# Hypothesis testing: two samples

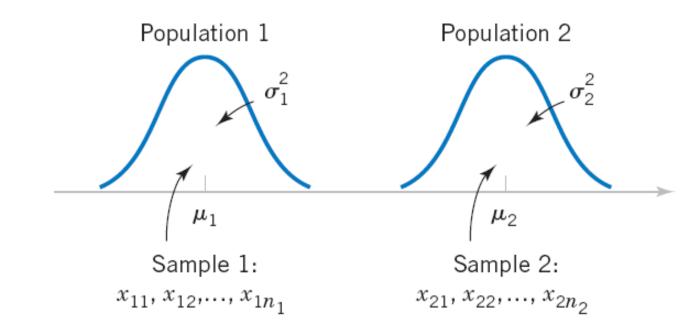


Figure 10-1 Two independent populations.

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#### **Assumptions**

- 1.  $X_{11}, X_{12}, \dots, X_{1n_1}$  is a random sample from population 1.
- 2.  $X_{21}, X_{22}, \ldots, X_{2n_2}$  is a random sample from population 2.
- 3. The two populations represented by  $X_1$  and  $X_2$  are independent.
- **4.** Both populations are normal.

$$E(\overline{X}_1 - \overline{X}_2) = E(\overline{X}_1) - E(\overline{X}_2) = \mu_1 - \mu_2$$

$$V(\overline{X}_1 - \overline{X}_2) = V(\overline{X}_1) + V(\overline{X}_2) = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}$$

The quantity

$$Z = \frac{\overline{X}_1 - \overline{X}_2 - (\mu_1 - \mu_2)}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$
(10-1)

has a N(0, 1) distribution.

10-2.1 Hypothesis Tests for a Difference in Means,

**Variances Known** 

Null hypothesis: 
$$H_0$$
:  $\mu_1 - \mu_2 = \Delta_0$ 
Test statistic:  $Z_0 = \frac{\overline{X_1} - \overline{X_2} - \Delta_0}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$  (10-2)

Alternative Hypotheses	<i>P</i> -Value	for Fixed-Level Tests		
$H_1$ : $\mu_1 - \mu_2 \neq \Delta_0$	Probability above $ z_0 $ and probability below $- z_0 $ ,	$z_0 > z_{\alpha/2} \text{ or } z_0 < -z_{\alpha/2}$		
$H_1$ : $\mu_1 - \mu_2 > \Delta_0$	$P = 2[1 - \Phi( z_0 )]$ Probability above $z_0$ ,	$z_0 > z_{\alpha}$		
$H_1$ : $\mu_1 - \mu_2 < \Delta_0$	$P = 1 - \Phi(z_0)$ Probability below $z_0$ , $P = \Phi(z_0)$	$z_0 < -z_{\alpha}$		

Dejection Cuitarian For

### 10-2.1 Hypotheses Tests on the Difference in Means, Variances Unknown

Case 2:
$$\sigma_1^2 \neq \sigma_2^2$$

If  $H_0$ :  $\mu_1 - \mu_2 = \Delta_0$  is true, the statistic

$$T_0^* = \frac{\overline{X}_1 - \overline{X}_2 - \Delta_0}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$
 (10-15)

is distributed as t-distribution with degrees of freedom given by

$$v = n_1 + n_2 - 2,$$

or more generally

### Multiple null hypotheses: Bonferroni correction

- What if you have m independent null hypotheses?
   Say you have m=25,000 genes in a genome?
- What is the probability that at least one of the null-hypotheses will be shown to be false at significance threshold  $\alpha_1$ ?
- Answer:
   Family-Wise Error Rate or FWER=1-(1- α₁)<sup>m</sup> ≈mα₁
- If m=20 and  $\alpha_1$ =0.05, FWER= 0.6415

Carlo Emilio Bonferroni (1892 –1960) Italian mathematician who worked on probability theory.



• If you want to get FWER<  $\alpha$ , use  $\alpha_1 = \alpha/m$ 

Did you know that M&M's® Milk Chocolate Candies are supposed to come in the following percentages: 24% blue, 20% orange, 16% green, 14% yellow, 13% red, 13% brown?

#### http://www.scientificameriken.com/candy5.asp

"To our surprise M&Ms met our demand to review their procedures in determining candy ratios. It is, however, noted that the figures presented in their email differ from the information provided from their website (http://us.mms.com/us/about/products/milkchocolate/). An email was sent back informing them of this fact. To which M&Ms corrected themselves with one last email:

In response to your email regarding M&M'S CHOCOLATE CANDIES

Thank you for your email.

On average, our new mix of colors for M&M'S® Chocolate Candies is:

M&M'S® Milk Chocolate: 24% blue, 20% orange, 16% green, 14% yellow, 13% red, 13% brown.

M&M'S® Peanut: 23% blue, 23% orange, 15% green, 15% yellow, 12% red, 12% brown.

M&M'S® Kids MINIS®: 25% blue, 25% orange, 12% green, 13% yellow, 12% red, 13% brown.

M&M'S® Crispy: 17% blue, 16% orange, 16% green, 17% yellow, 17% red, 17% brown.

M&M'S® Peanut Butter and Almond: 20% blue, 20% orange, 20% green, 20% yellow, 10% red, 10% brown.

Have a great day!

Your Friends at Masterfoods USA A Division of Mars, Incorporated



How to accept or reject the null hypothesis that these probabilities are correct from a finite sample?

#### Pearson chi<sup>2</sup> Goodness of Fit Test

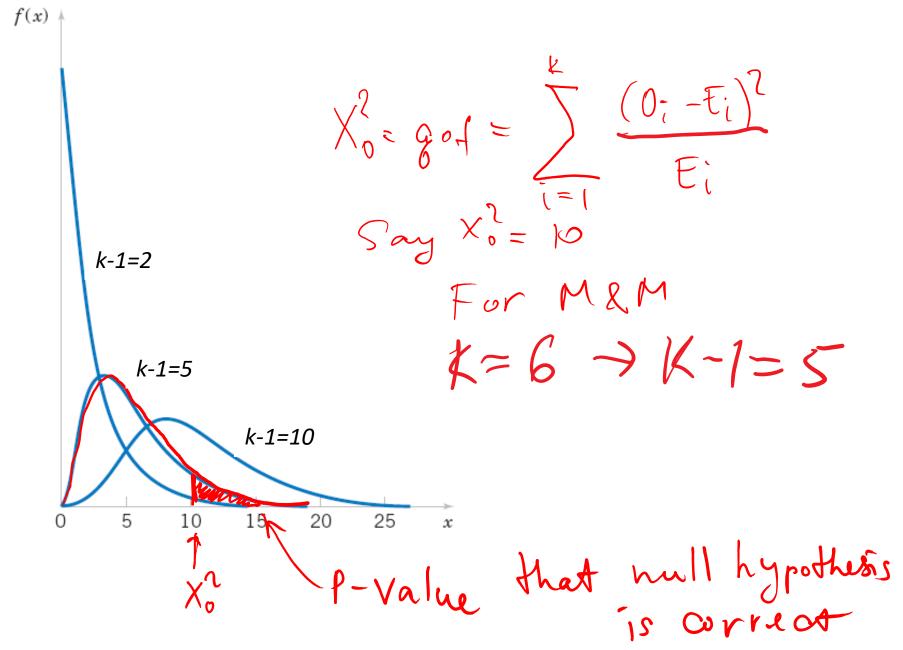
- Assume there is a sample of size n from a population with k classes (e.g. 6 M&M colors)
- Null hypothesis H<sub>0</sub>: class *i* has frequency *f<sub>i</sub>* in the population
- Alternative hypothesis  $H_1$ : some population frequencies are inconsistent with  $f_i$
- Let  $O_i$  be the observed number of sample elements in the *i*th class and  $E_i = n f_i$  be the expected number of sample elements in the *i*th class.
- Group any bin with  $E_i$  <3 with
- a) if numerical value of i is important, group it with its neighbor (k=i-1 or k=i+1) which has the smallest  $E_k$  until  $E_{group} >=3$ ;
- b) If numerical value of i is irrelevant, group together all  $E_i$  <3 bins until  $E_{qroup}$  >=3
- The test statistic is

$$X_0^2 = \sum_{i=1}^k \frac{(O_i - E_i)^2}{E_i} \tag{9-47}$$

P-value is calculated based on the chi-square distribution with k-1 degrees of freedom:

P-value = Prob(H<sub>0</sub> is correct) =1-CDF\_chi-squared( $X_0^2$ , k-1)

# chi<sup>2</sup> Goodness of Fit Test is a <u>one-sided</u> hypothesis



### M&M group exercise

- DO NOT EAT CANDY BEFORE COUNTING IS FINISHED! THEN, PLEASE, DO.
- We will be testing three null hypotheses one after another:
  - M&M official data: 24% blue, 20% orange, 16% green, 14% yellow, 13% red, 13% brown
  - Website (fan collected) data from
     http://joshmadison.com/2007/12/02/mms-color-distribution-analysis:
     18.36% blue, 20.76% orange, 18.44% green, 14.08% yellow, 14.20% red, 14.16% brown
  - Uniform distribution: 1/6~16.67% of each candy color
- You will estimate P-values for <u>each one of these null</u> <u>hypotheses</u>
- Hints:  $O_i$  is the observed # of candies of color i; calculate the expected #  $E_i$ =(# candies in your sample)\* $f_i$

Use 1-chi2cdf(X0squared, 5) for P-value

$$X_0^2 = \sum_{i=1}^k \frac{(O_i - E_i)^2}{E_i}$$

### M&M matlab exercise

```
observed=mm table(group,:); group % use when analyzing one group
f mm=[0.24,0.2,0.16, 0.14, 0.13,0.13];
f u=1./6.*ones(1,6);
f website=[18,21,18,14,14,14,14];
f website=f website./sum(f website);
%p website=[0.1836, 0.2076, 0.1844, 0.1408, 0.1420, 0.1416]
%p u=[0.1500, 0.2200, 0.2100, 0.1200, 0.1600, 0.1500];
n=sum(observed)
expected u=n.*f u;
expected mm=n.*f mm;
expected website=n.*f website;
gf mm=0; gf u=0; gf website=0;
for m=1:6;
  gf mm=gf mm+(observed(m)...
    -expected_mm(m)).^2./expected_mm(m);
  gf u=gf u+(observed(m)-expected u(m)).^2./expected u(m);
  gf website=gf website+(observed(m)...
    -expected website(m)).^2./expected website(m);
end:
disp('goodness of fit of MM ='); disp(num2str(gf mm));
disp('p-value of MM ='); disp(num2str(1-chi2cdf(gf mm,5))); disp(' ');
disp('goodness of fit of website ='); disp(num2str(gf website));
disp('p-value of MM ='); disp(num2str(1-chi2cdf(gf website,5))); disp(' ');
disp('goodness of fit of uniform ='); disp(num2str(gf_u));
disp('p-value of uniform='); disp(num2str(1-chi2cdf(gf u,5)));
```

### Statistical tests of independence

# How to <u>test the hypothesis</u> if multiple sample are drawn from the same population?

- Table: samples (Student groups) rows, classes (M&M colors) – columns
- Test if color fractions are <u>independent</u> from group
- P(Group 1 and Color = green) =
   P(Group 1)\*P(Color green)
- Compute for all groups/colors 6\*4=24 in our case

$$E_{green}(group 1) = n_{tot} *(group 1/n_{tot}) *(green/n_{tot})$$

• 
$$\chi^2 = \sum_{groups \& colors}^{n_{tot}} \frac{\left(o_{color} (group) - E_{color} (group)\right)^2}{E_{color} (group)}$$

# degrees of freedom=(colors-1)\*(groups-1)

- M&M exercise Spring 2024
- Was the M&M box from Costco well mixed?
   Let's compare the first two groups' data

Title -	Blue 🔽	Oran	Greer	Yello₁▼	Red 🔽	<b>Brow</b>	Samp  -	<b>Origit</b>
group 1	29	22	34	45	41	14	185	Costco
group 2	30	28	25	43	44	27	197	Costco
all Costco	59	50	59	88	85	41	382	
							0	
group 3	44	30	52	10	50	27	213	Schnuc
group 4	53	31	58	17	41	30	230	Schnuc
all Schnuc	97	61	110	27	91	57	443	

• Using 
$$\chi^2 = \sum_{groups \& colors}^{24} \frac{\left(O_{color}\left(group\right) - E_{color}\left(group\right)\right)^2}{E_{color}\left(group\right)}$$
with # degrees of freedom (colors 1)\*(groups 1)

with # degrees of freedom (colors-1)\*(groups-1)

Find P-value of null hypothesis H<sub>0</sub> that samples are independent from each other

### Was the Costco box well mixed?

```
    clear mm table

 mm table=mm table all(1:2,:);
 ngroups=2;
 ncolors=6;
 sumt=sum(sum(mm table))
 sum color=sum(mm table, 1)
 sum group=sum(mm table, 2)
 mm exp=kron(sum group,sum color)./sumt
 gof=sum(sum((mm table-mm exp).^2./mm exp))
 P value gof=1-chi2cdf(gof, (ngroups-1)*(ncolors-1))
• %gof = 6.0121; P value gof = 0.3050
```

 The null model that samples are independent is not rejected → The Costco box was well mixed!

### Batch effect

### Does color composition vary between Costco and Schnucks

- Costco: 59 50 59 88 85 41
- Schnucks: 97 61 110 27 91 57
- Test if they are significantly different from each other:
- Same statistical independence test: ngroups=2; ncolors=6;
- Results:

Goodness of Fit = 56.7101 P-value = 5.8028e-11

Batch effect is highly statistically significant!
 Costco and Schnucks do nor represent the same population

# Do Costco (groups 1 and 2) and Schnucks (groups 3 and 4) data come from the same population (factory?)

```
clear mm table
mm table (1,:) = sum (mm \text{ table all } (1:2,:));
mm table (2,:) = sum (mm \text{ table all } (3:4,:));
ngroups=2;
ncolors=6;
sumt=sum(sum(mm table))
sum color=sum(mm table, 1)
sum group=sum(mm table, 2)
mm exp=kron(sum group,sum color)./sumt
gof=sum(sum((mm table-mm exp).^2./mm exp))
P_value_gof=1-chi2cdf(gof, (ngroups-1)*(ncolors-1))
% Goodness of Fit = 56.7101
P-value = 5.8028e-11
```

- The null model that samples are independent is <u>rejected</u>
- Costco and Schnucks get candy from different factories

