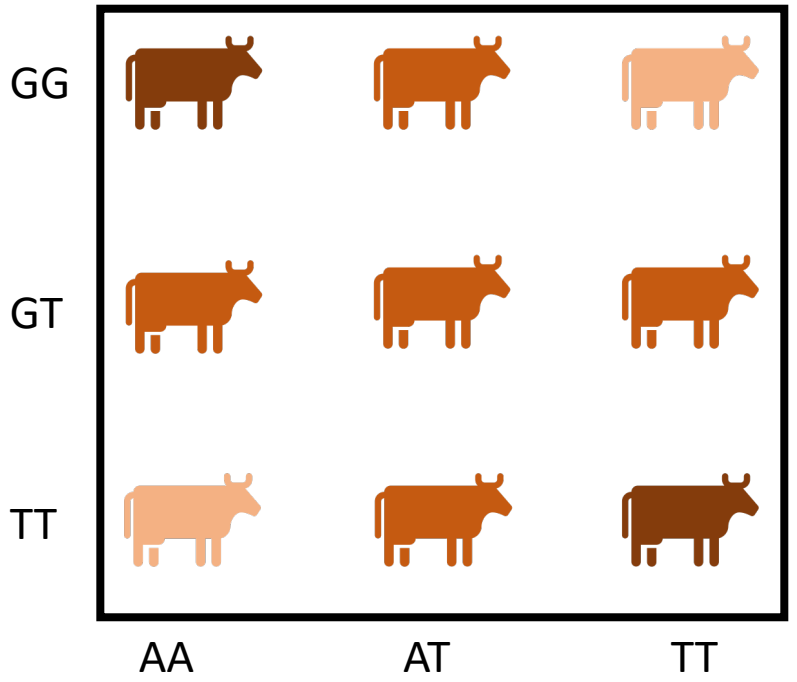
What is epistasis?

It is the case where the impact of a genotype at one locus depend on the genotype at another locus!

To find this type of effect you would need to look at all pairs of genotypes. How many tests would we need to do then?

About 6 orders of magnitude more tests would be required.

Humans have around 4,000,000 sites that are variable like this which equates to 7,999,998,000,000



GWAS - Problems

What about the environment?

Many diseases have a strong environmental component (heart disease, diabetes, cancer, etc.)

If these are left out of the study often what is discovered is actually genetic variation that happens to coincide with environmental factors?

If a disease is more common in Europeans than Africans or Asians but it is because of a lifestyle characteristic any genetic variation that is common in Europeans but rare in Africans and Asians could appear associated with the disease.

Mixed models

Mixed models are models that include fixed and and random effects.

Fixed effects can be repeated by other researchers. These are the variables that you are interested in studying.

Random effects are usually nuisance parameters. These are variables that other researchers cannot replicate and you are not interested in inferring anything about them.

Mixed models

Fixed effects are the variables whose impact we wish to determine

- Characteristics of the media or habitat
- Experimental treatments
- Age groups
- Time points
- Mutant genotypes

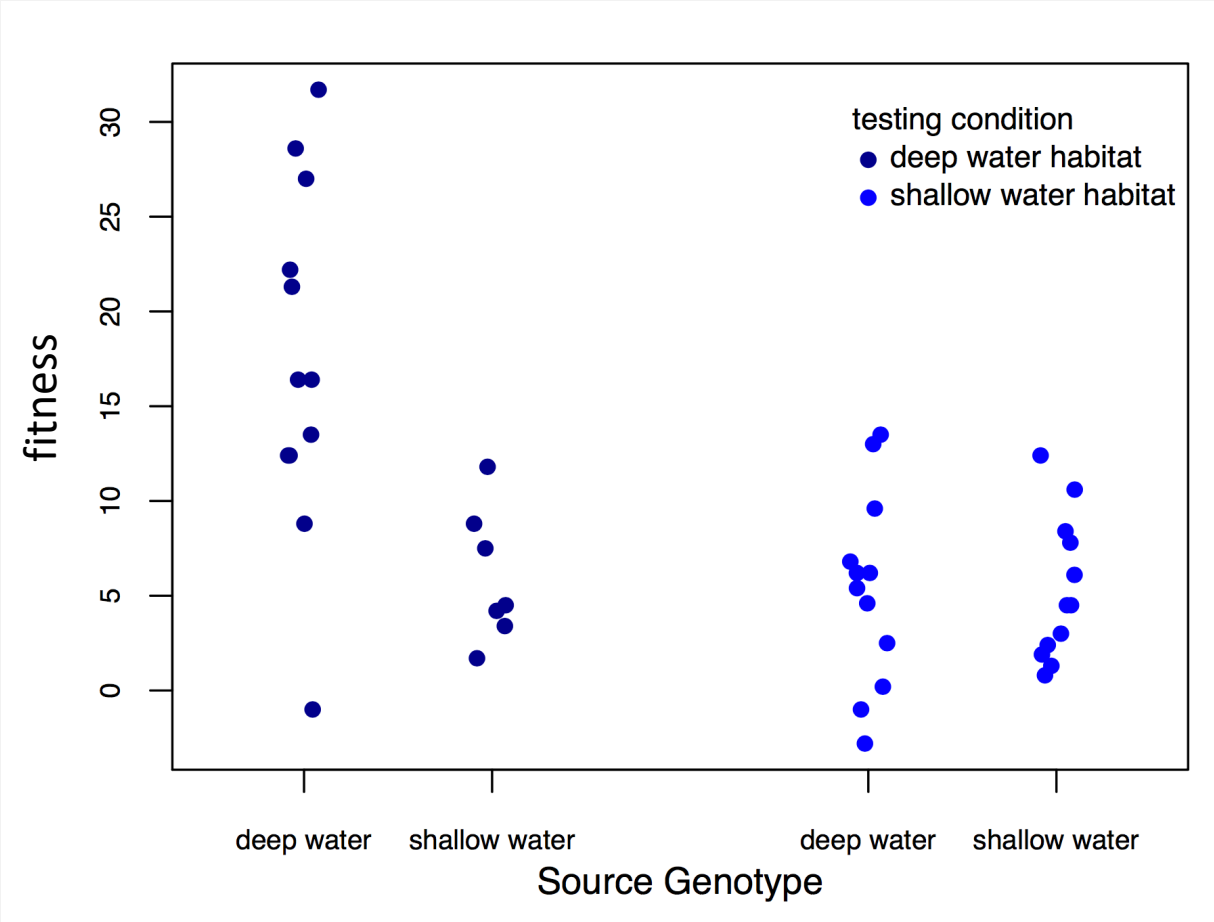
Conclusions that you reach are only applicable to the groups or treatments you include in the study

Example of fixed effects

Reciprocal relocation experiment to investigate how genotype and habitat interact to determine the fitness of stickleback fish (Rundle 2002).

		Source habitat	
		Shallow	Deep
Test habitat	Shallow	12 fish	11 fish
	Deep	7 fish	11 fish

Example of fixed effects (two factor ANOVA)




```
> anova(lm(fitness ~ genotype * test.habitat))
Analysis of Variance Table

Response: fitness
                      Df  Sum Sq Mean Sq F value    Pr(>F)
genotype               1  363.49  363.49  9.7045 0.0034403 **
test.habitat           1  550.55  550.55 14.6986 0.0004485 ***
genotype:test.habitat  1  333.58  333.58  8.9059 0.0048864 **
Residuals             39 1460.77   37.46
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> summary(lm(fitness ~ genotype * test.habitat))

Call:
lm(formula = fitness ~ genotype * test.habitat)

Residuals:
     Min       1Q   Median       3Q      Max
-18.4750  -3.6917  -0.8083   3.4583  14.2250

Coefficients:
                                Estimate Std. Error t value Pr(>|t|)
(Intercept)                       17.475      1.767   9.891 3.49e-12 ***
genotypeshallow                  -11.489      2.911  -3.947 0.000321 ***
test.habitatshallow              -12.125      2.499  -4.853 1.99e-05 ***
genotypeshallow:test.habitatshallow  11.448      3.836   2.984 0.004886 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 6.12 on 39 degrees of freedom
Multiple R-squared:  0.4606,    Adjusted R-squared:  0.4192
F-statistic:  11.1 on 3 and 39 DF,  p-value: 2.093e-05
```

Interpreting Coefficients

```
> summary(lm(fitness ~ genotype * test.habitat))

Call:
lm(formula = fitness ~ genotype * test.habitat)

Residuals:
     Min       1Q   Median       3Q      Max
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Coefficients:
                                 Estimate Std. Error t value Pr(>|t|)
(Intercept)                        17.475      1.767   9.891 3.49e-12 ***
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test.habitatshallow               -12.125      2.499  -4.853 1.99e-05 ***
genotypeshallow:test.habitatshallow  11.448      3.836   2.984 0.004886 **
---
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Residual standard error: 6.12 on 39 degrees of freedom
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```
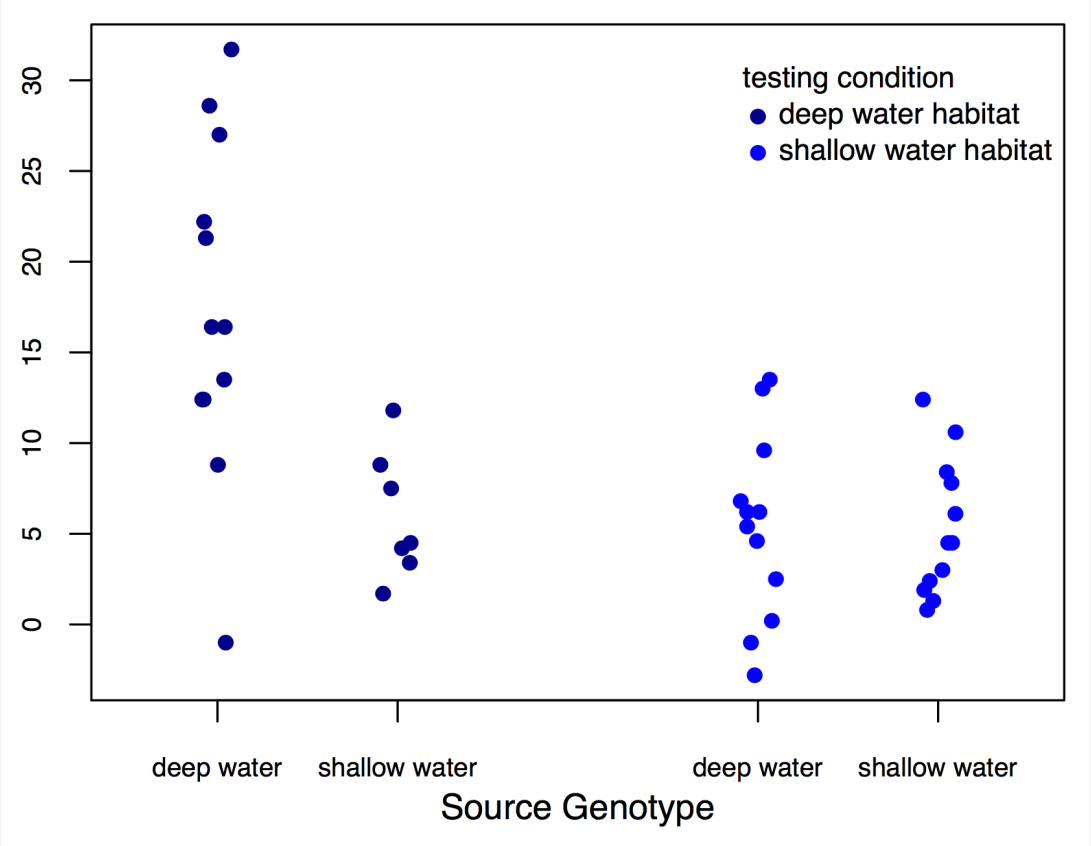



What is a random effect

These are randomly sampled categories of a variable that represent groups of individual measurements. Usually random effects are not repeatable.

- Study sites
- Environmental chambers
- Families made up of siblings
- Measurements within individuals

Conclusions that you reach are applicable only to the sample being studied.

What is a random effect

Sometimes random effects are a nuisance

- Field sites
- Environmental chambers
- Field plots
- Repeated measures

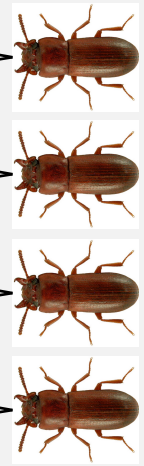
Occasionally random effects are of great interest

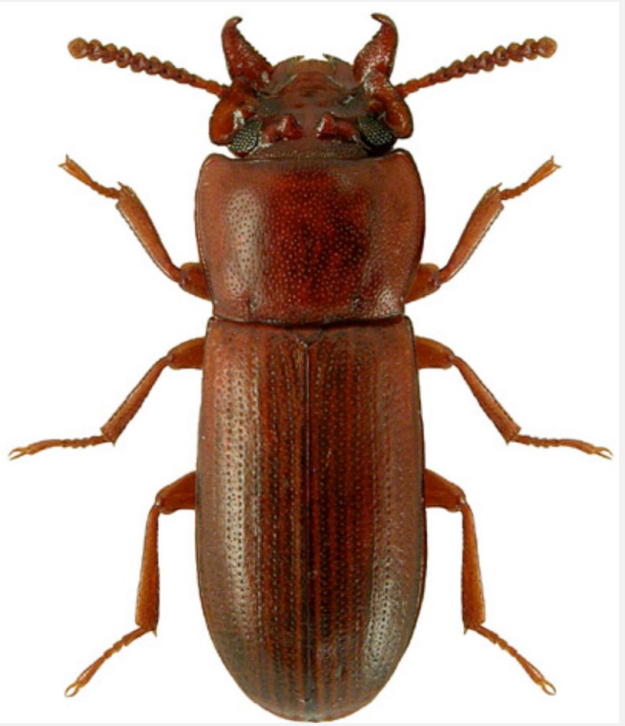
- Families - Heritability
- Individuals – Breeding value

Example of random effects

Impact of selective regime on horn size.
Measure both the left and right horn in 25 beetles from two different selective regimes.

Horn size	Beetle	Selective regime
256	1	High
276	1	High
321	2	High
321	2	High
423	3	Low
401	3	Low
381	4	Low
409	4	Low





Example of random effects

Identifying the predictors for the presence or absence of Chrysina beetles.

Number collected	oak	juniper	site	date	trip
8	1	0	21	210	A
2	1	1	13	210	A
1	0	1	31	211	A
5	0	1	15	212	A
4	1	1	21	242	B
6	1	0	13	242	B
0	1	1	31	245	B
7	1	1	15	245	B

Implementing a mixed effects model

```
library(nlme)
fit <- lme(sqrt(beyeri) ~ oaks + jun + elev,
           random = list(~1|site,~1|trip),
           data=dat)
summary(fit)
```

Fixed effects

Random effects

Repeated measures at sites can't be treated as independent

Random Intercept Model (~1 | site)

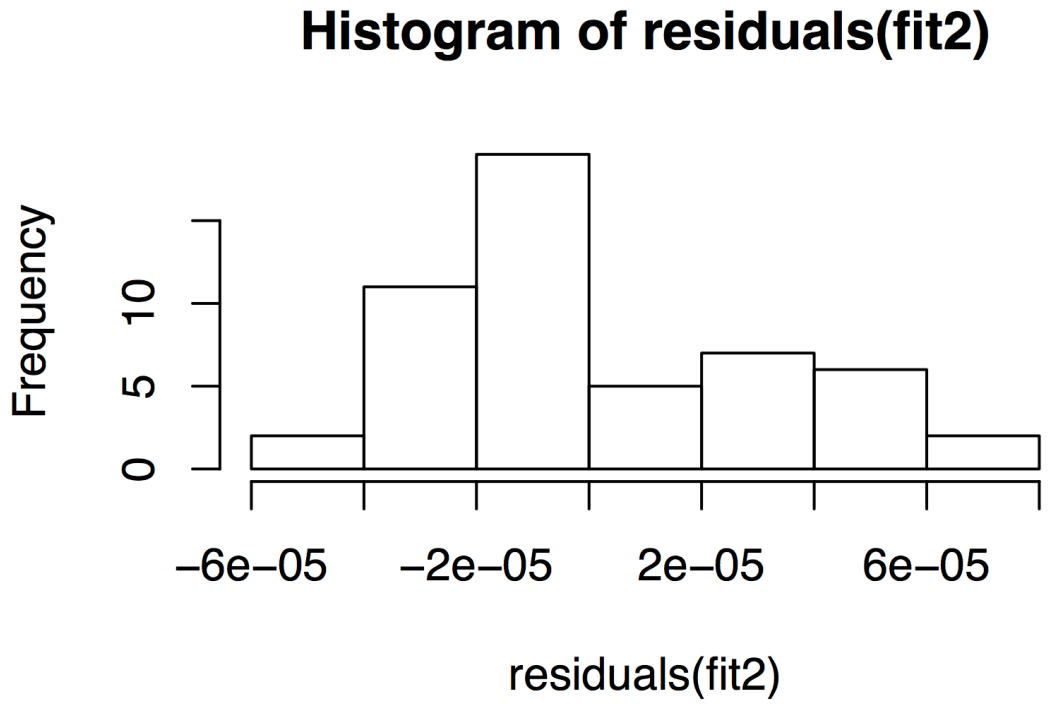
- Only intercepts vary across groups.
- Assumes that the effect of predictors is the same across all groups.

Random Slope Model (~ X | site)

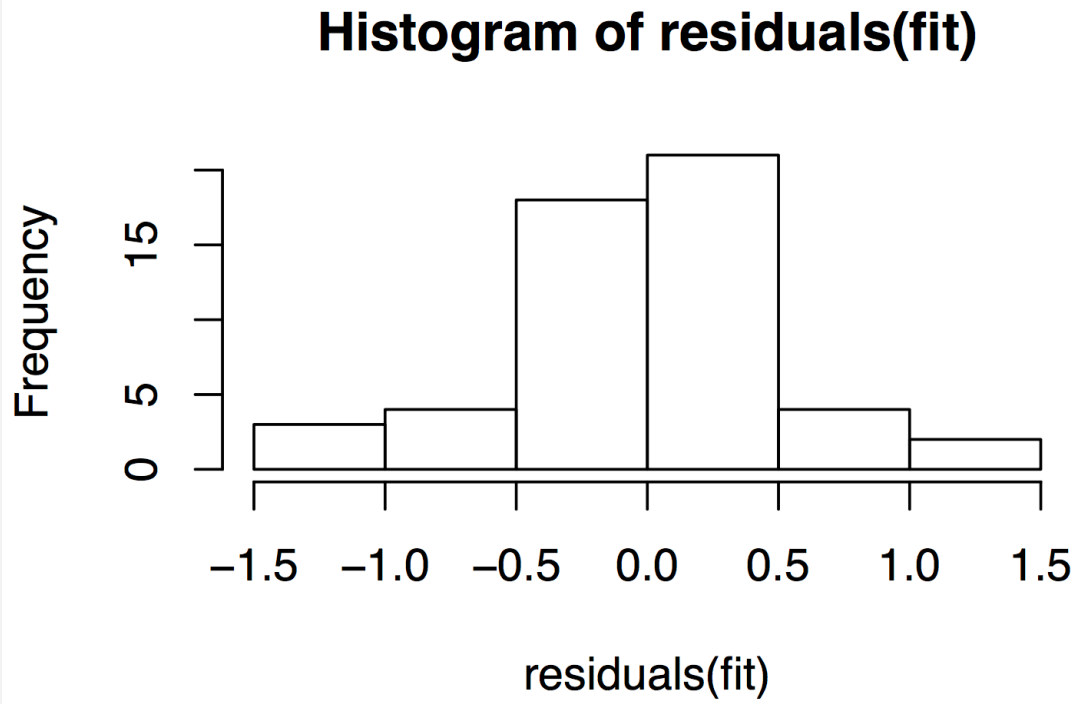
- Both intercepts and slopes for predictor X vary across groups.
- Captures group-specific trends in the effect of X.

Implementing a mixed effects model

```
fit2 <- lme(beyeri ~ oaks + jun + elev,
            random = list(~1|site, ~1|trip),
            data=dat)
```




```
fit <- lme(sqrt(beyeri) ~ oaks + jun + elev,
           random = list(~1|site,~1|trip),
           data=dat)
```



Implementing a mixed effects model

Mixed effect models can be fit using the LME function from the package nlme.

```
fit <- lme(sqrt(beyeri) ~ oaks + jun + elev,
           random = list(~1|site,~1|trip),
           data=dat)
> summary(fit)
Linear mixed-effects model fit by REML
 Data: dat
       AIC      BIC    logLik
  153.6247 166.7231 -69.81233

Random effects:
 Formula: ~1 | site
        (Intercept)
StdDev: 2.272341e-05

 Formula: ~1 | trip %in% site
       (Intercept)   Residual
StdDev:   0.8169537 0.00264004

Fixed effects: sqrt(beyeri) ~ oaks + jun + elev
                Value Std.Error DF   t-value p-value
(Intercept) -1.6788177 1.5125428 26 -1.109931  0.2772
oaks         0.9707025 0.2943403 22  3.297892  0.0033
jun         -0.0860503 0.2460159 22 -0.349775  0.7298
elev         0.0012513 0.0008072 22  1.550167  0.1354
 Correlation:
     (Intr) oaks   jun
oaks  0.530
jun   0.197 -0.097
elev -0.993 -0.584 -0.250
```

```
fit <- lme(sqrt(beyeri) ~ oaks + elev,
           random = list(~1|site,~1|trip),
           data=dat)
> summary(fit)
Linear mixed-effects model fit by REML
 Data: dat
       AIC      BIC   logLik
  150.7722 162.1231 -69.3861

Random effects:
 Formula: ~1 | site
        (Intercept)
StdDev: 2.191491e-05

 Formula: ~1 | trip %in% site
       (Intercept)    Residual
StdDev:   0.8096043 0.002598713

Fixed effects: sqrt(beyeri) ~ oaks + elev
                Value Std.Error DF   t-value p-value
(Intercept) -1.5744919 1.4695032 26 -1.071445  0.2938
oaks         0.9607576 0.2903283 23  3.309211  0.0031
elev         0.0011807 0.0007746 23  1.524404  0.1410
 Correlation:
     (Intr) oaks
oaks  0.563
elev -0.994 -0.631
```

```
fit <- lme(sqrt(beyeri) ~ oaks,
           random = list(~1|site,~1|trip),
           data=dat)
> summary(fit)
Linear mixed-effects model fit by REML
 Data: dat
       AIC      BIC    logLik
  138.5902 148.1504 -64.29512

Random effects:
 Formula: ~1 | site
        (Intercept)
StdDev: 2.309943e-05

 Formula: ~1 | trip %in% site
       (Intercept)    Residual
StdDev:   0.8202517 0.002667513

Fixed effects: sqrt(beyeri) ~ oaks
                Value Std.Error DF  t-value p-value
(Intercept) 0.6514133 0.1674341 26 3.890566   6e-04
oaks        1.2400606 0.2281742 24 5.434710   0e+00
 Correlation:
     (Intr)
oaks -0.734
```

If you report your df with your F-statistic the reviewer will know if you did the right type of model

Considerations for models with random effects

- Most software will assume that all factors are fixed unless you specify them as mixed.
- Designating factors as random effects takes extra work.
- The lm function treats all predictors as fixed effects.
- Treating random effects as fixed effects is fundamentally wrong.

Links

MCMCMglmm: Fit mixed models with phylogenetic or pedigree information in a Bayesian framework.

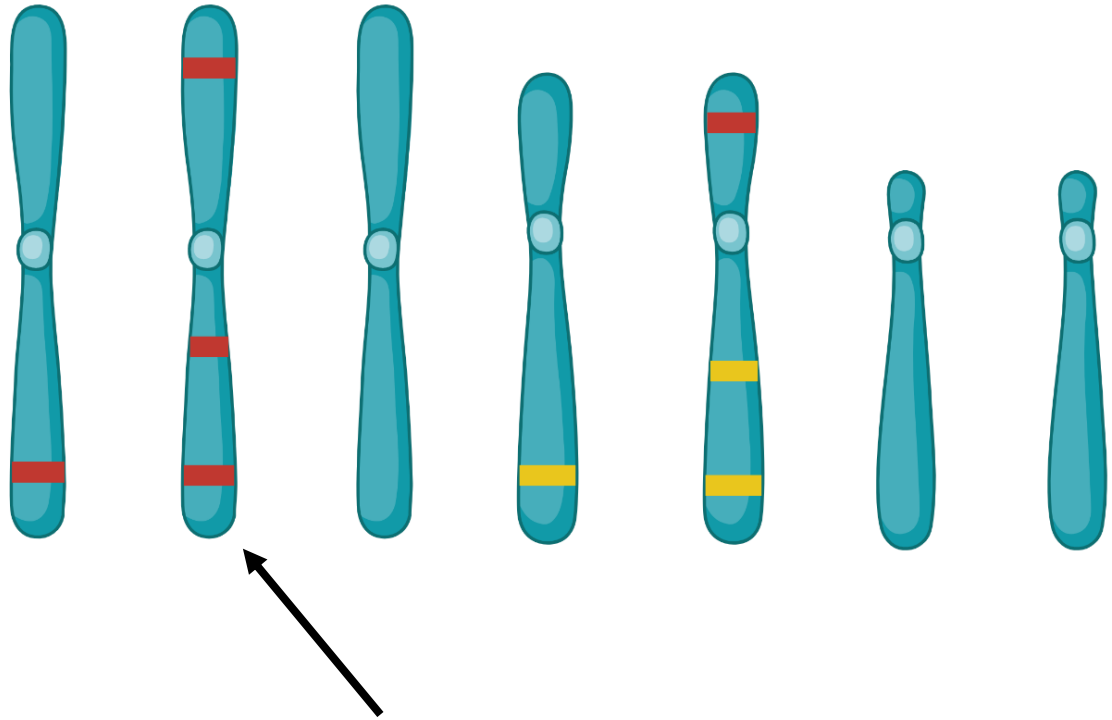
Outlier Package: Apply outlier tests to identify possible outlier datapoints - I don’t recommend this.

Monte Carlo Methods

The name "Monte Carlo" comes from the Monte Carlo Casino in Monaco, famous for its games of chance and association with gambling and randomness. The term was coined in the 1940s by scientists working on the Manhattan Project during World War II.

You will eventually be faced with questions where you can't find a clear statistical test that will be appropriate to your data.

Are these beetles randomly distributed, aggregating or avoiding each other?



Does chromosome 2 have more "red" genes than I would expect by chance?

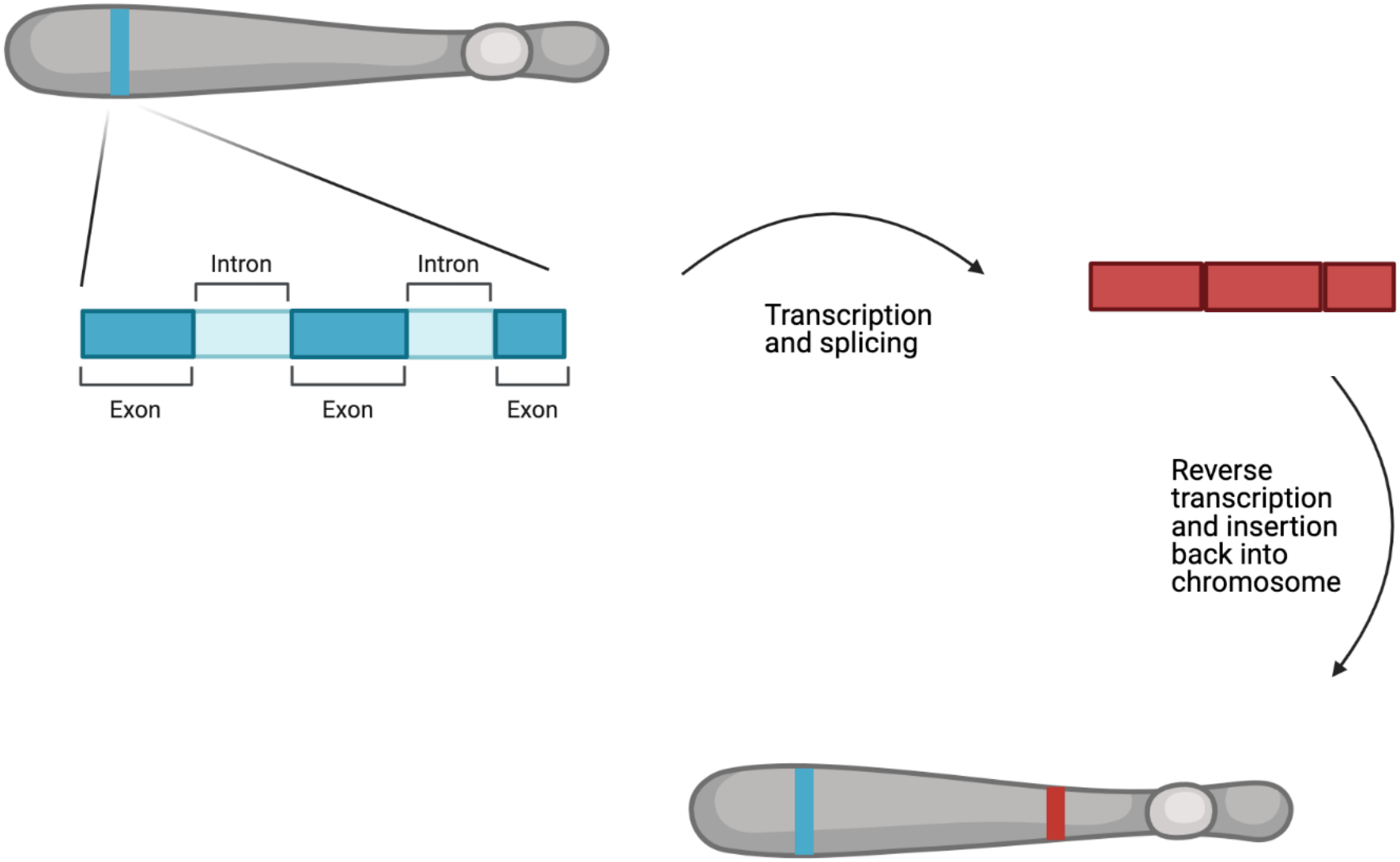
You will eventually be faced with questions where you can’t find a clear statistical test that will be appropriate to your data.

Monte Carlo methods are most similar to permutation methods that we have used previously. The distinction between the two is that with permutations we simply randomized data. In contrast, with Monte Carlo we will use a simulation process that represents the biology of a null hypothesis.

Monte Carlo methods offer an approach to answer difficult often complex questions.

Lets look at an example with retrogenes.

Lets look at an example with retrogenes.



Sexual antagonism hypothesis of retrogene distribution:

If some genes have variation that is beneficial to one sex but harmful to the other fitness could be increased in two ways.

1) make a duplicate of a gene and express one version in males and one in females
2) make a duplicate of a gene and move it from an autosome to an X or Y chromosome

Additionally in many species the sex chromosomes do not get expressed during meiosis if you have a gene that can benefit males during spermatogenesis moving this type of gene from a sex chromosome to an autosome will be beneficial.

From this sexual antagonism hypothesis we might expect to see more retrogenes on sex chromosomes and possibly more parental genes on sex chromosomes than we would expect by chance.

We identified retrogenes in a Tribolium beetle that has a recent fusion of chromosome 2 (lg2) to the sex chromosome. This means that the X in this species now include everything that is in lg2 as well as lgX.

	lg1	lg2	lgX	lg4	lg5	lg6	lg7	lg8	lg9	lg10	Total
GeneNumber	3254	2941	3705	1507	1543	1287	994	684	325	212	16512
PhysicalSize	45	41	38	30	20	15	14	11	12	9	235
Parents	22	45	39	9	8	7	8	3	1	0	142
Daughters	24	40	35	15	7	6	5	4	5	1	142

We identified retrogenes in a Tribolium beetle that has a recent fusion of chromosome 2 (lg2) to the sex chromosome. This means that the X in this species now include everything that is in lg2 as well as lgX.

	lg1	lg2	lgX	lg4	lg5	lg6	lg7	lg8	lg9	lg10	Total
GeneNumber	3254	2941	3705	1507	1543	1287	994	684	325	212	16512
PhysicalSize	45	41	38	30	20	15	14	11	12	9	235
Parents	22	45	39	9	8	7	8	3	1	0	142
Daughters	24	40	35	15	7	6	5	4	5	1	142

What is the probability that we would observe this many parents and daughters assuming that chromosome 2 is no different than any other chromosomes?

From this we might hypothesize that we should see more retrogenes on sex chromosomes and possibly more parental genes on sex chromosomes than we would expect by chance.

1) Size of the chromosome (determines probability of a daughter gene being on a chromosome)

2) Number of genes on each chromosome (determines the probability of a parent gene being on a a chromosomes)

1. First decide what we want to test
    a) the number of parents on lg2
    b) the number of daughters on lg2

2. Create an object to store a null distribution of these values

3. Simulate an expected number of parents and and an expected number of daughters on lg 2.
    a) when simulating account for chromosome size and gene number

4. Repeat step 3 thousands of times.

5. Compare our observation to the null distribution to calculate a p-value for our observation.