

# Lecture 09

10.21.21

# Paired or unpaired?

- Body temperature using two different thermometers on the same group of participants
- Average commute times for randomly selected individuals in New York and Chicago
- A psychological test where half the participants are assigned to the control group and half are assigned to the treatment group
- The before and after effect of a drug treatment on the same group of people
- Water samples upstream and downstream of a factory taken across a two year period

# Paired (dependent) or independent?

- Body temperature using two different thermometers on the same group of participants **PAIRED**
- Average commute times for randomly selected individuals in New York and Chicago **INDEPENDENT**
- A psychological test where half the participants are assigned to the control group and half are assigned to the treatment group **INDEPENDENT**
- The before and after effect of a drug treatment on the same group of people **PAIRED**
- Water samples upstream and downstream of a factory taken across a two year period **PAIRED**

# Paired or unpaired?

- **Water samples upstream and downstream of a factory taken across a two year period**

Collection date	Upstream	Downstream
Aug	2755	1872
Sept	3448	2481
Oct	2098	613
Nov	789	733
Dec	988	960
Jan	638	388
Feb	1187	833
March	2481	1421
April	2471	1076

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# Paired or unpaired?

- **Water samples upstream and downstream of a factory taken across a two year period**
- Paired analysis accounts for structure in the data
- Will often result in a lower p-value (more significant result)
- Often a more complicated study design

Collection date	Upstream	Downstream
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**Paired:**

**$P = 0.0049$**

**Unpaired:**

**$P = 0.0939$**

cAMP is a substance that can mediate cellular response to hormones. In a certain study, **oocytes from four *Xenopus* females were divided into two batches**: one batch was exposed to progesterone and the other was not. After 2 minutes, each batch was assayed for its cAMP content. Use a  $t$  test to investigate the effect of progesterone on cAMP. Let  $H_A$  be nondirectional and let  $\alpha = 0.10$

Frog	Control	Progesterone
1	6.01	5.23
2	2.28	1.21
3	1.51	1.40
4	2.12	1.38

***Because the oocytes came from the same frog, we could treat this as paired to reduce frog-to-frog variation***

# Assumptions for parametric $t$ tests

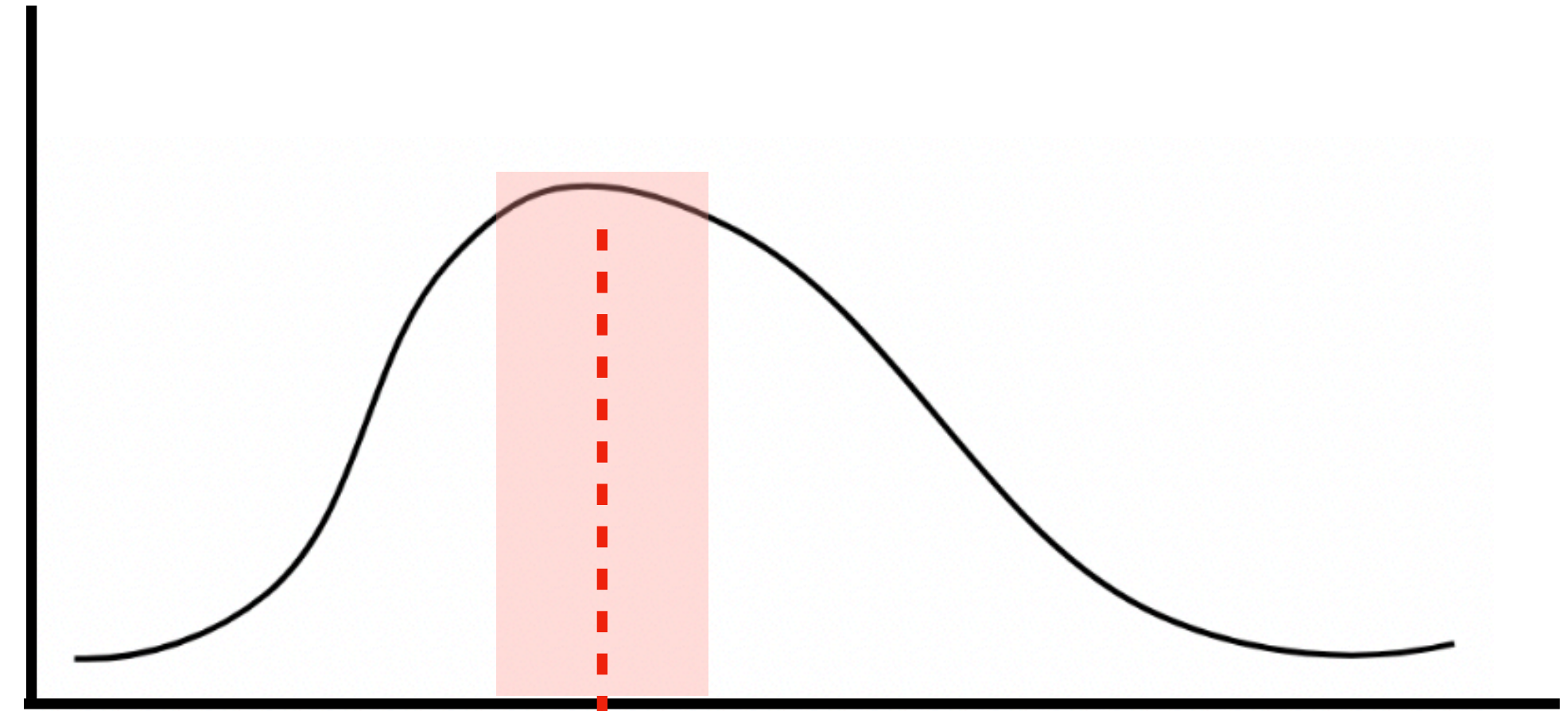
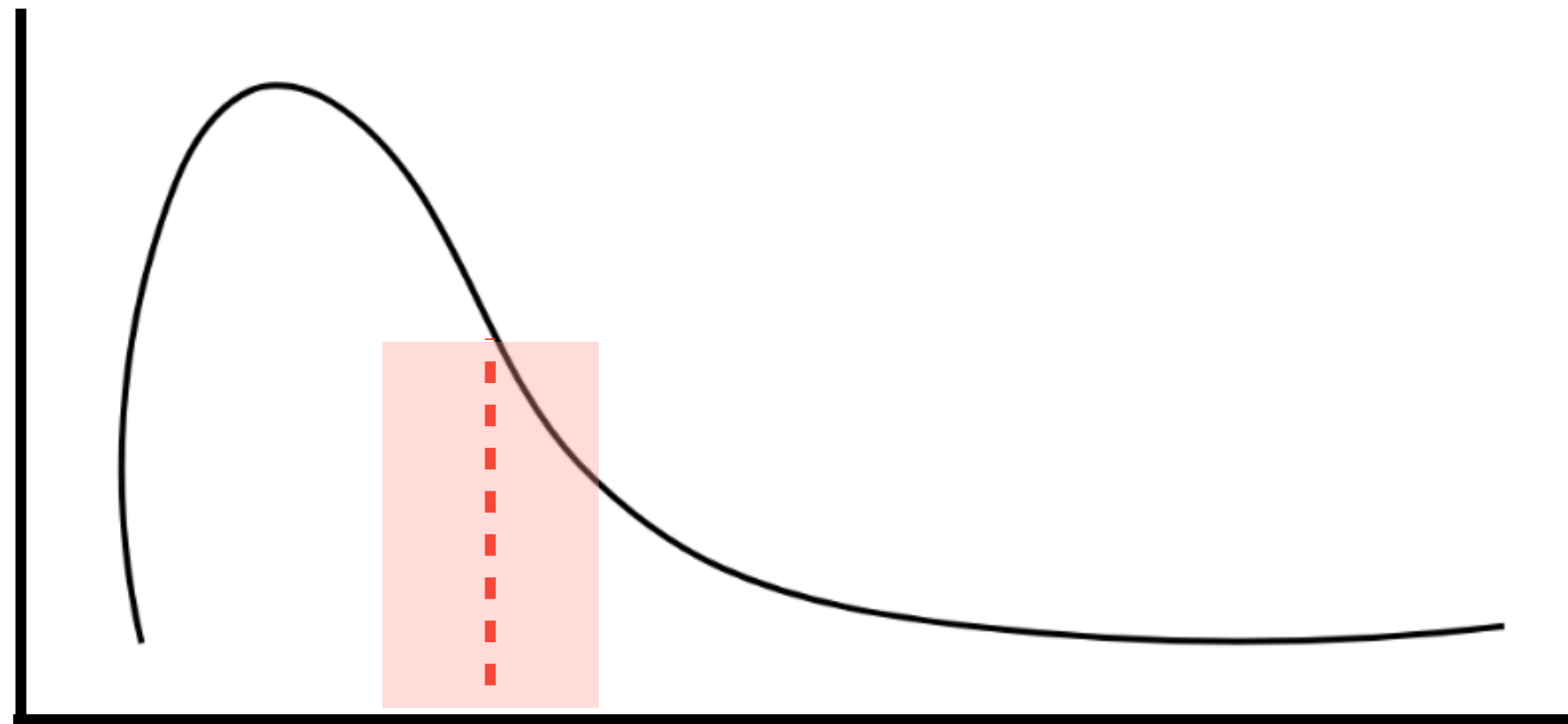
- **Conditions on the design of the study:**
  - (1) Data is a random sample from a large population
  - (2) Observations in the sample must be independent of each other
- **Conditions on the form of the population distribution**
  - (3) If  $n$  is small, the population distribution must be ~normal
  - (4) If  $n$  is large, the population distribution doesn't have to be normal



# Non-parametric alternatives

- Where **parametric** tests focus on a specific **parameter** (i.e. mean), **non-parametric** tests do not
- In general, there is a non-parametric alternative for every parametric test
- Non-parametric tests will also allow us to perform hypothesis testing with non-quantitative (nominal/ordinal) data
- Many non-parametric tests are based on the idea of **rank**

# Non-parametric alternatives



**Is the parameter mean even a meaningful measure  
for a skewed population?**

**Randomization**

**Wilcoxon-Mann-  
Whitney (Rank Sum)**

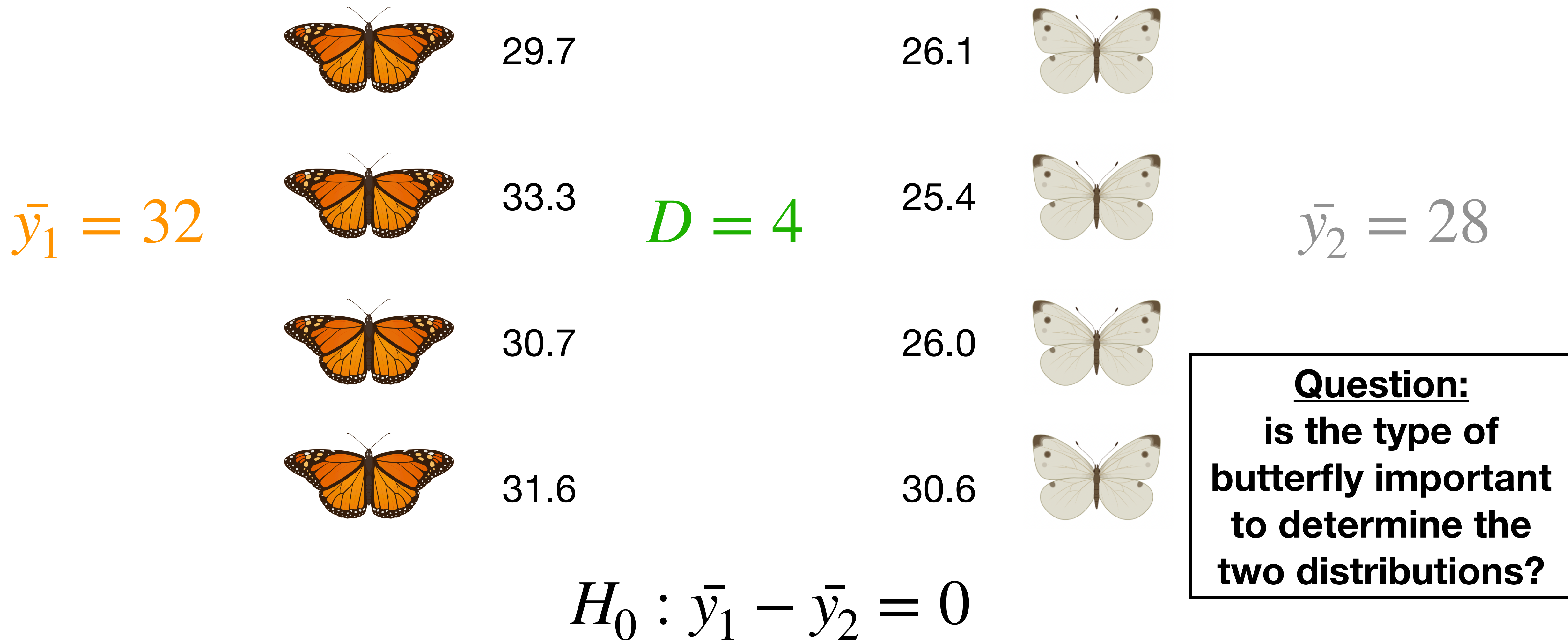
**Sign test**

**Wilcoxon  
signed-rank test**

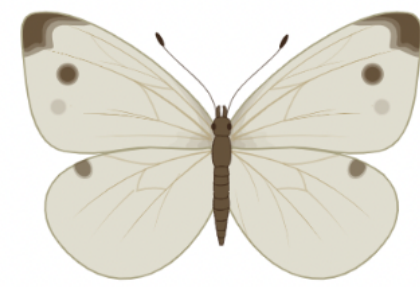
# Randomization (or permutation) as an alternative to the $t$ test

- How different do two samples have to be in order for us to infer that the populations that generated them are actually different?
- One way: compare actual difference in means to the hypothetical expected difference of means by chance
- (1) Scramble the data, (2) calculate some value (i.e. mean,  $t$ , etc.), (3) repeat 1000s times, (4) calculate the exact P-value (i.e. how often do you expect to see a value as extreme as your observed data)

# Randomization (or permutation) as an alternative to the $t$ test

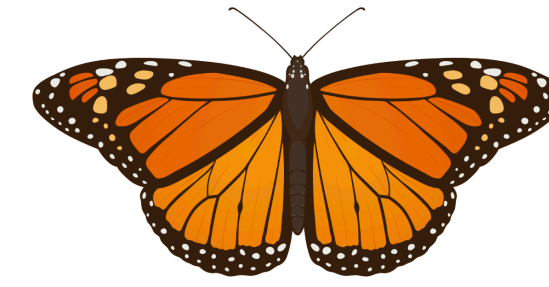


# Randomization (or permutation) as an alternative to the $t$ test



29.7

26.1



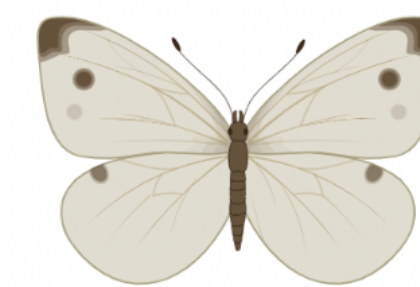
$$\bar{y}_1 = 29.3$$



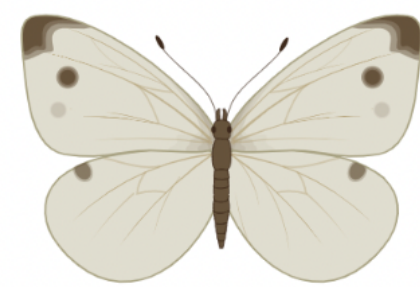
33.3

$$D = 0.2$$

25.4

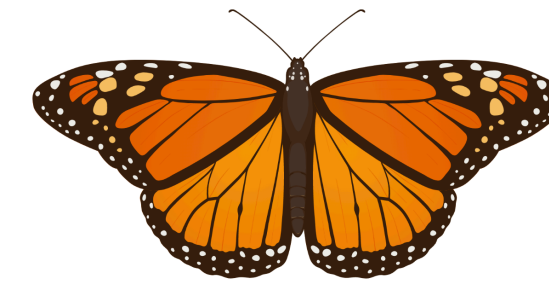


$$\bar{y}_2 = 29.1$$



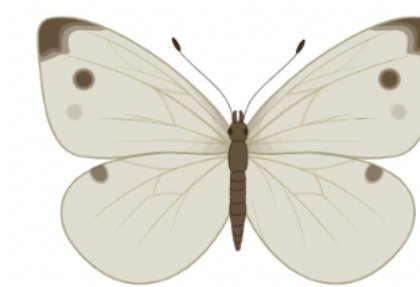
30.7

26.0



31.6

30.6

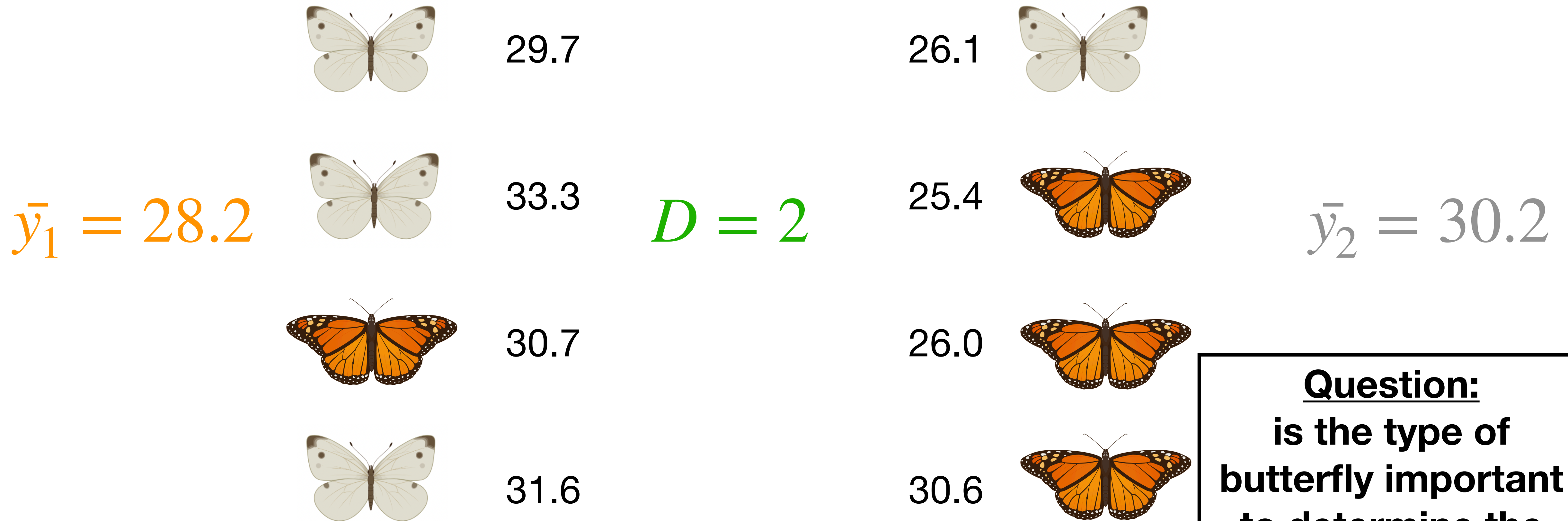


$$H_0 : \bar{y}_1 - \bar{y}_2 = 0$$

**Question:**  
is the type of  
butterfly important  
to determine the  
two distributions?



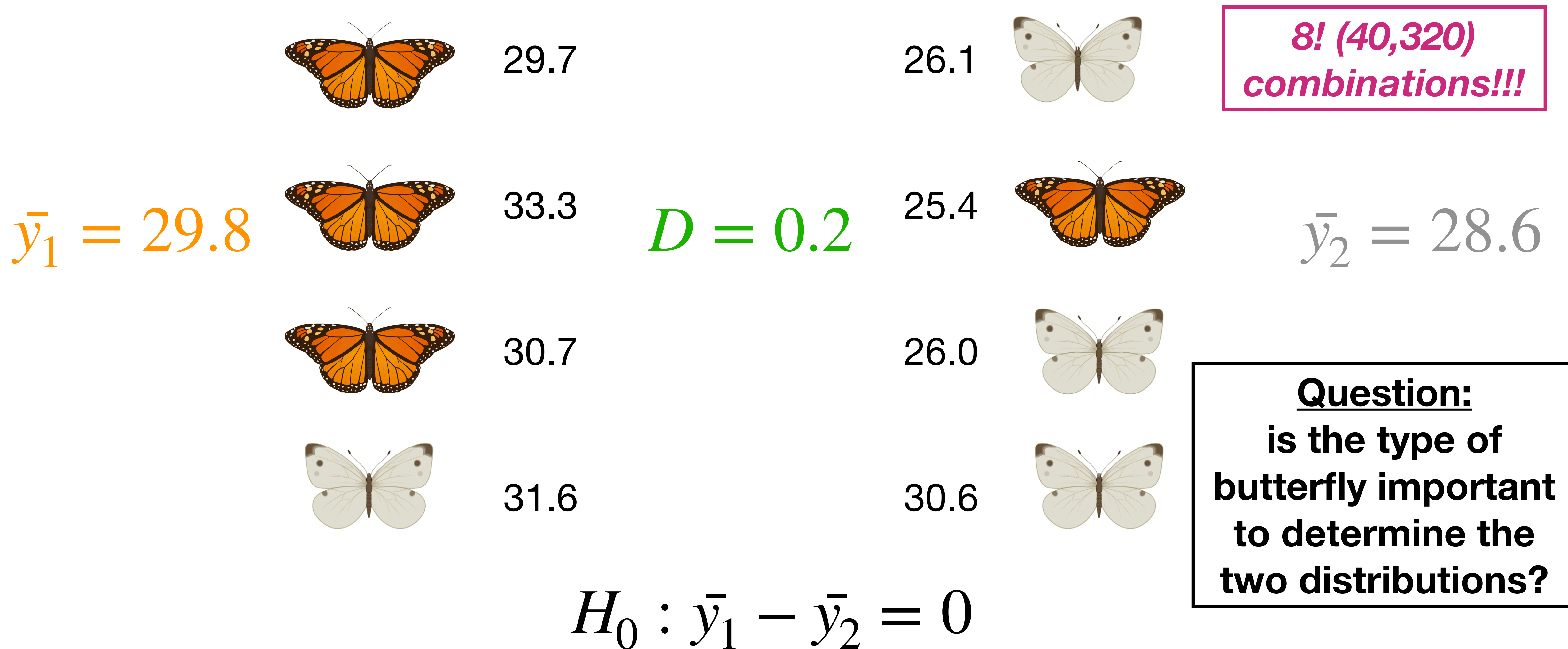
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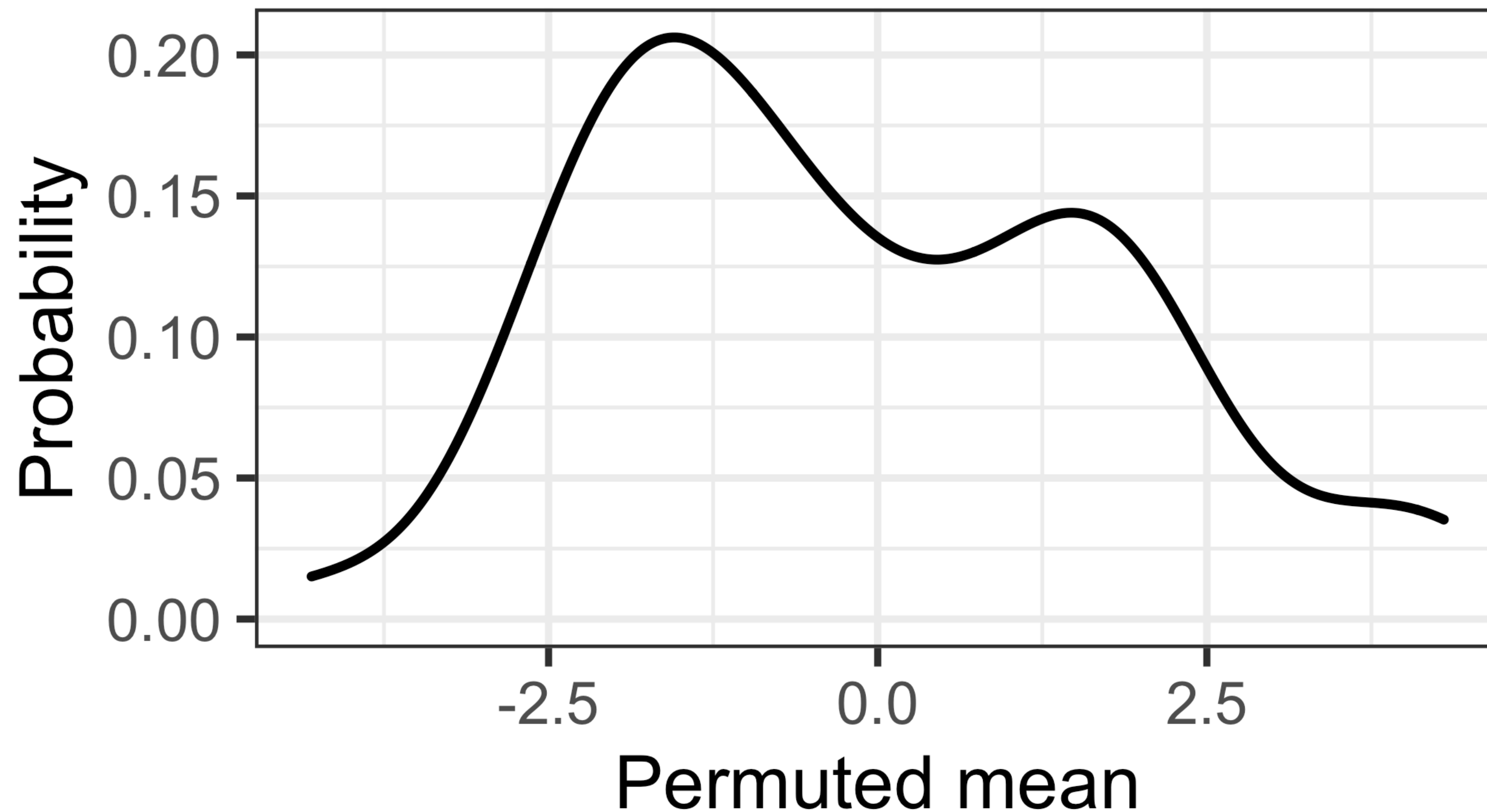
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# Randomization (or permutation) as an alternative to the $t$ test



# Randomization (or permutation) as an alternative to the $t$ test





# Randomization (or permutation) as an alternative to the $t$ test

3 values out of 100 were  $\geq |4|$

$$P = \frac{3 + 1}{100 + 1}$$

Add one to both numerator and denominator to avoid  $P = 0$

Probability

0.10  
0.05  
0.00

$\alpha = 0.05$



Probability = 0.039 that we observed our real data by chance

Observed value: 4

summary(new\_mean)

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
-4.300	-1.575	-0.625	-0.137	1.512	4.300

-2.5

0.0

2.5

Permuted mean

# Randomization (or permutation) as an alternative to the $t$ test

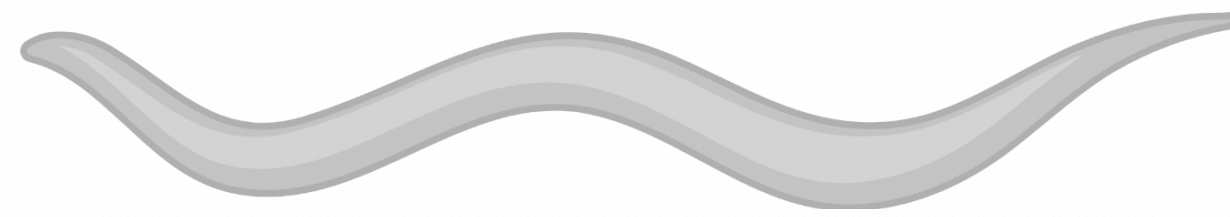
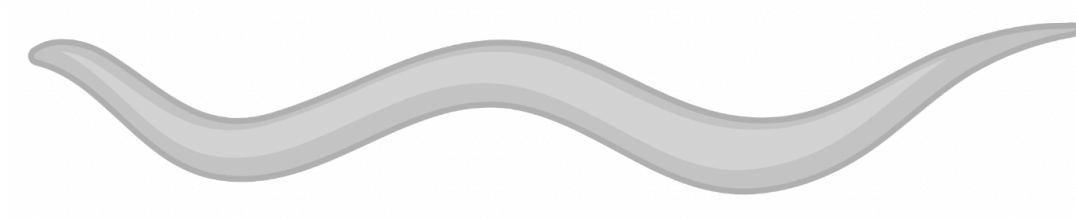
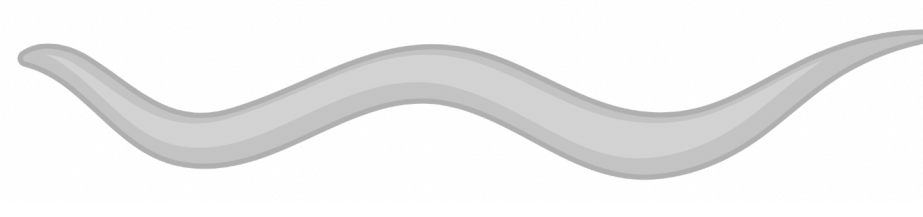
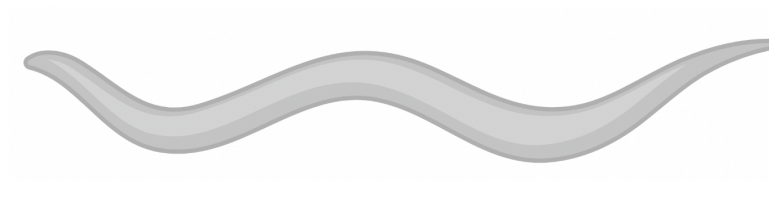
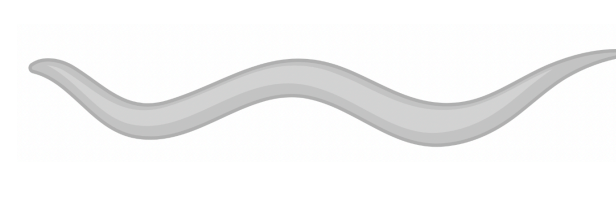
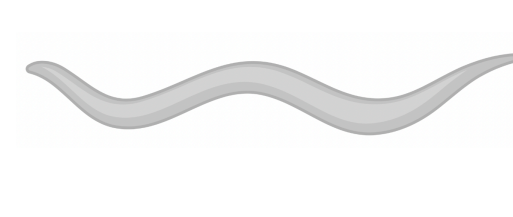
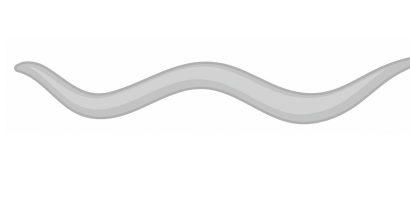

## Question:

Is it statistically significant that worms with the “A” genotype are longer than worms with the “C” genotype?

$$\bar{y}_A = 407.5$$

$$\bar{y}_C = 361.25$$

$$D = 46.25$$

	A	450
	A	439
	A	412
	C	400
	C	378
	C	356
	A	329
	C	311

# Randomization (or permutation) as an alternative to the $t$ test

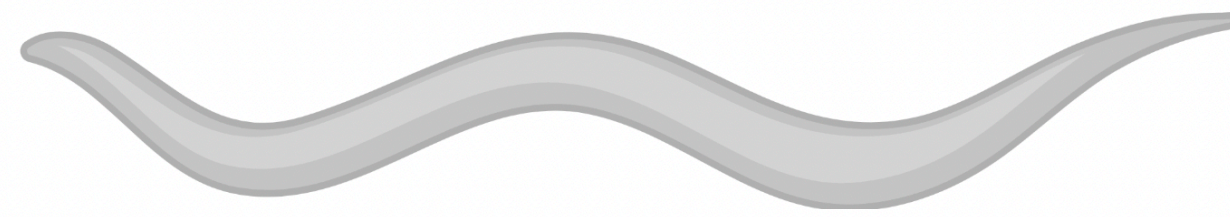
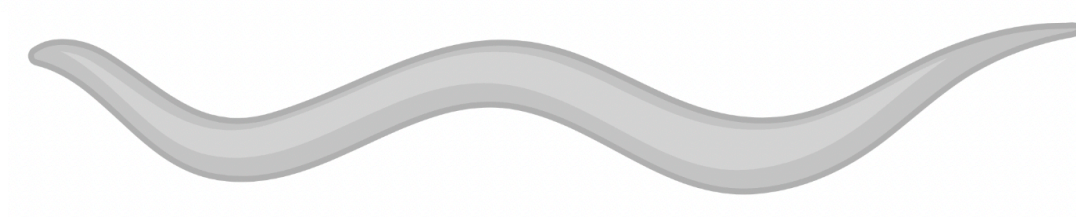
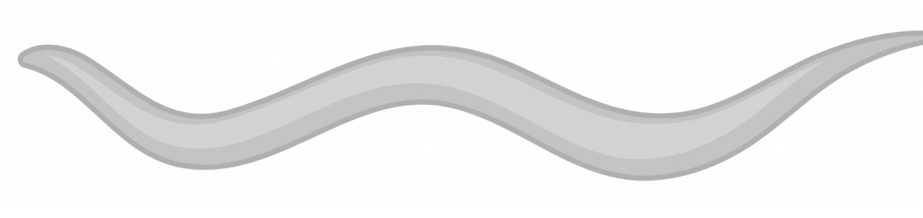
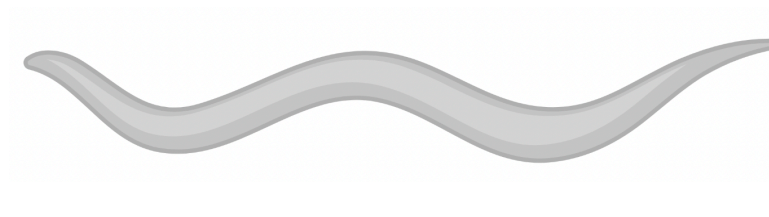
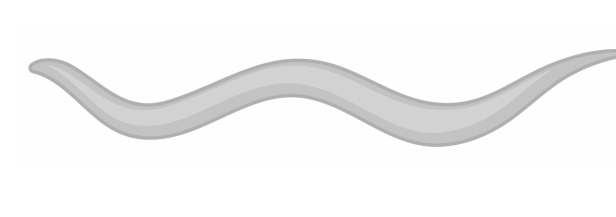
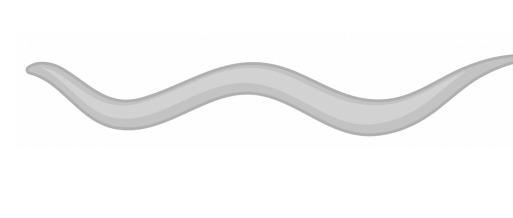
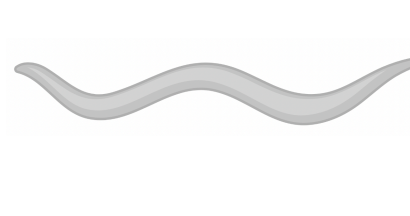

## Question:

Is it statistically significant that worms with the “A” genotype are longer than worms with the “C” genotype?

$$\bar{y}_A = 364.25$$

$$\bar{y}_C = 404.5$$

$$D = -40.25$$

	C	450
	A	439
	C	412
	C	400
	A	378
	C	356
	A	329
	A	311

1000x



# Randomization (or permutation) as an alternative to the $t$ test

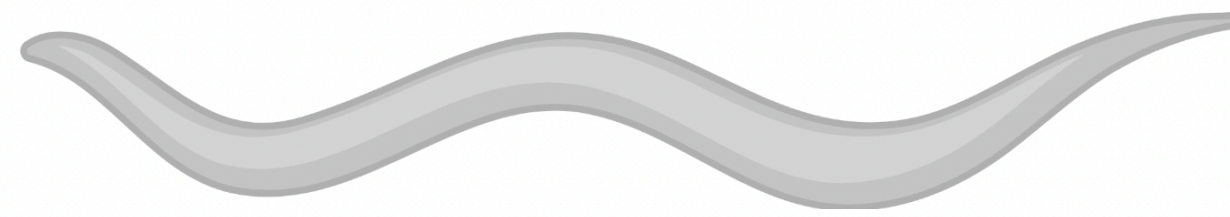
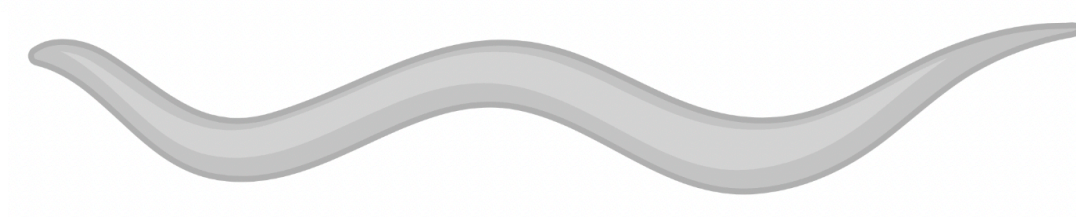
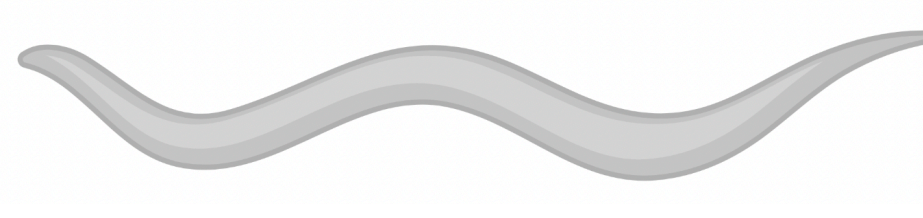
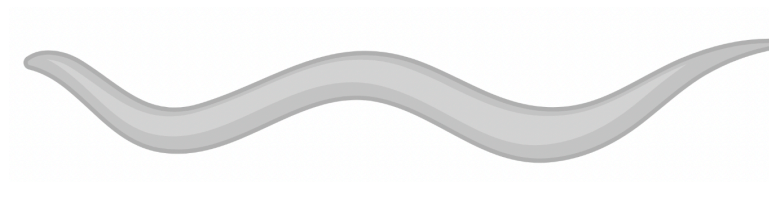
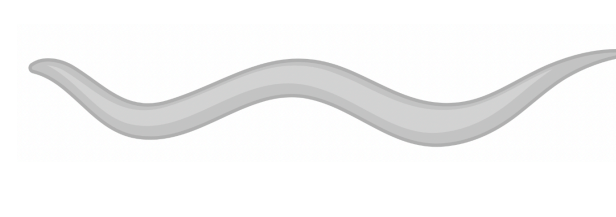
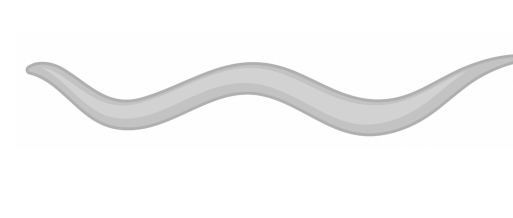
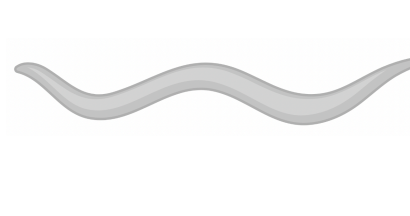

## Question:

Is it statistically significant that worms with the “A” genotype are longer than worms with the “C” genotype?

$$\bar{y}_A = 376.5$$

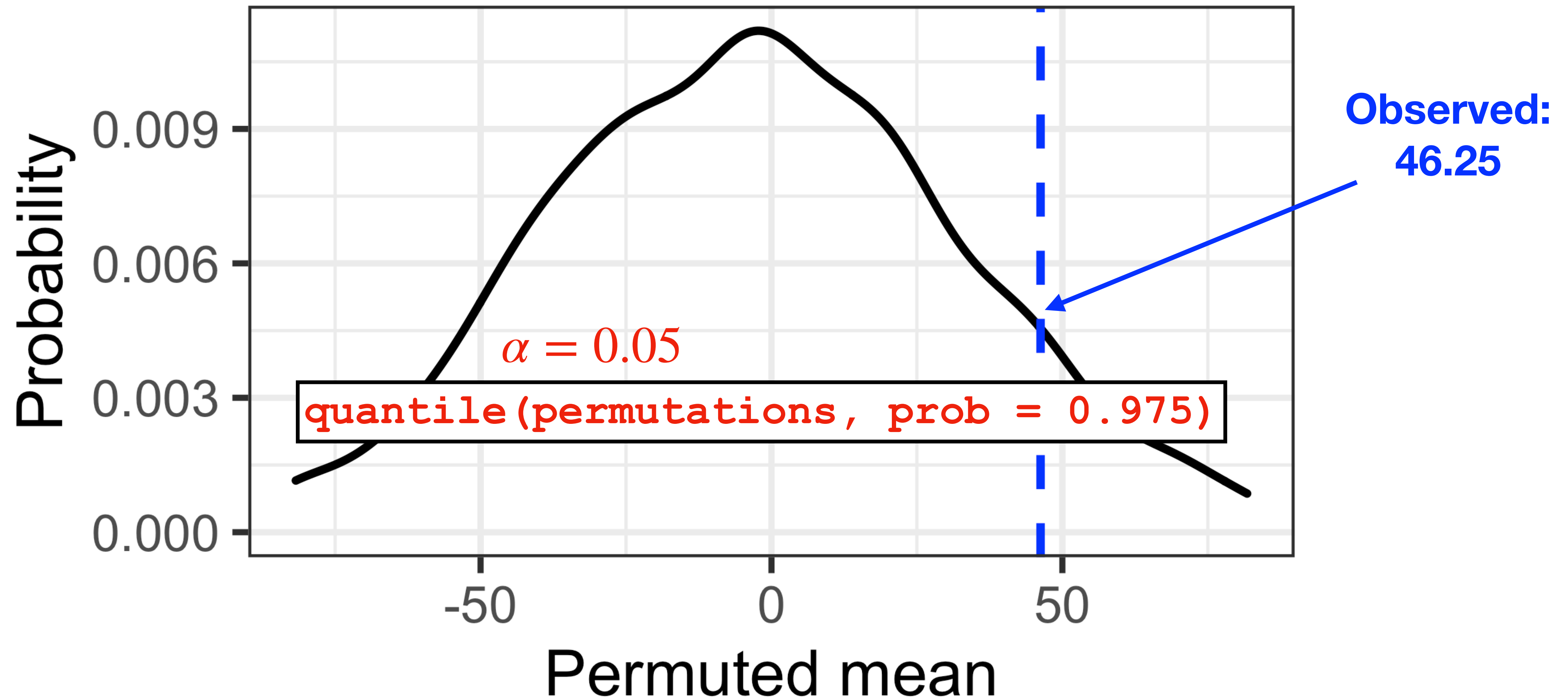
$$\bar{y}_C = 392.25$$

$$D = -15.75$$

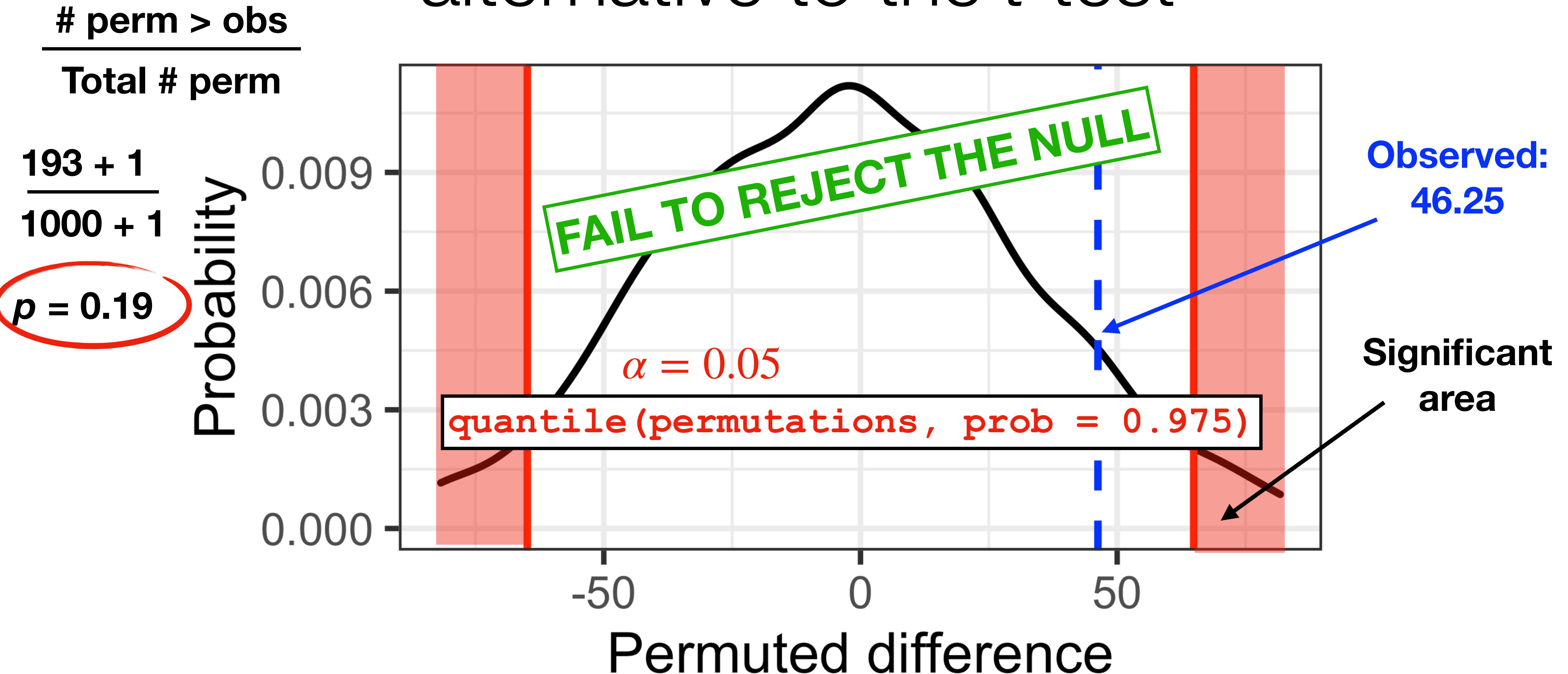
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1000x

# Randomization (or permutation) as an alternative to the $t$ test



# Randomization (or permutation) as an alternative to the $t$ test



# Application: boot strapping CI

- Boot strapping is a process of multiple re-sampling (permutations) **WITH replacement** of a single set of observations

```
# generate random variable x  
x <- rnorm(100)
```

- Central limit theorem tells us that the sampling distribution will be approximately normal
- We can use the sampling distribution of the bootstrap to define a 95% confidence interval

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- Central limit theorem tells us that the sampling distribution will be approximately normal
- We can use the sampling distribution of the bootstrap to define a 95% confidence interval

```
# generate random variable x  
x <- rnorm(100)
```

```
# what is the mean of x?  
mean(x)
```

```
# [1] 0.002912563
```



```
# create variable to hold permutation means  
perm_means <- c()
```

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```
# do 10,000 permutations  
for(i in 1:10000) {
```

```
}
```

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perm_means <- c()

# do 10,000 permutations
for(i in 1:10000) {

  # sample from x with replacement (perm is same size as x)
  perm <- sample(x, replace = T)

}
```

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# create variable to hold permutation means
perm_means <- c()

# do 10,000 permutations
for(i in 1:10000) {

  # sample from x with replacement (perm is same size as x)
  perm <- sample(x, replace = T)

  # calculate mean and add to perm_means
  perm_means <- c(perm_means, mean(perm))

}
```

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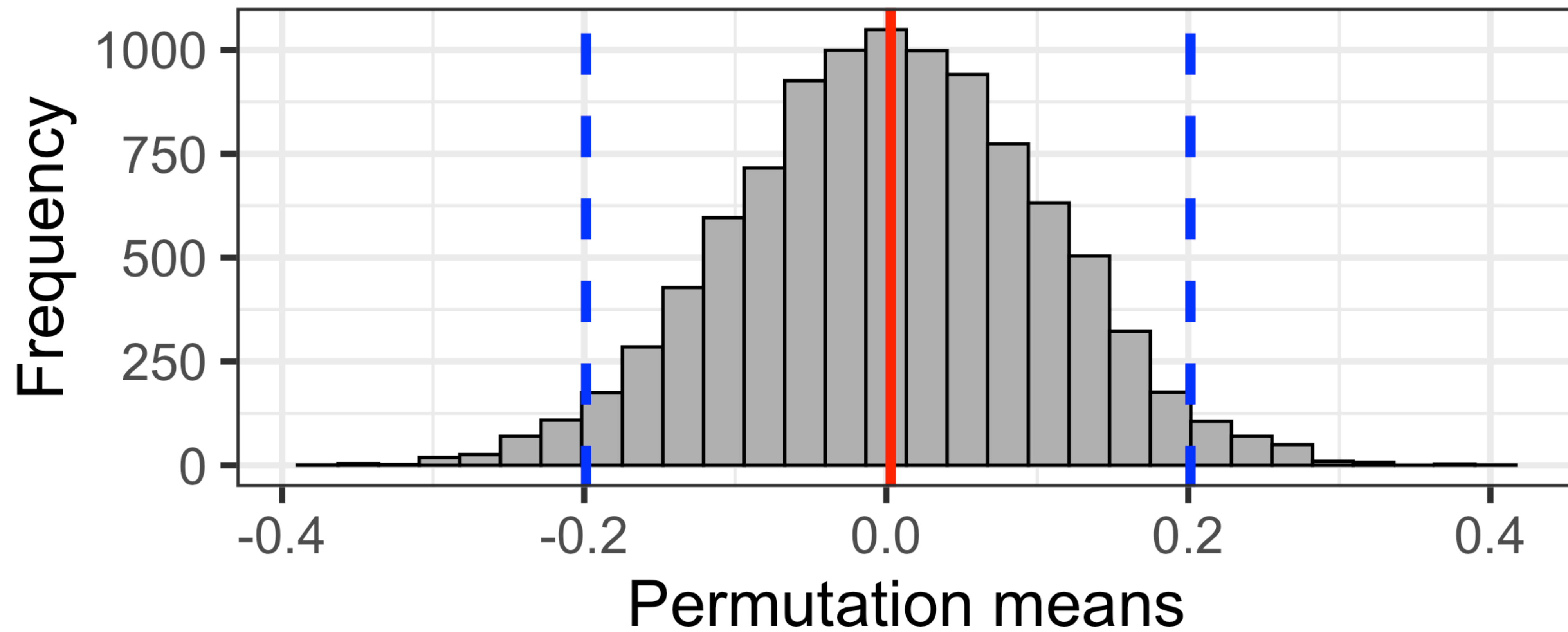
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  # calculate mean and add to perm_means
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}

# get the middle 95% of the mean of the perms distribution
quantile(perm_means, c(0.025, 0.975))

#           2.5%           97.5%
# -0.1987355  0.2013981
```



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quantile(perm_means, c(0.025, 0.975))
```

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#           2.5%           97.5%  
# -0.1987355  0.2013981
```

```
mean(x)
```

```
# [1] 0.002912563
```

# Non-parametric alternatives

## **Randomization**

- Any population distribution
- Does not even assume random or independent samples!
- High power
- Difficult to perform\*

## **Wilcoxon-Mann-Whitney (Rank Sum)**

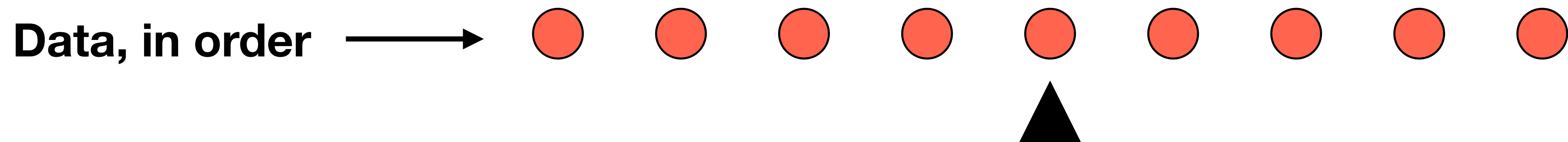
## **Sign test**

## **Wilcoxon signed-rank test**

# Wilcoxon-Mann-Whitney (U) test

(Wilcoxon Rank Sum test)

- Analogous to  $t$ -test for one- or two- (independent) samples
- Valid even if the population distributions are not normal (**distribution free**)
- Uses **ranks** of the data, not the real data, to compare populations

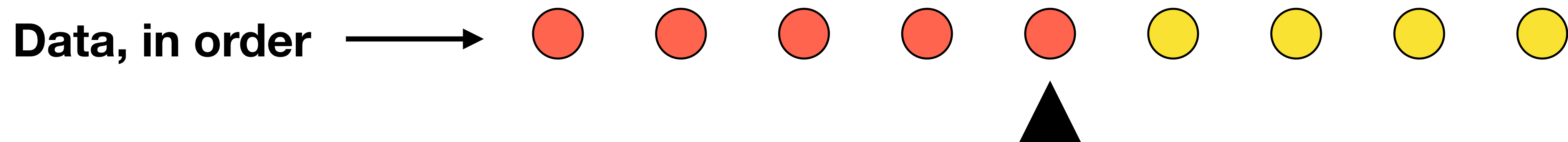




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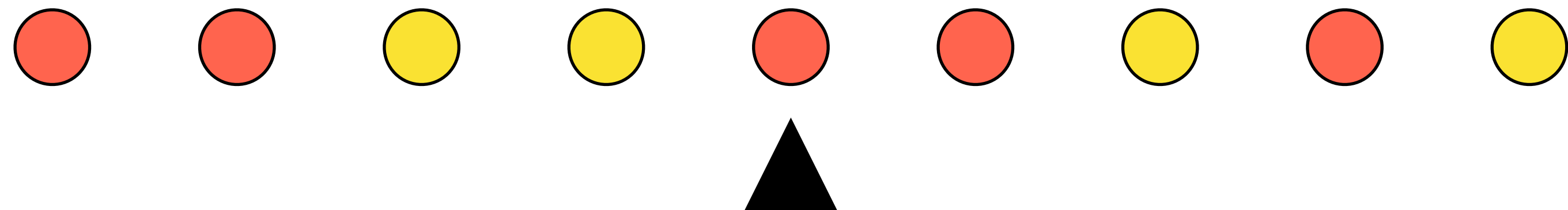


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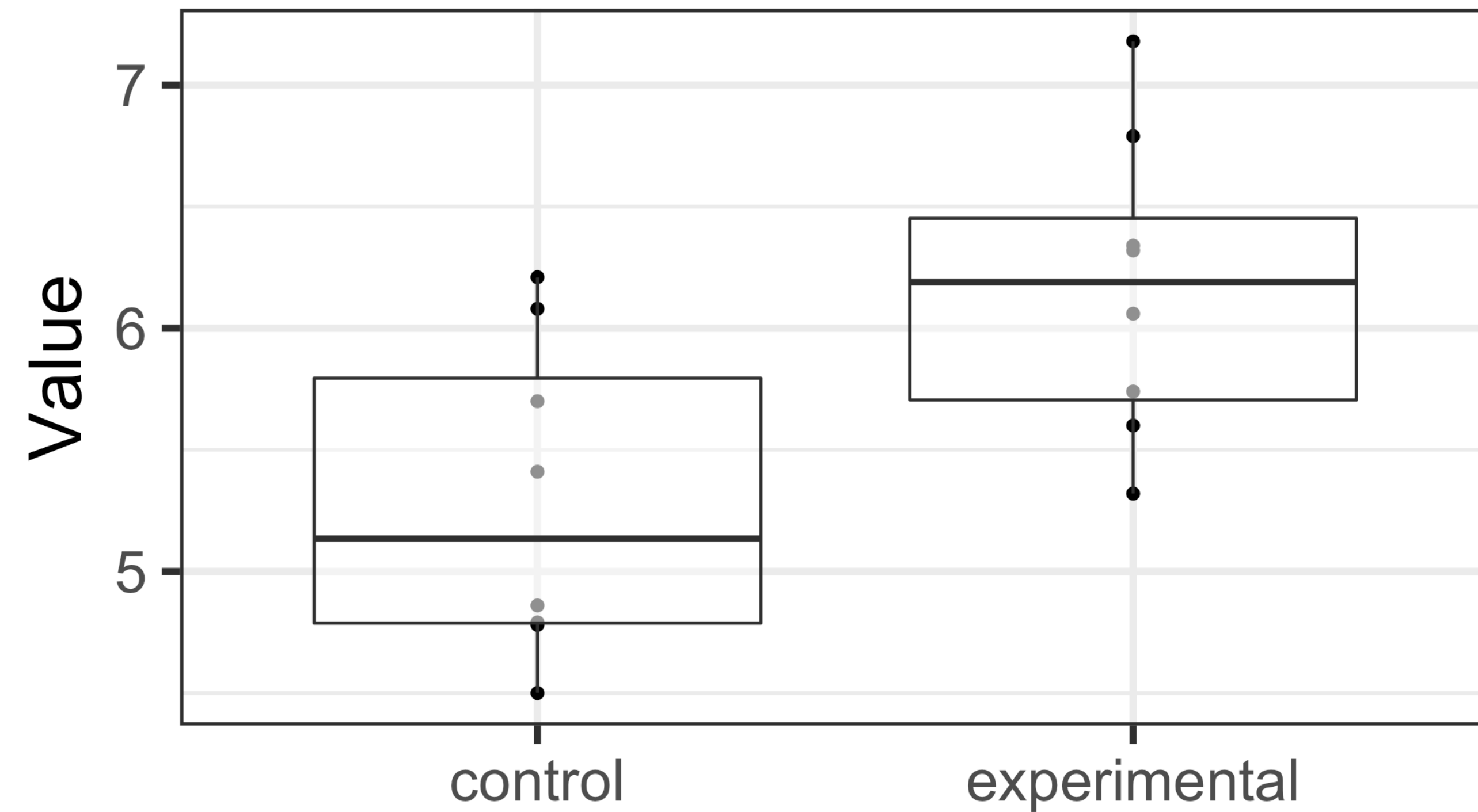
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Data, in order →



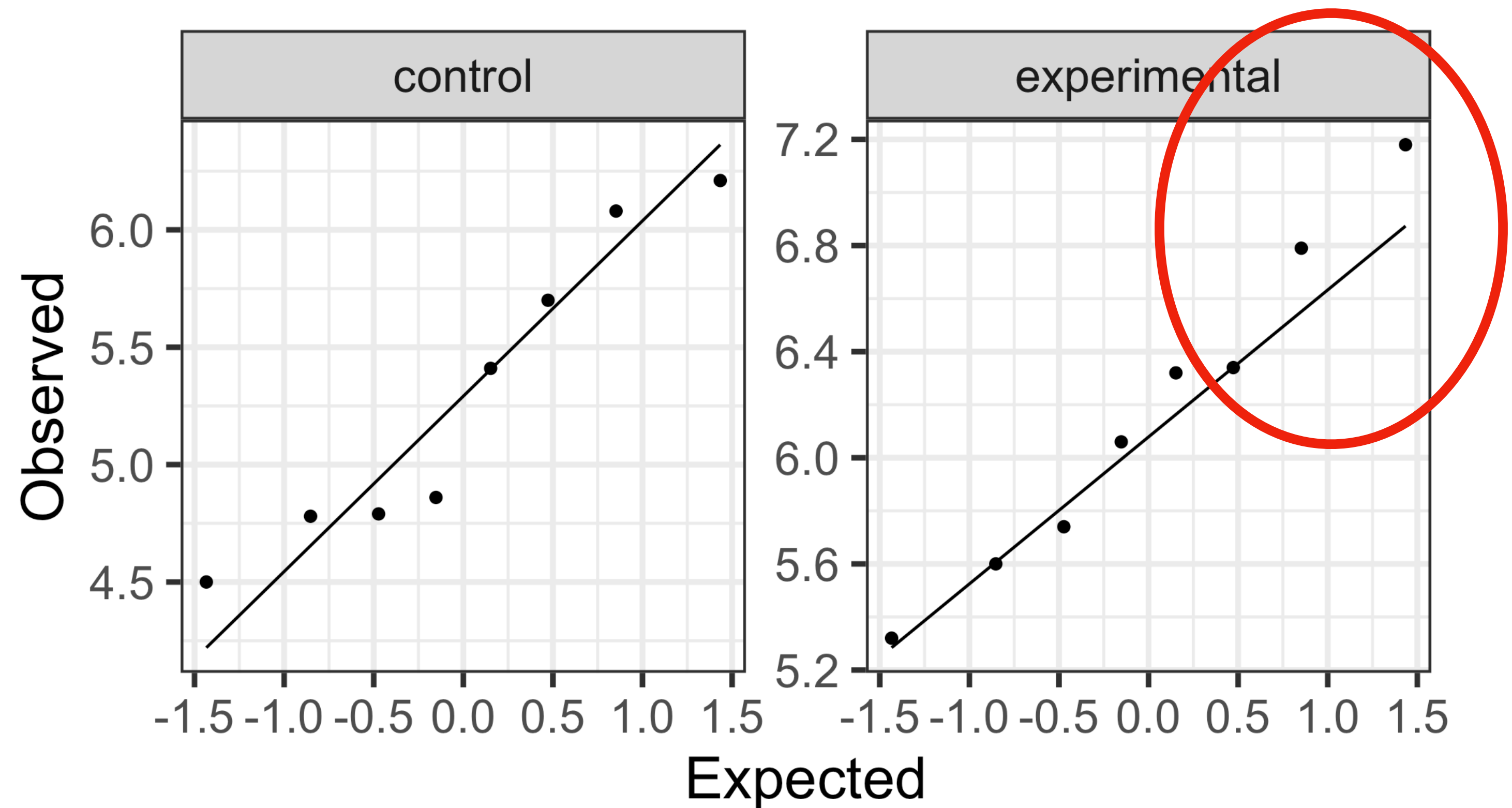
# Wilcoxon-Mann-Whitney (U) test

experimental	control
5.32	4.50
5.60	4.78
5.74	4.79
6.06	4.86
6.32	5.41
6.34	5.70
6.79	6.08
7.18	6.21



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$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different



# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, focus on the ideas !!**

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**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large for well separated populations with little overlap}$ )

1. Calculate test statistic
2. Look at distribution of test statistic under assumption of null hypothesis
3. Calculate the  $P$ -value based on significance level  $\alpha$

$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different

# Wilcoxon-Mann-Whitney (U) test

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1. Calculate test statistic
  1. Determine  $K_1$  and  $K_2$
  2.  $U_S$  is the larger of  $K$ s

$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different



# Wilcoxon-Mann-Whitney (U) test

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experimental		control
4	5.32	✓ 4.50
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	5.74	✓ 4.79
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6	6.06	✓ 4.86
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8	7.18	✓ 6.21

**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large for well separated populations with little overlap}$ )

1. Calculate test statistic  $K_1 = 4+5+6+6+8+8+8+8 = 53$ 
  1. Determine  $K_1$  and  $K_2$
  2.  $U_S$  is the larger of  $K$ s

$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different

# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, focus on the ideas !!**

experimental		control
4	5.32	0 4.50
5	5.60	4.78
6	5.74	4.79
6	6.06	4.86
8	6.32	5.41
8	6.34	5.70
8	6.79	6.08
8	7.18	6.21

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experimental		control	
4	5.32	0	4.50
5	5.60	0	4.78
6	5.74	0	4.79
6	6.06	0	4.86
8	6.32	1	5.41
8	6.34	2	5.70
8	6.79	4	6.08
8	7.18	4	6.21

**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large for well separated populations with little overlap}$ )

1. Calculate test statistic  $K_1 = 4+5+6+6+8+8+8+8 = 53$

1. Determine  $K_1$  and  $K_2$

$$K_2 = 11$$

$$K_1 + K_2 = n_1 n_2$$

2.  $U_S$  is the larger of  $K$ s

$$U_S = 53$$

$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different

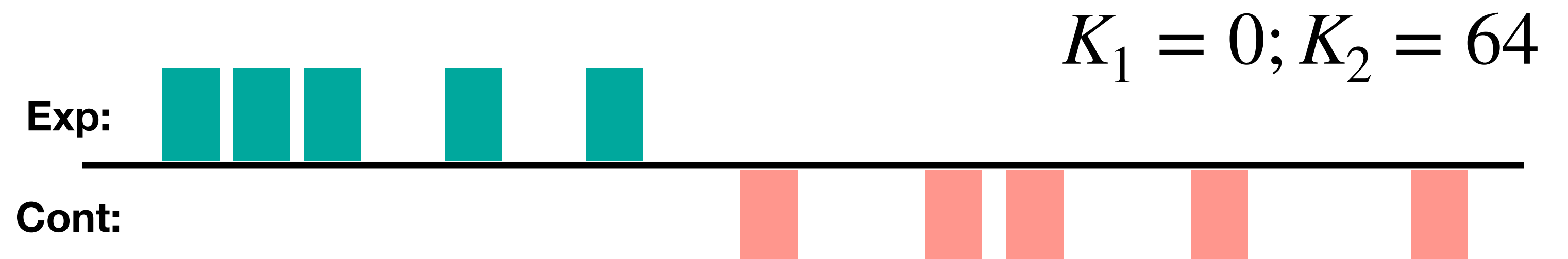


# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, focus on the ideas !!**

experimental	control
5.32	4.50
5.60	4.78
5.74	4.79
6.06	4.86
6.32	5.41
6.34	5.70
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7.18	6.21

**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large}$  for well separated populations with little overlap)



$H_0$  : The population distributions for control and experiment are the same

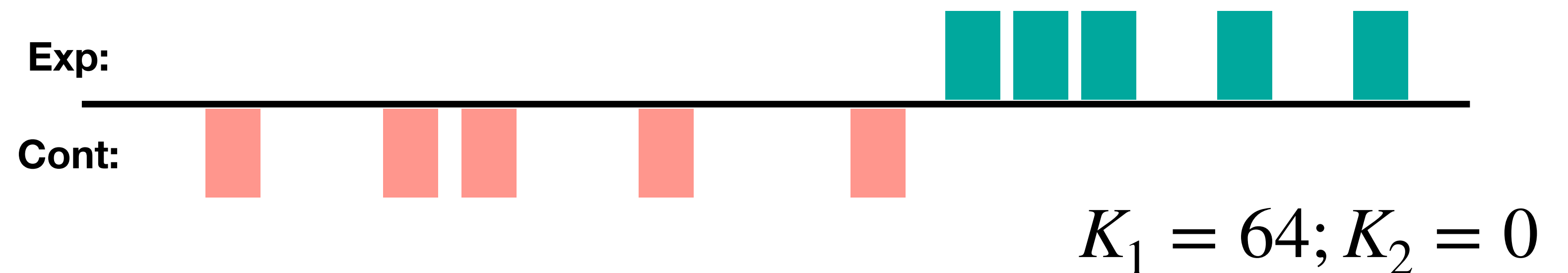
$H_A$  : The population distributions for control and experiment are different

# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, focus on the ideas !!**

experimental	control
5.32	4.50
5.60	4.78
5.74	4.79
6.06	4.86
6.32	5.41
6.34	5.70
6.79	6.08
7.18	6.21

**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large}$  for well separated populations with little overlap)



$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different

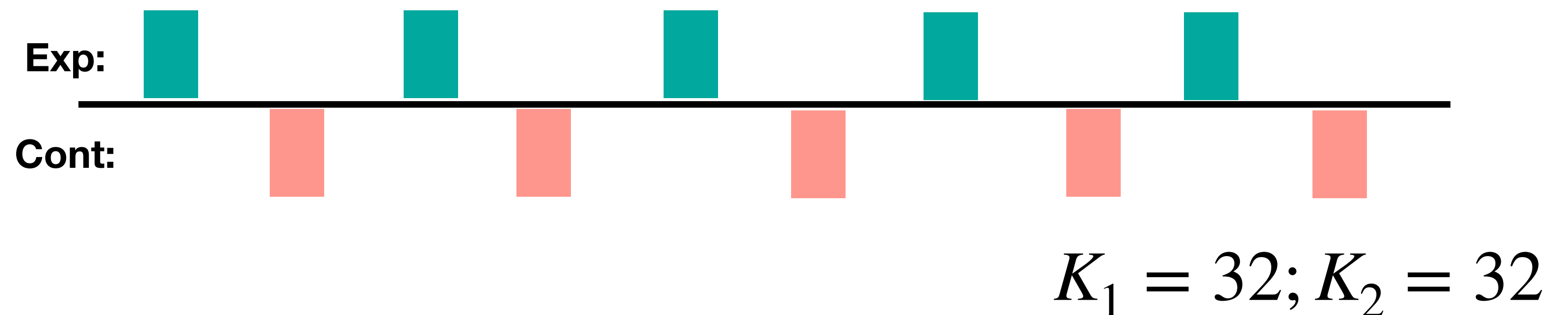


# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, focus on the ideas !!**

experimental	control
5.32	4.50
5.60	4.78
5.74	4.79
6.06	4.86
6.32	5.41
6.34	5.70
6.79	6.08
7.18	6.21

**Test statistic  $U_S$  measures degree of separation between two samples** ( $U_S = \text{large}$  for well separated populations with little overlap)



$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different



# Wilcoxon-Mann-Whitney (U) test

**!! Don't focus on the math, remember the R code !!**

$$U_S = 53$$

```
> wilcox.test(experiment, control)
```

Wilcoxon rank sum exact test

data: study\$experimental and study\$control

W = 53, p-value = 0.02813

alternative hypothesis: true location shift is not equal to 0



$H_0$  : The population distributions for control and experiment are the same

$H_A$  : The population distributions for control and experiment are different

# Non-parametric alternatives

## Randomization

- Any population distribution
- Does not even assume random or independent samples!
- High power
- Difficult to perform\*

## Wilcoxon-Mann-Whitney (Rank Sum)

- **Random samples**
- **Independent observations**
- **Independent samples**
- Any population distribution
- Lower power
- Easy to perform

## Sign test

## Wilcoxon signed-rank test

# The sign test

- Analogous to the **paired *t*-test** (differences) OR **one-sample *t*-test**
- Simplest (and least powerful) test
- Only uses the sign/direction of the data (i.e. + or -) which can be useful for non-quantitative data (i.e. survival, increase/decrease, yes/no... etc)

# The sign test

Sample	Survival
1	+
2	+
3	+
4	+
5	+
6	+
7	-
8	+
9	+
10	+
11	-

1. Calculate test statistic
2. Look at distribution of test statistic under assumption of null hypothesis
3. Calculate the  $P$ -value based on significance level  $\alpha$

$H_0$  : There are equal numbers of survival and non-survival

$H_A$  : There are not equal numbers of survival and non-survival

# The sign test

Sample	Survival
1	+
2	+
3	+
4	+
5	+
6	+
7	-
8	+
9	+
10	+
11	-

1. Calculate test statistic

1. Calculate the number of positives ( $N_+$ ) and negatives ( $N_-$ )

2. Test statistic  $B_S = \text{larger of } N_s$

**Binomial distribution!!**

$$N_+ = 9$$

$$N_- = 2$$

$$B_S = 9$$

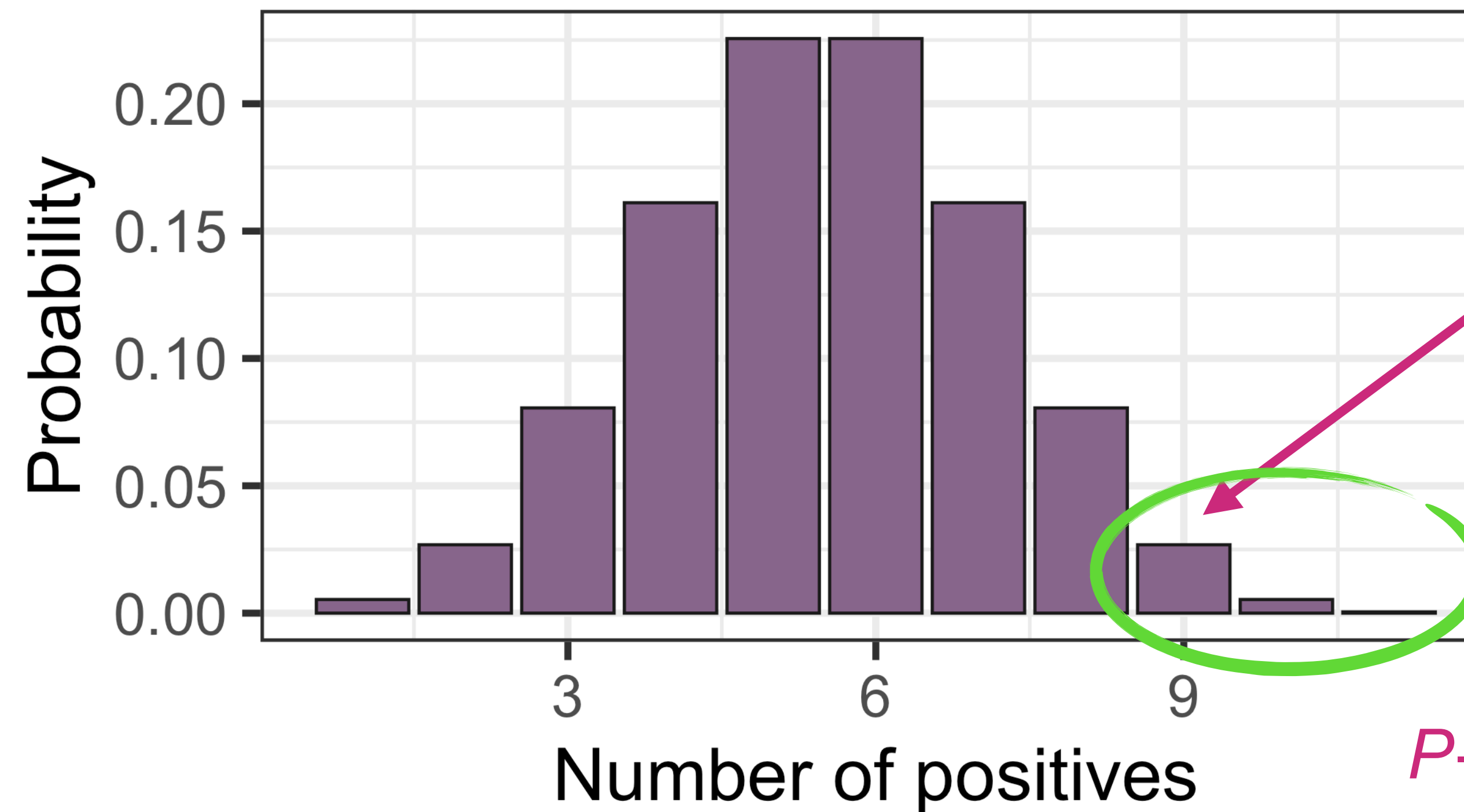
$H_0$  : There are equal numbers of survival and non-survival

$H_A$  : There are not equal numbers of survival and non-survival

# The sign test

$$H_0 : Pr[survival] = 0.5$$

Sample	Survival
1	+
2	+
3	+
4	+
5	+
6	+
7	-
8	+
9	+
10	+
11	-



$P\text{-value} = 0.032$



$H_0$  : There are equal numbers of survival and non-survival

`pbinom(q, n, p)`

$H_A$  : There are not equal numbers of survival and non-survival

`pbinom(2, 11, 0.5)`

# Non-parametric alternatives

## Randomization

- Any population distribution
- Does not even assume random or independent samples!
- High power
- Difficult to perform\*

## Wilcoxon-Mann-Whitney (Rank Sum)

- **Random samples**
- **Independent observations**
- **Independent samples**
- Any population distribution
- Lower power
- Easy to perform

## Sign test

- **Random samples**
- **Independent observations**
- **Independent samples**
- Any population distribution
- Lower power
- Easy to perform

## Wilcoxon signed-rank test



# Wilcoxon signed-rank test

- Analogous to the **paired *t*-test** (differences) OR **one-sample *t*-test**
- Can mostly be used in same scenario as sign test, but more powerful (and more difficult to perform)
- Uses **sign**/direction AND **rank**

# Wilcoxon signed-rank test

**!! Don't focus on the math, focus on the ideas !!**

Animal	Site I	Site II
1	50.6	38
2	39.2	18.6
3	35.2	23.2
4	17.0	190
5	11.2	6.6
6	14.2	16.4
7	24.2	14.4
8	37.4	37.6
9	35.2	24.4

1. Calculate test statistic
  1. Calculate the difference
  2. Take absolute value of difference
  3. Rank values from smallest to largest
  4. Restore + and - to ranks
  5. Sum positive signed ranks ( $W_S$ ) and sum absolute values of negative signed ranks ( $W_-$ )
  6. Test statistic  $W_S = \text{larger of } W_s$

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

# Wilcoxon signed-rank test

## 1. Calculate the difference

Animal	Site I	Site II	Diff
1	50.6	38	12.6
2	39.2	18.6	20.6
3	35.2	23.2	12.0
4	17.0	190	-2.0
5	11.2	6.6	4.6
6	14.2	16.4	-2.2
7	24.2	14.4	9.8
8	37.4	37.6	-0.2
9	35.2	24.4	10.8

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

# Wilcoxon signed-rank test

## 2. Take the absolute value of each difference

Animal	Site I	Site II	Diff	Abs(Diff)
1	50.6	38	12.6	12.6
2	39.2	18.6	20.6	20.6
3	35.2	23.2	12.0	12.0
4	17.0	190	-2.0	2.0
5	11.2	6.6	4.6	4.6
6	14.2	16.4	-2.2	2.2
7	24.2	14.4	9.8	9.8
8	37.4	37.6	-0.2	0.2
9	35.2	24.4	10.8	10.8

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

# Wilcoxon signed-rank test

## 3. Rank absolute value of differences from smallest to largest

Animal	Site I	Site II	Diff	Abs(Diff)	Rank
1	50.6	38	12.6	12.6	8
2	39.2	18.6	20.6	20.6	9
3	35.2	23.2	12.0	12.0	7
4	17.0	190	-2.0	2.0	2
5	11.2	6.6	4.6	4.6	4
6	14.2	16.4	-2.2	2.2	3
7	24.2	14.4	9.8	9.8	5
8	37.4	37.6	-0.2	0.2	1
9	35.2	24.4	10.8	10.8	6

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II



# Wilcoxon signed-rank test

## 4. Restore + and - to produce “signed ranks”

Animal	Site I	Site II	Diff	Abs(Diff)	Rank	Signed rank
1	50.6	38	12.6	12.6	8	8
2	39.2	18.6	20.6	20.6	9	9
3	35.2	23.2	12.0	12.0	7	7
4	17.0	190	-2.0	2.0	2	-2
5	11.2	6.6	4.6	4.6	4	4
6	14.2	16.4	-2.2	2.2	3	-3
7	24.2	14.4	9.8	9.8	5	5
8	37.4	37.6	-0.2	0.2	1	-1
9	35.2	24.4	10.8	10.8	6	6

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

# Wilcoxon signed-rank test

## 5. Sum signed ranks and choose test statistic $W_S$

Animal	Site I	Site II	Diff	Abs(Diff)	Rank	Signed rank
1	50.6	38	12.6	12.6	8	8
2	39.2	18.6	20.6	20.6	9	9
3	35.2	23.2	12.0	12.0	7	7
4	17.0	190	-2.0	2.0	2	-2
5	11.2	6.6	4.6	4.6	4	4
6	14.2	16.4	-2.2	2.2	3	-3
7	24.2	14.4	9.8	9.8	5	5
8	37.4	37.6	-0.2	0.2	1	-1
9	35.2	24.4	10.8	10.8	6	6

$$W_+ = 8+9+7+4+5+6 = 39$$

$$W_- = 2+3+1$$

$$W_S = 39$$

# Wilcoxon signed-rank test

**!! Don't focus on the math, focus on the ideas (and remember R code) !!**

```
wilcox.test(siteI, siteII, paired = T)
```

$$W_S = 39$$

Wilcoxon signed rank exact test

data: siteI and siteII

V = 39, p-value = 0.05469

alternative hypothesis: true location shift is not equal to 0



$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

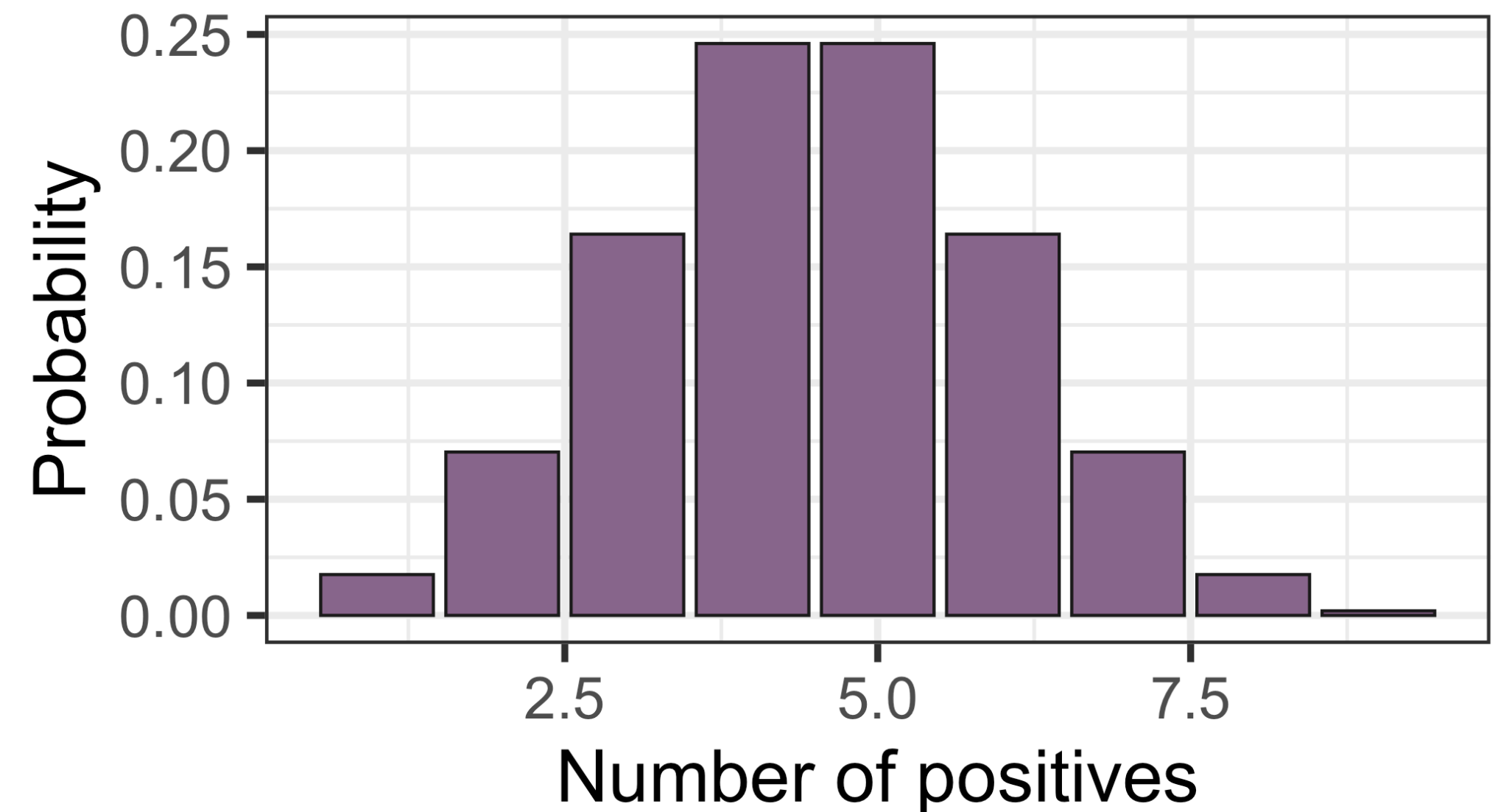
# Wilcoxon signed-rank test

( $P = 0.054$ ;  
signed-rank test)

How does this compare to the sign test?

Animal	Site I	Site II	Diff
1	50.6	38	12.6
2	39.2	18.6	20.6
3	35.2	23.2	12.0
4	17.0	190	-2.0
5	11.2	6.6	4.6
6	14.2	16.4	-2.2
7	24.2	14.4	9.8
8	37.4	37.6	-0.2
9	35.2	24.4	10.8

$W_+ = 6$        $W_- = 3$        $W_S = 6$



$\text{pbinom}(3, 9, 0.5) \longrightarrow 0.25$

$H_0$  : No difference in nerve cell density between site I and site II

$H_A$  : There is a difference in nerve cell density between site I and site II

# Non-parametric alternatives

## Randomization

- Any population distribution
- Does not even assume random or independent samples!
- High power
- Difficult to perform\*

## Wilcoxon-Mann-Whitney (Rank Sum)

- **Random samples**
- **Independent observations**
- **Independent samples**
- Any population distribution
- Lower power
- Easy to perform

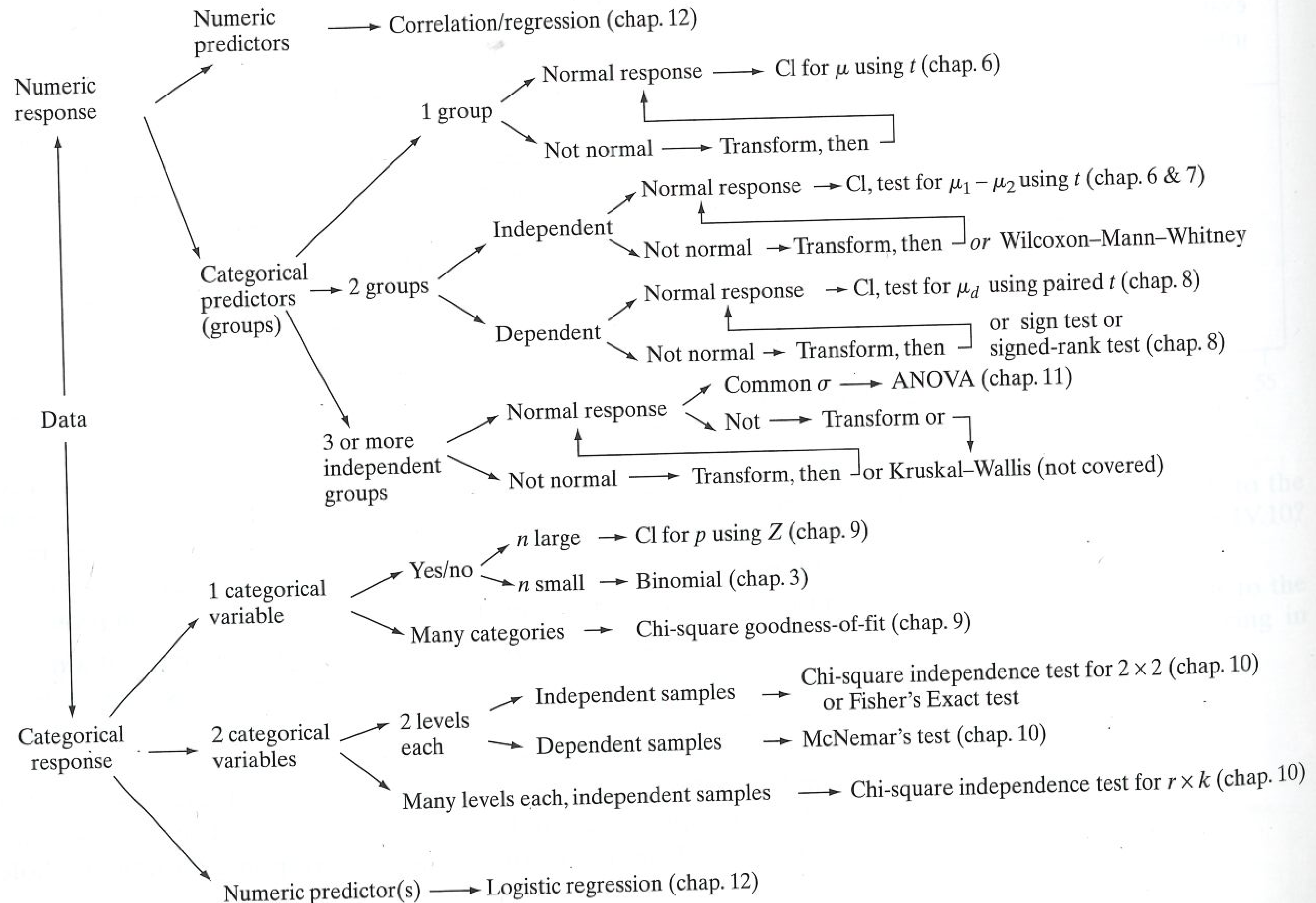
## Sign test

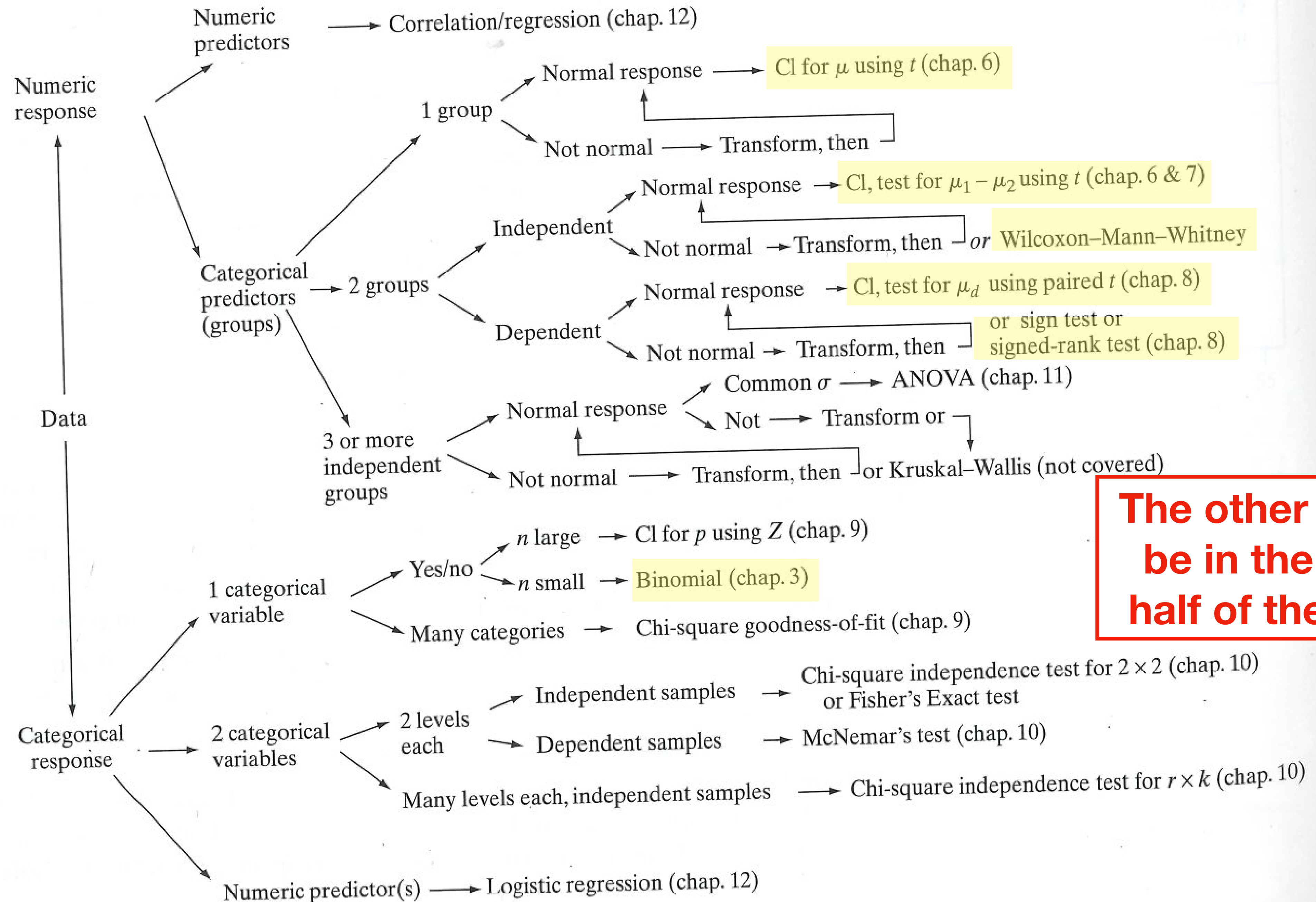
- **Random samples**
- **Independent observations**
- **Independent samples**
- Any population distribution
- Lower power
- Easy to perform

## Wilcoxon signed-rank test

- **Random samples**
- **Independent observations**
- **Symmetric distribution (not normal)**
- Can use with incomplete data
- Higher power
- More difficult to perform







**The other tests will be in the second half of the course!**

# Announcements

- Midterm is one week from today (**Thursday, October 28, 1:30-3:00, Hughes Auditorium**)
- Tuesday will be a review day, please plan to attend as we will cover important information about the midterm and final project, but you may leave early if you wish
- HW grading announcement