Things we haven't covered that are of general interest
- Two-way factorial ANOVA
- Randomized Complete Block Designs (RCBD) Quick introduction to these topics
  - Goal is that you know some names if you want to pursue any topic
  - Stat 5710 (Intro to Expt. Design) covers factorial ANOVA and RCBD in great depth

Two-way factorial designs:
- Treatments are classified by two different factors
  - My language: A treatment is a unique thing "done" to an experimental unit
- Collard study: 4 methods to measure riboflavin in cooked collards
  - Experimental unit: 100 gm of collard leaves
  - Observational unit: 100 gm of collard leaves

Treatment	Sample size	Chemical Clarification	Mean
A	Small (0.25 gm)	No	μ_a
B	Small (0.25 gm)	Yes	μ_b
C	Large (1.00 gm)	No	μ_c
D	Large (1.00 gm)	Yes	μ_d

Two-way factorial designs: cell means model
- Cell-means model: Fitting a mean for each treatment, followed by linear contrasts
- We already know how to do this:
  - Fit an ANOVA model where each treatment has a different mean
  - Know how to answer various relevant questions

- Q: Do all treatments have the same mean?
  - Overall F test (3 df) comparing 4 different means model to equal means model

- Q: Which means differ from which others?
  - Pairwise comparisons, almost certainly with Tukey adjustment for multiple comparisons

- Q: What is the difference between Small and Large, averaged over Clarification
  - Linear contrast: $(\mu_a + \mu_b)/2 - (\mu_c + \mu_d)/2$

- Q: What is the difference between Yes and No, averaged over Sample size
  - Linear contrast: $(\mu_b + \mu_d)/2 - (\mu_a + \mu_c)/2$

- Q: Is the effect of sample size (large - small) the same for Yes and No?
  - Linear contrast: $(\mu_d - \mu_b) - (\mu_c - \mu_a)$

Collard analysis: cell means approach:

Cell means:	a (sn)	b (sy)	c (ln)	d (ly)	Pooled sd: $s_p = 2.57$, 8 df
	42.6	25.1	37.0	23.7	

Contrasts:

	Coefficients						
Effect	a (sn)	b (sy)	c (ln)	d (ly)	Estimate	se	p
Sample size	0.5	0.5	-0.5	-0.5	3.48	1.48	0.047
Clarify	0.5	-0.5	0.5	-0.5	-15.45	1.48	<0.0001
Interaction	1	-1	-1	1	-4.17	2.97	0.20

Interpretation / conclusions:
- No evidence of an interaction between Sample size and Clarification
- Smaller sample size has 3.48 larger concentration
- Clarifying the sample reduces concentration by 15.45

Factorial designs: Vocabulary
- Factor: Variable being manipulated
  - 2 factors: Sample size and Chemical Clarification
- Levels: each value of a factor
  - Small or Large for Size, No or Yes for Clarification
- Two-way factorial: 2 factors
  - 2 x 2 factorial: 2 factors, each with 2 levels
  - 3 x 5 factorial: 2 factors, one with 3 levels, other with 5
- Four-way factorial: 4 factors, e.g., 2 x 2 x 3 x 5 factorial
- Complete: treatments are all combinations of all levels of the factors
  - Complete 2 x 2 factorial will have 4 treatments (like the Collard study)
  - Complete 3 x 5 factorial will have 15 treatments
- Balanced: all cells have same sample size
- Focus on the treatments
  - label them by the combination of levels, and
  - organize in two rows and two columns
- Dot in the subscript represents averaging over that subscript
  - $\mu_{.n} = (\mu_{sn} + \mu_{ln})/2$

	Chemical Clarification		Marginal Mean
Sample Size	No	Yes	
Small	μ_{sn}	μ_{sy}	$\mu_{s.}$
Large	μ_{ln}	μ_{ly}	$\mu_{l.}$
Marginal Mean	$\mu_{.n}$	$\mu_{.y}$	

More vocabulary:
- Cell mean: mean for one treatment
- Marginal mean (or LSMEAN): mean for a level **averaged over all levels of other factors**
- Main effect: difference in marginal means
- Simple effect: difference between cell means

Two-way factorial designs: factor-based analyses
- Simpler way to get the same results
  - Avoids writing contrast coefficients

Based on the 2x2 organization of treatment means
Use 2 subscripts to identify treatments,
i: which level of sample size (s or l), j which level of clarification (n or y)

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

α_i main effects for factor A: α_s shared by both μ_{sn} and μ_{sy}
β_j main effects for factor B: β_n shared by both μ_{sn} and μ_{ln}
$\alpha\beta_{ij}$ interaction effects: unique to each cell of the treatment structure
Get SS and tests of A (main effect of A), of B (main effect of B) and AB (interaction)
Use type III SS
Answer useful questions even if data aren't balanced
Same results as using linear contrasts, but much simpler to write code
Follow up with contrasts or multiple comparisons between marginal means
e.g., between levels of A or levels of B

Collard example: factor-based analysis

Effect	p	LSMEANS		Difference	se
Sample size	0.047	S: 33.8	L: 30.4	3.48	1.48
Clarify	<0.0001	N: 39.8	Y: 24.4	15.4	1.48
Interaction	0.20			-4.17	2.97

Same test results, same estimates, same standard errors
as the cell means approach

Interpretation / usual practice:
Why consider main effects instead of simple effects?
When no interaction or interaction is small, definitely use main effects:
Main effects are more precise:
marginal means are averages of cell means
main effects are averages of simple effects
Main effect: Small - Large, se = 1.48.
Simple effect: Small - Large in No Clarify: se = 2.10
Main effect has double the sample size (6 obs get Small sample size, 3 get Small/No)
Main effects give simpler summaries:
one estimate of effect of Small - Large, not two simple effects
Main are estimates of each simple effect
Usual practice: test interaction
If n.s., interpret main effects, both as averages and simple effects
If signif. interaction, think about whether average is interpretable
Don't interpret main effects as estimates of simple effects
My practice: always leave interactions in the model
Maintains connection to contrasts in cell means analysis
and pooled error sd, s_p, estimated only from within-cell variability
There are other opinions and practices
usually based on regression interpretations, not comparisons of means

Randomized complete block designs (RCBD):
- Same idea as pairing, but with more than 2 treatments
- Identify groups of similar experimental units (blocks)
- Examples: Parts of a field, litters of pigs, age& sex groups
  - Want small variability within block, large differences between blocks
- Randomly assign treatments **within** blocks
- Want small blocks, so usually each trt only once per block
  - Better to have more small blocks, than fewer large blocks

Analysis of data from an RCBD:
- Model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

  - i identifies blocks, α_i are block main effects
  - j identifies treatments, β_j are treatment main effects
- When 2 eu's per block, equivalent to pairing
- Similar but not same as 2 way factorial ANOVA:
  - No interaction term.
    - Common to have each treatment only once in each block
    - When only one rep, can't separate error and interaction
- Fundamental difference in interpretation
  - Don't really care about blocks
    - In model because that's how the study was designed
  - Blocks not randomly assigned;
  - Treatments are randomly assigned (within each block)
  - In a 2 way factorial, combinations of factors are randomly assigned

General practice:
- Block effects are always included in the model
  - Even when appear to be small
- Don't test significance of block effects
  - You use blocks because you expect them to be different
- Can quantify effectiveness of your blocks (see 5710)
  - Can guide design for your next study

Treatment design and experimental design:
- Every study has two separate design components
  - Treatment design: What is done to an eu: the treatments and their structure
  - Experimental design: How treatments are randomly assigned to eu's
- Most of the studies we've seen: treatments randomly assigned to eu's
- Paired and block designs: treatments assigned **within** pairs or blocks
- These are two different experimental designs.
  - Different ways of randomly assigning trts to eu's
  - Many experimental designs (see 5710)
- Treatment design is the one-way, two-way, or something else structure of trts
  - Again, many others (see 5710)

Why care about the difference
Allow you to combine ideas, e.g., two-way factorial trt with blocks
Guide constructing a model for **your** study

Mixed models:
Models with more than one "source" of variability or more than one e.u.
e.u. = Experimental unit: "thing" randomly assigned to a treatment
Everything this semester has focused on studies with one size of e.u.
subsampling (multiple observation units per e.u.)
dealt with by averaging
Example: pens with multiple cows per pen
ou = cow
if treatments only assigned to pens, eu = pen
Can average cows within pen to get one obs per pen, so ou now = pen
A mixed model explicitly has two sources of variability
among pens and among cows
Estimate both.
Useful for study planning: how many pens? how many cows per pen?
What if two treatment factors and two sizes of eu?
feed amount randomly assigned to pen
hormone stimulus randomly assigned to cow
Called a split-plot study - modern analyses use mixed models
Similar issues (different models) when each eu observed repeatedly
2nd course, Stat 5710, spends a lot of time on mixed models
To estimate multiple sources of variation (variance components)
To analyze data from split plot studies
To analyze repeated measures data
To analyze multi-site or multi-year studies

Overview of the entire semester:
Statistical analysis is a set of principles, not a set of recipes

- A good analysis is motivated by three things:
  your question(s)
  the study design, and
  the characteristics of the data
- Diagram from first lecture: Question → Data → Answers
  Design is how to collect data that answers your question
  Analysis is how to provide appropriate answers to your questions
- Goal is answers that are defensible because the statistical method is appropriate
  At least approximately
- Different models answer different questions
  Construct a model that answers **your** questions
- Estimates and confidence intervals are more useful answers than p-values
- Check your assumptions,
  but remember that real data only approximately matches assumptions
- Violating some assumptions doesn't make an analysis wrong
  Better fit to assumptions is almost always a better analysis
- Violating independence is usually trouble
  But random assignment of treatments can provide independence
- Almost everything else is details
- We have covered the core methods and principles
  There are lots of specialized areas of statistics
  each with their own sets of methods