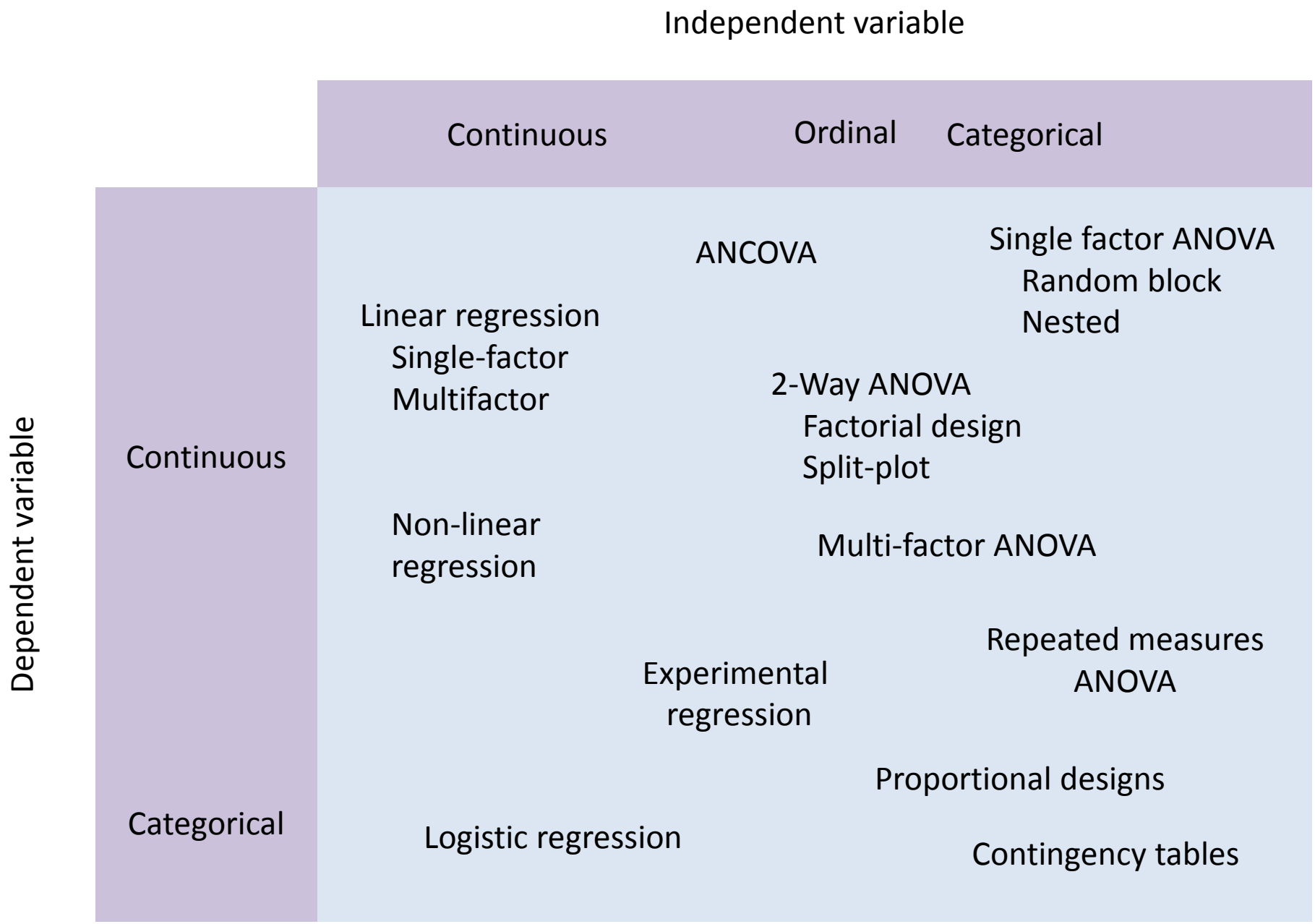
NRES_798_6_2015

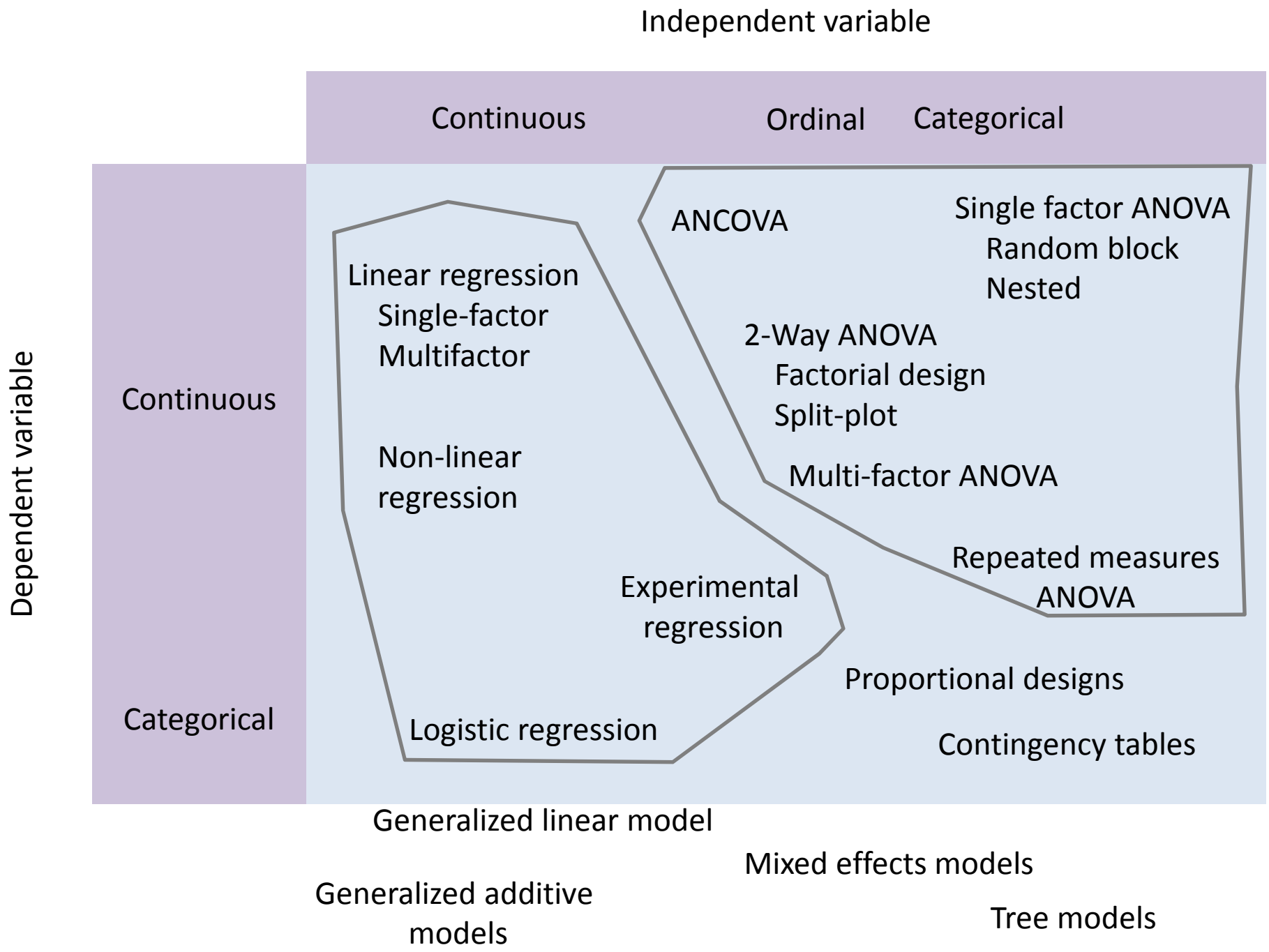
Sampling design and analysis frameworks

Landscape of experimental design

Field experiment	Natural experiment	Observational
Control	Replication	Randomization
Independence		Blocking

		Independent variable: Continuous	Independent variable: Categorical
Dependent variable	Continuous	Regression	ANOVA
	Categorical	Logistic regression	Tabular


Independent variable
Continuous
Ordinal
Categorical
Dependent variable
Continuous
Categorical
ANCOVA
Single factor ANOVA
Random block
Nested
Linear regression
Single-factor
Multifactor
2-Way ANOVA
Factorial design
Split-plot
Non-linear
regression
Multi-factor ANOVA
Repeated measures
ANOVA
Experimental
regression
Proportional designs
Logistic regression
Contingency tables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Logistic regression
Contingency tables
Generalized linear model
Mixed effects models
Generalized additive
models
Tree models

Experiment design

- How replicates are physically arranged in space, and how those replicates are sampled through time

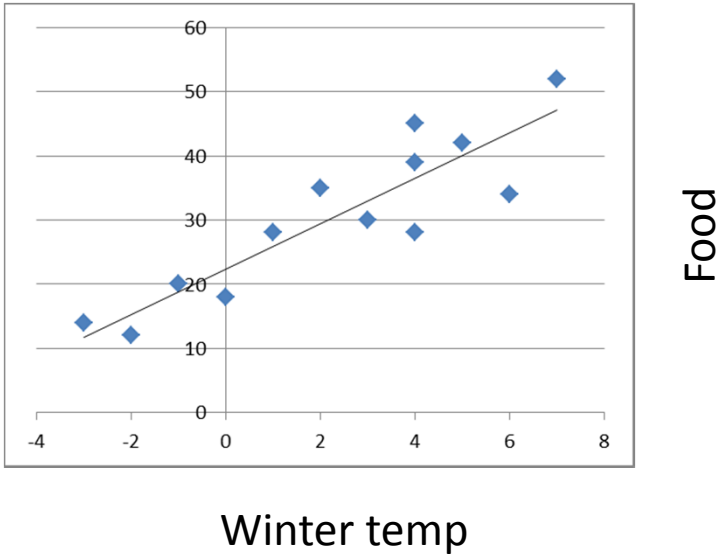
- Focus on the costs and benefits (advantages, disadvantages) of each of the design frameworks
- The most appropriate design will often depend on the nature of the system, the focus of the study, and constraints on sampling

- Underlying question that most experimental design and statistical analysis is how to best assess an partition various sources of variation (uncertainty)

Regression

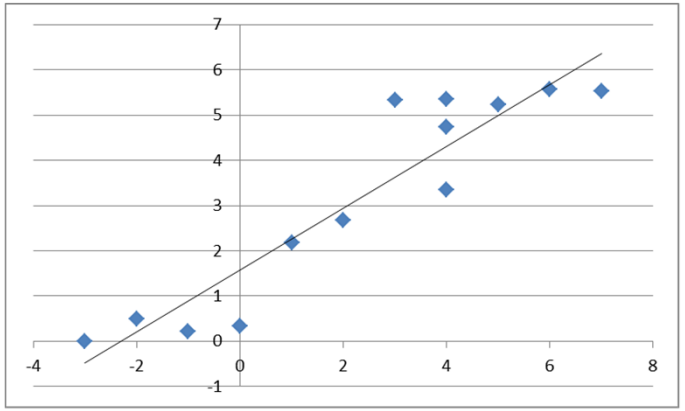
- Correlation vs. regression
  - Correlation
    - Evaluate the relationship between two variables
    - No causality assumed
    - Evaluation of correlations between all variable should be performed during the data exploration stage, prior to performing an analysis

Winter temperature	Food available during the previous summer	Offspring weight

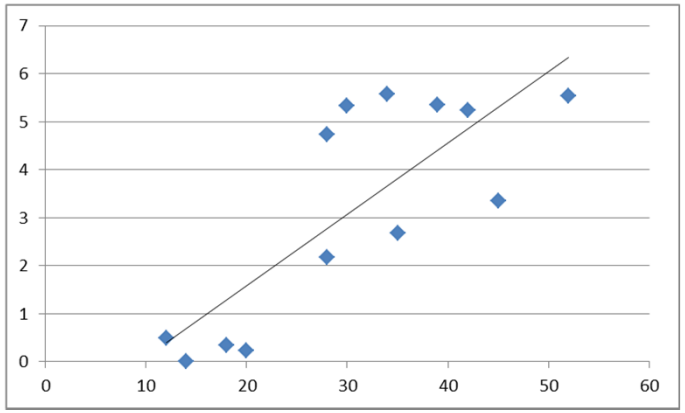


Regression

- Correlation vs. regression
  - Regression
    - Assumed asymmetric functional dependence between variables
    - X value influence Y value
    - Assumes that the value of the independent variable is known exactly
    - regression lines are fitted by only minimizing residual deviations only along the Y axis, while a correlation is fit by minimizing residual deviations along both X and Y



Winter temp.

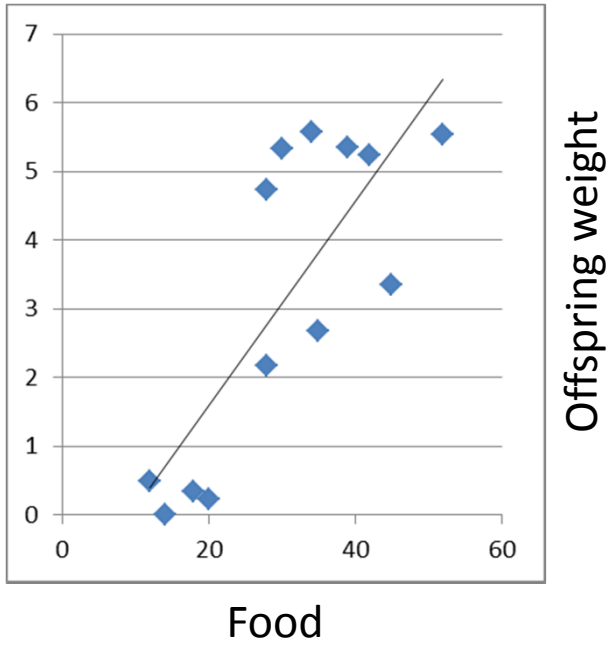


Food

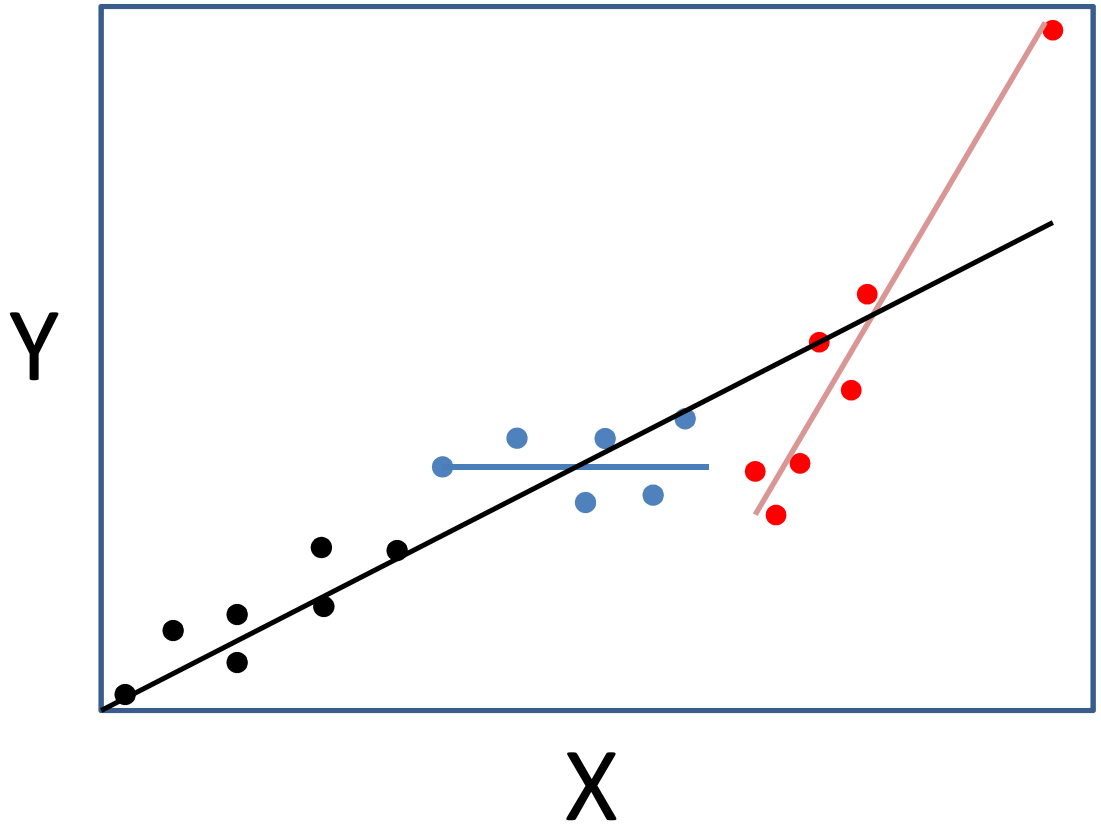
Offspring weight

Regression

- Single-factor regression
  - One continuous independent variable, one continuous dependent variable
    - Causality of independent variable state influencing the dependent variable is assumed
    - Observational study
      - neither variable is manipulated, natural variance in both is used
    - Experimental study
      - levels of the predictor variable are altered (e.g. food addition, predator exclusion), but the realized value of the variable is still measured.
  - Assume
    - Independence of data
    - Replication



Simple regression



Designing regression studies

- Ensure that the range of values sampled for the predictor variable is large enough to capture a full range of responses by the response variable.
- Ensure that the distribution of the predictor values is approximately uniform within the sampled range (What distribution would you expect?)
- Beware of extreme predictor values that can drive fit

Multiple regression

- Two or more continuous predictor variables with one continuous response variable
- Multiple predictor variables should be independent of one another (ideally)
- All predictor variables should be evaluated across the full range of possible values (usually not achievable)

Winter temperature	Food available during the previous summer	Offspring weight

Multiple regression

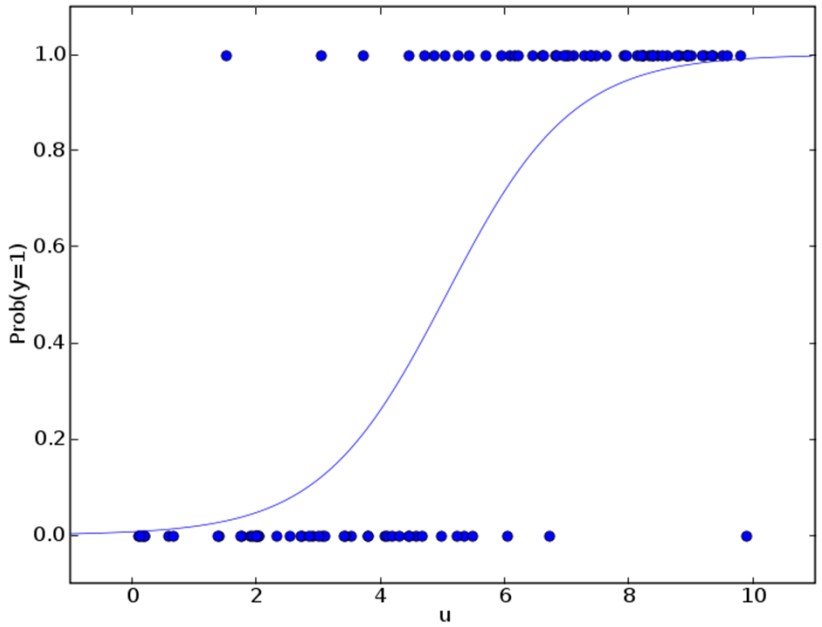
- Collinearity
  - Approximate linear relationship between two predictor variables
  - E.g. Rodents ~ Seeds + Veg Cover
    - In high Veg Cover, seed density is low
- Multicollinearity
  - Many of the predictor variables are correlated with one another

- **Designing multiple regression studies**
  - Replication important: at least 10 replicates for each variable in your study
  - Avoid the temptation to measure and include everything in the multiple regression model

Logistic regression

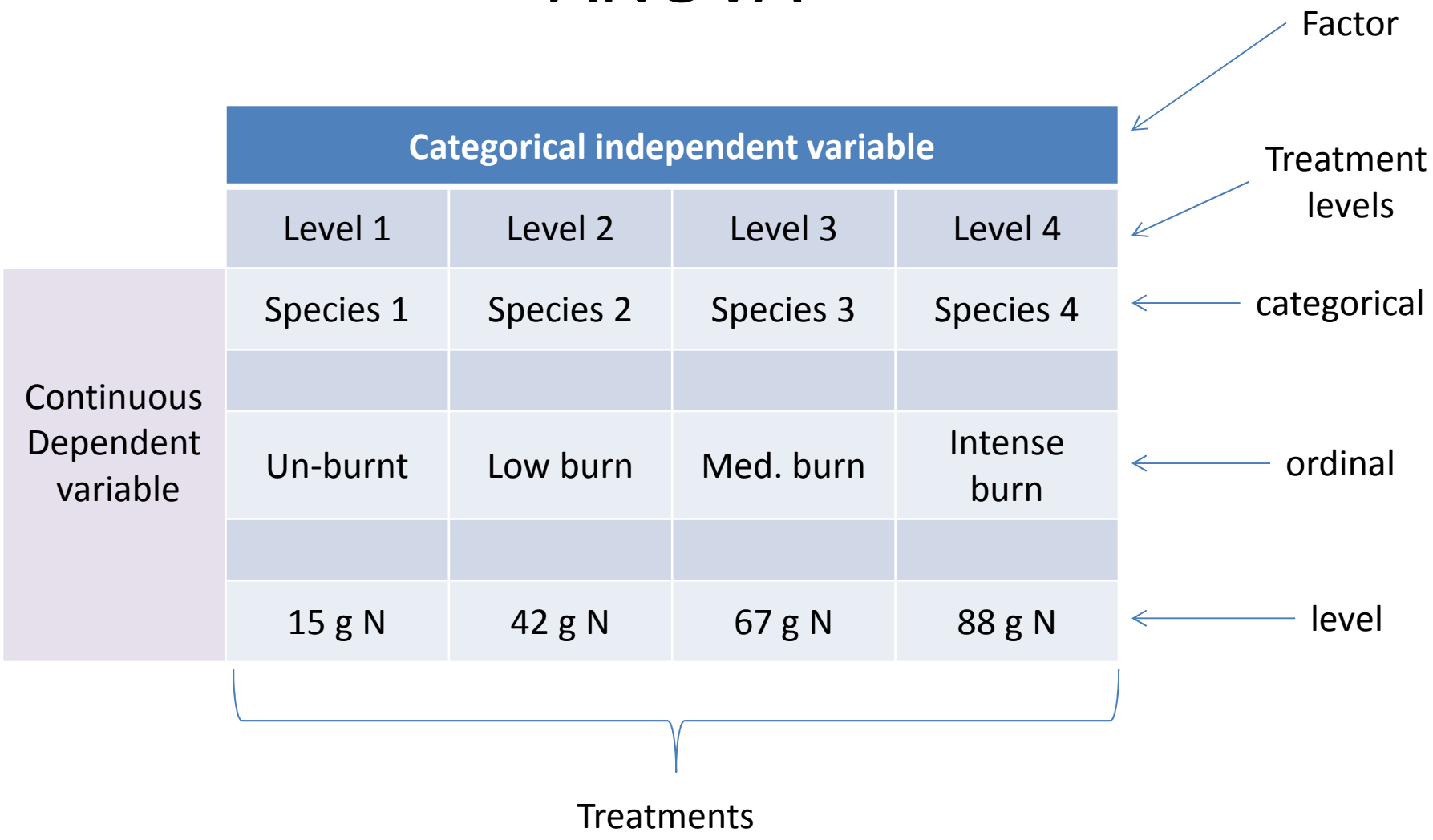
- Dependent variable categorical, independent variable continuous
- Type of generalized linear model
- Uses a “link” function (Logit) to allow the categorical variable to be evaluated within a linear model (regression) framework

Binary Response variable



Continuous predictor variable

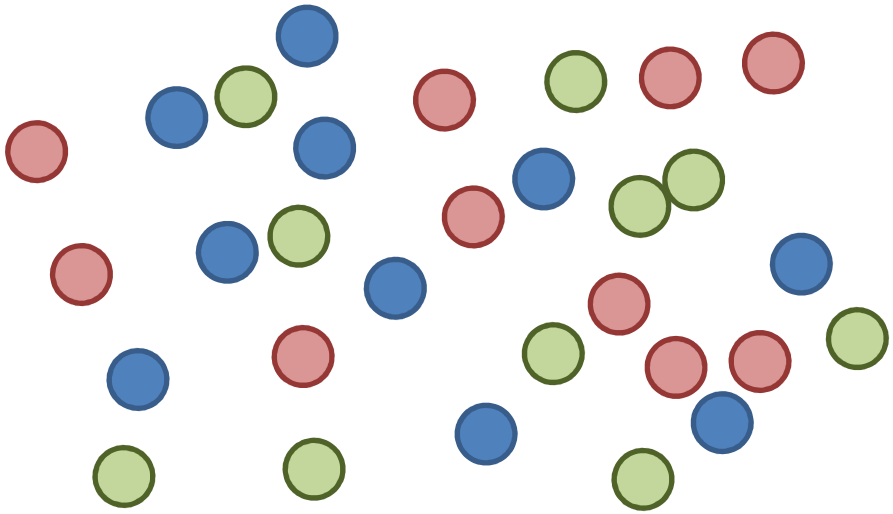
ANOVA


Factor
Categorical independent variable
Treatment levels
Level 1
Level 2
Level 3
Level 4
Continuous Dependent variable
Species 1
Species 2
Species 3
Species 4
categorical
Un-burnt
Low burn
Med. burn
Intense burn
ordinal
15 g N
42 g N
67 g N
88 g N
level
Treatments

ANOVA

- Single factor (one-way) ANOVA

	Treatment (Factor A)		
	0	25	75
Replicates	10	10	10

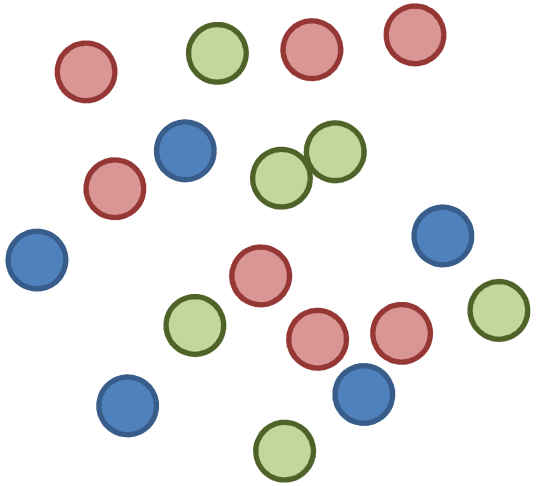


- Aim for equal replication across treatments
- Replicates should be independent
- Randomly allocated on environment

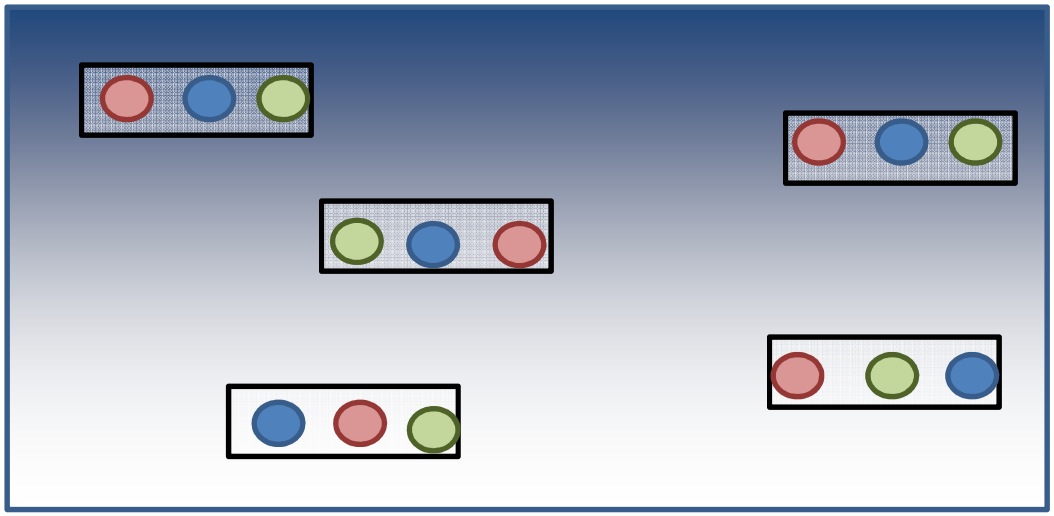
ANOVA

- Single factor (one-way) ANOVA

  - **Advantages of a one-way layout:**
    - Can accommodate unequal replicate number per treatment
    - Powerful test for differences among treatments
  - **Disadvantages of a one-way layout:**
    - Does not explicitly deal with environmental heterogeneity
      - Large unaccounted noise, can reduce power
      - But results can more likely be applied to other areas (i.e. stronger biological effect size, but weaker statistical effect size)
    - Explicitly implies that treatments are of only one factor (i.e. no colinearity with other factors)



ANOVA: Randomized block

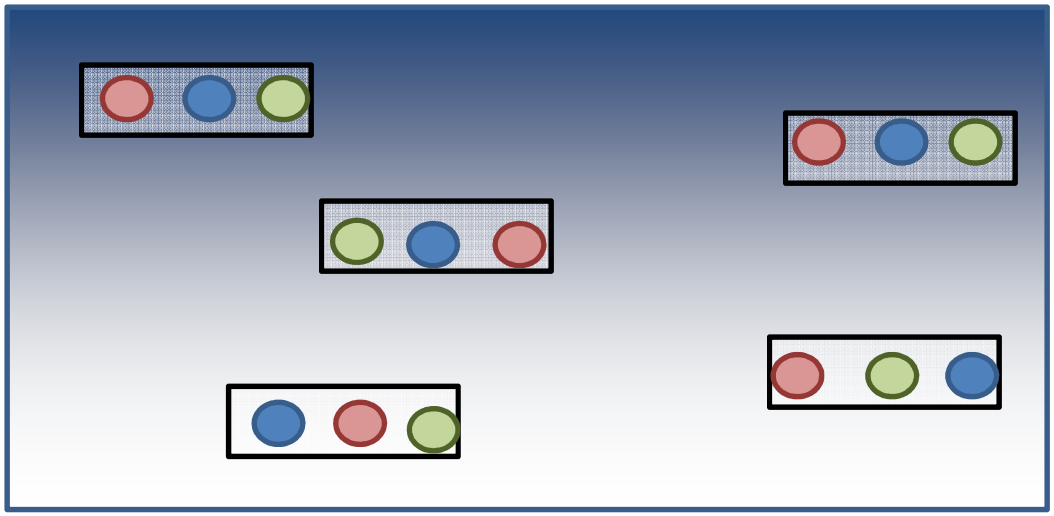


- Effective way of incorporating environmental homogeneity into a one-way ANOVA

- Block: a delineated are or time period within which the environmental conditions are relatively homogeneous.
- Blocks should be randomly distributed across the landscape.
- Environmental conditions should be more similar within blocks than between them
- One treatment replicate per block, random allocation of treatments within block

- **Within a block**
  - Block small enough that environmental conditions are homogeneous (i.e. gradients
  - Block large enough that one replicate of each treatment fits
  - Block large enough that each replicate is independent
  - Blocks randomly assigned is spatial environmental heterogeneity is unknown

ANOVA: Randomized block

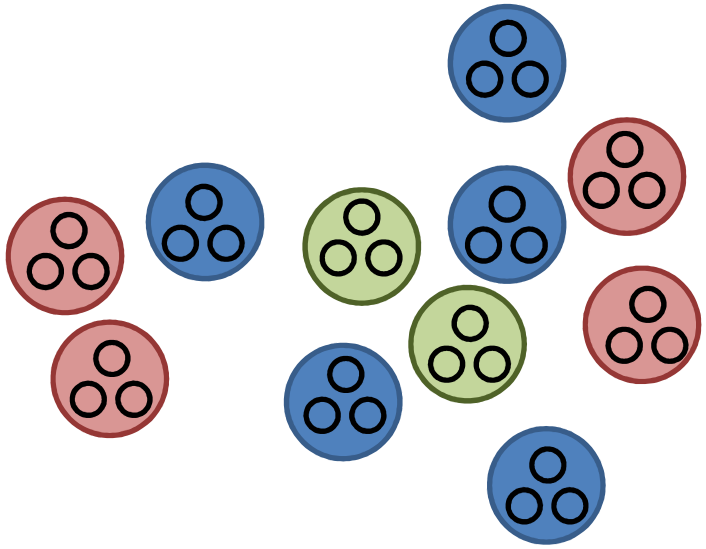


- **Advantages to a Random block design**
  - Effective means for dealing with spatial environmental heterogeneity, or temporal heterogeneity (i.e. necessity to run replicates through time)

- **Disadvantages to a Random block design**
  - If the “block” effect is weak, the statistical power of the test is less
  - Risk of non-independence if blocks are small
  - If data from one replicate is lost, the data from the whole block is compromised
  - The design assumes no interaction between the blocking factor and the treatment. (there is the risk that treatment does interact with environmental state)

ANOVA: Nested design

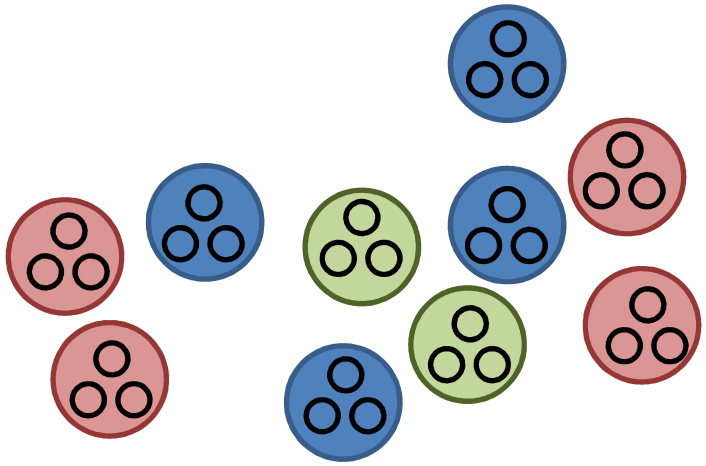
- Sub-sampling within replicates to increase precision
- Sub-samples not independent (nested)



ANOVA: Nested design

- **Advantages to a Nested design**
  - Increase in precision of the estimate for the replicate
  - Allows to test for variation within a treatment (within vs. between)
  - Design is beginning of a hierarchical design (design that allows partitioning of variance within and between hierarchical levels: e.g. within replicate, within river reach, within river, within watershed.

- **Disadvantages to a Nested design**
  - Risks not being analyzed properly (no nesting included in analysis framework, and pseudoreplication)
  - Sample sizes should be equal within each group.
  - Investment in obtaining more replicates is often better than increasing power within a replicate.



Multiple factor ANOVA

		Treatment (Factor A: 3 levels)		
		0	25	75
Treatment (Factor B: 4 levels	0	10	10	10
	0.2	10	10	10
	0.4	10	10	10
	0.6	10	10	10

- In a proper factorial design treatments are fully crossed
- If treatments are not fully crossed design is described as being confounded (i.e. can't properly partition the variance between the factors)

Multiple factor ANOVA

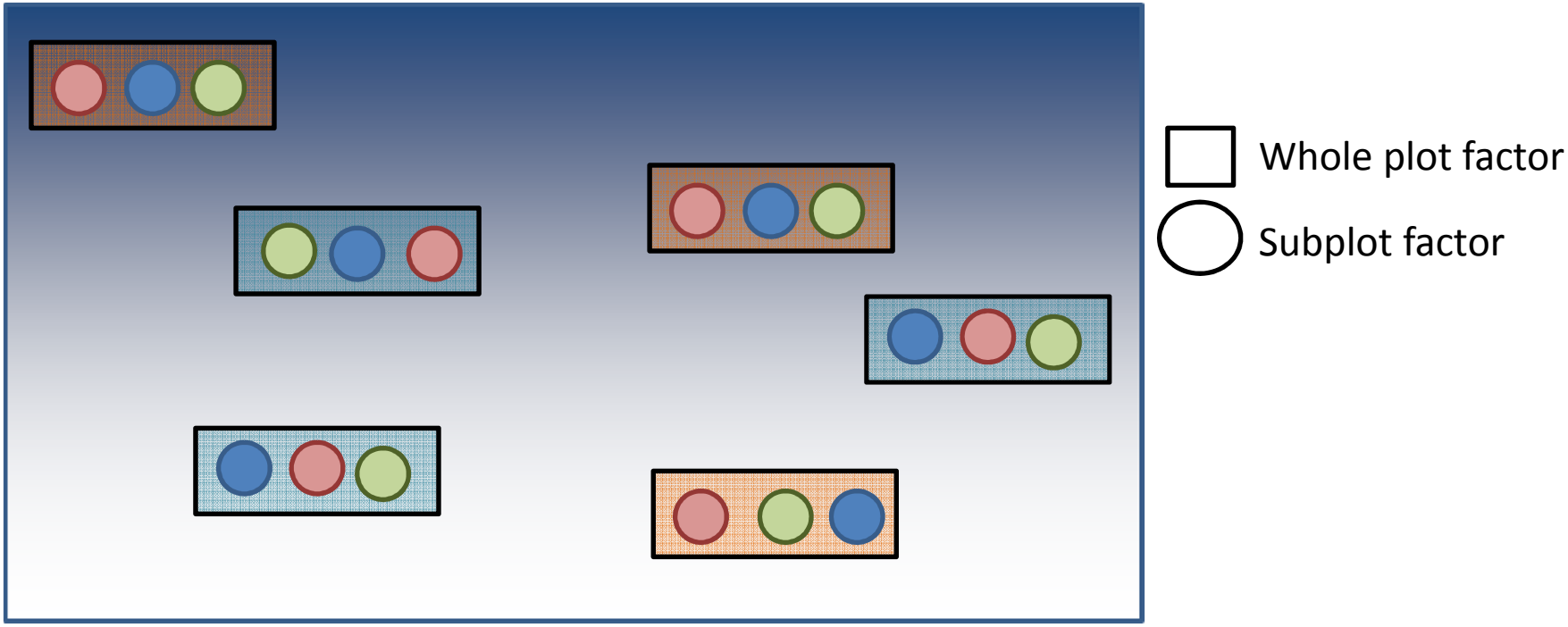
		Treatment (Factor A: 3 levels)		
		0	25	75
Treatment (Factor B: 4 levels	0	10	10	10
	0.2	10	10	10
	0.4	10	10	10
	0.6	10	10	10

- **Advantages of a two-way design**
  - Allows for the main effect and interaction effects to be examined
  - Interaction effect represent non-additive components of response
  - Interaction measures the extent to which treatment combinations act additively, synergistically, or antagonistically.

- **Disadvantages of a two way design**
  - Number of treatment combinations can quickly become too large (for adequate replication)
  - In some ecological circumstances all treatment combinations may not be feasible (all treatment combinations not possible or realistic)

ANOVA: split plot design

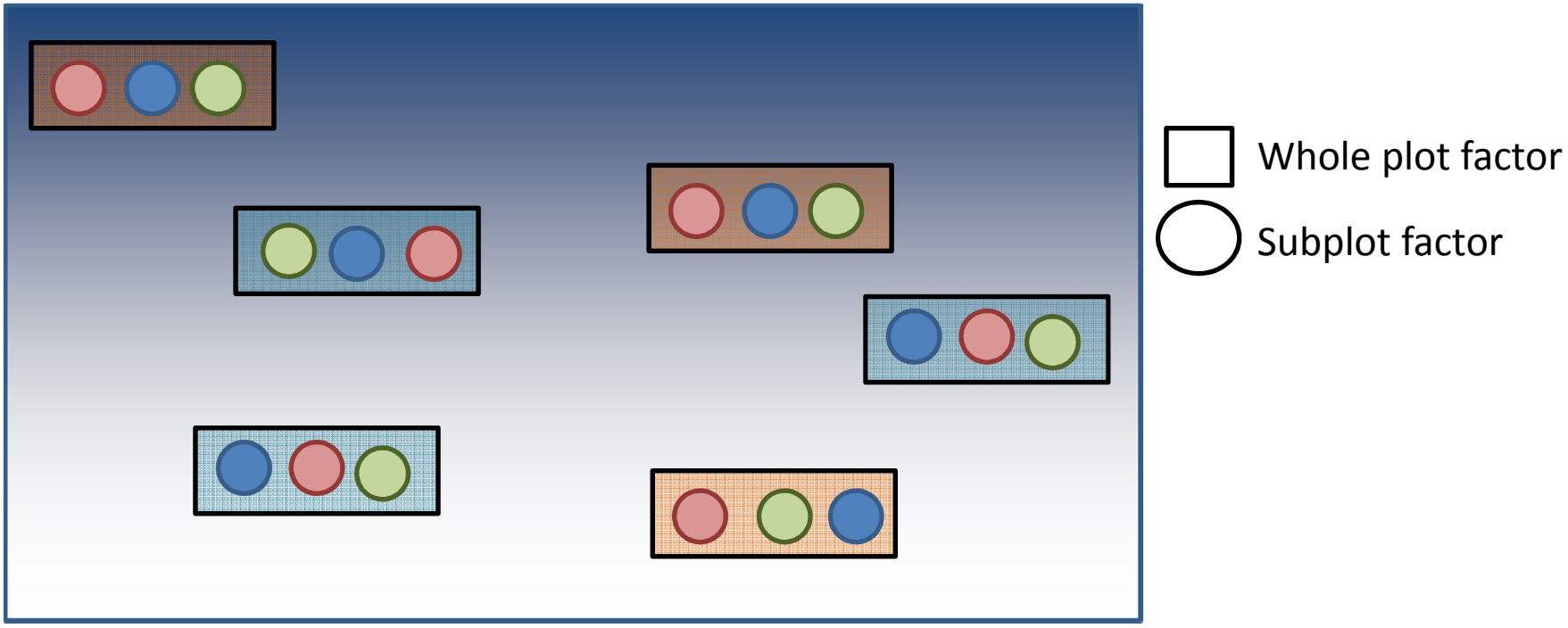
- Extension of the random block design to two experimental treatments.
- Second factor is applied at the level of a block.



ANOVA: split plot design

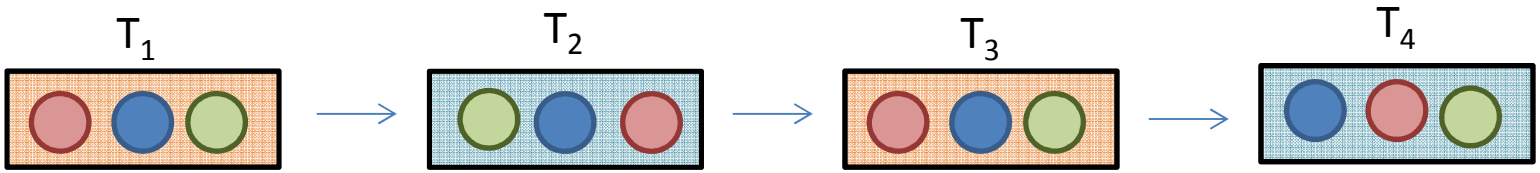
- **Advantages of a split-plot design**
  - Can control for environmental heterogeneity
  - Removes the additive effect of the blocks, and allows for test of the main effects and interactions between the two factors

- **Disadvantages of a split-plot design**
  - You can't test for interactions between blocks and subplot factors



ANOVA: repeated measures

- Repeated measure of replicate through time
- Measures of replicate not independent



- **Advantages of repeated measures**
  - Efficient use of sampling if getting high replicate number is problematic
  - Each replicate functions as its own "block" or control
    - i.e. controlling for size, age, life history etc.
  - Allows for testing of time by treatment interactions
    - i.e. test if differences between treatments change through time

- **Disadvantages of repeated measures**
  - Lack of independence

Environmental impact assessment
BACI design

- Type of repeated measures
- BACI: Before, After, Control, Impact

- Can include replication (spatial and temporal) before and after the perturbation, and blocking factors

Experimental Regression ~ ANOVA

- ANOVA good, but limited and can be divorced from ecological process of interest
- Is the predictor variable naturally categorical, or is just applied that way?

- Experimental regression, increase the number of treatment levels, at the expense of replication

- Range and spacing of treatment levels needs to be well designed
- Allows for a “response surface design”
- Design makes it easier to detect non-linear impacts and thresholds
- A regression framework allows model parameters (intercept, slope) to be estimated

		Treatment (Factor A)						
		0	1	2	3	4	5	6
Treatment (Factor B)	0.0	1	1	1	1	1	1	1
	0.1	1	1	1	1	1	1	1
	0.2	1	1	1	1	1	1	1
	0.3	1	1	1	1	1	1	1
	0.4	1	1	1	1	1	1	1
	0.5	1	1	1	1	1	1	1

Tabular designs

- Categorical independent variable, categorical response variable

- Contingency table analysis
  - Significance tested using chi-square, G-test, etc.

	Right-handed	Left-handed	Total
Males	43	9	52
Females	44	4	48
Totals	87	13	100