Behavioral Ecology of Vertebrates

Unit 2. Testing hypotheses

Module 1 Introduction

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Learning, Discovering and Sharing Knowledge

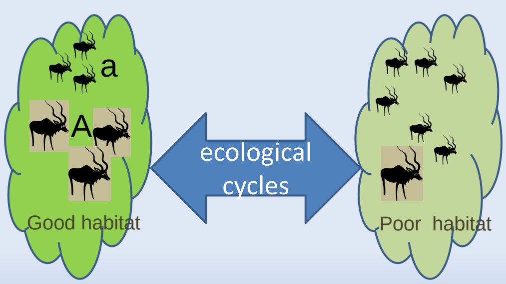
In the previous unit, we covered 4 major themes that run throughout behavioral ecology: natural selection, trade-offs, social context, ecological context. We will be revisiting these themes in each of the modules that follow. In this unit 2 we continue with building the foundation for the course. We cover three approaches to testing hypotheses, which you will also see reappear in modules 2-5.

Learning Objectives (Davies et al. 2012:24,49)

Three ways to formulate and test hypotheses:

1. **Comparison between individuals <u>within a species</u>**: e.g. compare tactics within groups, compare across diverse groups, compare across diverse populations
2. **Experiments (lab & field)**: e.g. manipulate one factor ; compare behavior of “controls” vs. “treatment”
3. **Comparisons <u>among species</u>**: e.g. analyze the results of “natural experiments” over evolutionary time

These are three ways to examine hypotheses in Behavioral Ecology. | I like to emphasize that formulating hypotheses is just as important as testing hypotheses. As we will see, comparisons may be more useful for formulating hypotheses. The emphasis is on “testable”. My mentor, Konrad Lorenz, used to tell us student interns “It is good to test and throw out a hypothesis every morning before breakfast!” He was referring to his time with Tinbergen. Lorenz was good at coming up with hypotheses and Tinbergen at testing hypotheses. They made quite a team. Lorenz thought it was a cultural bias, that the English speaking scientists valued testing more than formulating. But if you do not have good field observations, you may come up with outlandish hypotheses that do not relate to the reality of the system you are studying!



Phenotypic variation within one species

1. COMPARE INDIVIDUALS

Remember in Unit 1, we talked about the ecological setting, and clarified how two groups might occupy two habitat fragments that differed in quality. Furthermore, the quality might change over time with ecological cycles. I want to return to this idea to illustrate what is meant by testing hypotheses based on comparing individuals. When comparing individuals in this manner, we would be asking questions about the phenotypic variation within a species. So if a species behaves the same in two very different habitat fragments, then we would say the phenotype is relatively resistant to environmental influences (remember the non-plastic phenotype in great tits). If the phenotype of individuals differs between the two habitats, then we would infer that variation in the behavioral trait is influenced by the environmental variables (plastic phenotype in great tits). By comparing habitats, we can come up with hypotheses about what are those environmental variables that influence individuals.

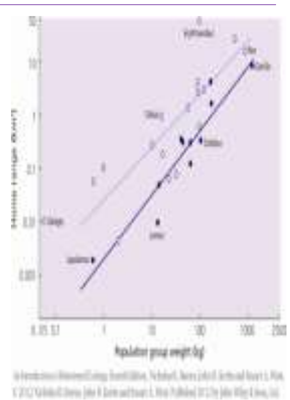
1.1 Application (Davies et al. 2012:34, Fig. 2.4)

- Home range size varies across populations of colobus monkeys
- Folivores- feed on leaves
- Leaves vary in nutrients (new growth) and toxins
- Distribution and abundance varies with successional stage

Unfortunately, Chapt 2 is lacking in good examples of comparing individuals within the same species. To illustrate the idea, take a look at the example of how home range size varies within (as well as between) species. Colobus monkeys are folivores (leaf eaters). The nutritional quality of leaves vary with the growth stage. New leaves have lots of yummy proteins. Old leaves defend themselves from herbivores (toxins and tough fibers give tummy aches). Depending on the successional stage of a particular habitat fragment, the quality of the leaves available is likely to vary between the home ranges of different groups of colobus.

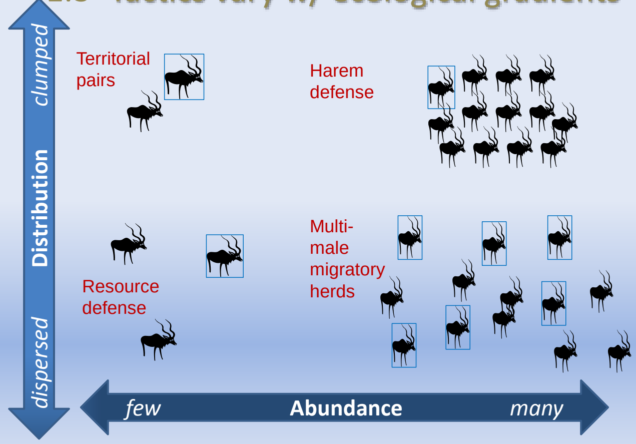
1.2 Compare groups (Davies et al. 2012:35, Fig. 2.5)

- **H1**: Individuals in larger groups have larger home ranges
- **H2**: Individuals with more specialized diets have relatively larger home ranges than generalists
- Varies across & within species



From previous observations, we see a positive correlation between home range size and group size. Do you see the scatter of points in the middle of the graph for 5 populations of Colobus? This illustrates the variation within the species. So to illustrate the concept of formulating testable hypotheses based on individual comparisons, we could come up with these two alternative hypotheses: H1, H2. | To test H1, you could go out to two habitat fragments, measure home ranges of individuals and compare them statistically based on group size. To test H2, you could analyze fecal material from individuals and score it based on the variety of leaves, as an index of diet breadth. You would predict individuals with larger home ranges would have a relatively narrow diet. This could have important implications for how you manage habitat in forest fragments for this species.

1.3 Tactics vary w/ ecological gradients



To elaborate further on how we compare individuals in order to test hypotheses in Behavioral Ecology, I want to go back to this slide that I introduced in the last unit. I will relate it to what you will be seeing on the field trip to Fossil Rim. We are using this approach of comparing individuals in our ongoing studies of the addax, more about that later.

This slide is meant to illustrate that the behavior of one individual might change depending on the distribution and abundance of resources in the environment. (blue boxes = males). In ungulates, females are more likely to clump together where resources are clumped. So, a male may be expected to switch between territoriality at low population density and harem defense at high population density. Where resources are widely dispersed, a male may be expected to switch between defense of a resource that attracts females (like a water source) and moving with migrating groups of females as they move from patches with few resources to patches with abundant resources (like the migrations between highlands and lowlands of the Serengetti plains).

So what? Why is this useful…

The addax is one of three species that we are currently studying at Fossil Rim. We will come back to comparison of these three species later. In this part of the lecture about comparing variation of individuals within one species, I want to tell the story of how the behavior of the sable antelope varied between two habitat fragments. Since sable are not endangered, we are exploring whether they could serve as a surrogate for studies that will help us understand the addax and oryx, which are both at risk of extinction. I add this example from personal experience to help you understand why this approach is important from a practical perspective, even though there may not be a lot of good practical examples in Chapt 2.

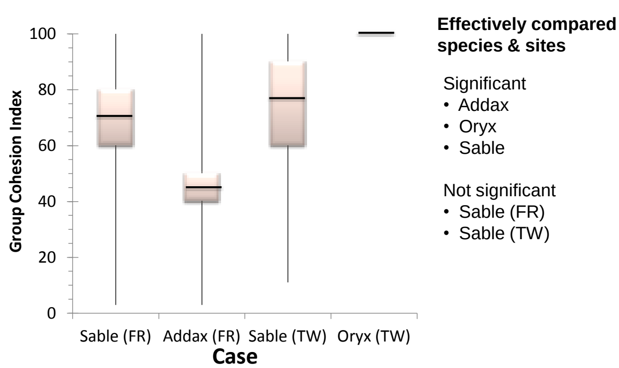
Tactics vary within & between sites

Fossil Rim- Main Pasture (417 acres)

The Wilds- Pasture E (166 acres)

Last summer, our graduate course compared these species at two very different habitat fragments, the main pasture at Fossil Rim, which you will experience on the field trip, and at the Wilds of Ohio, which is a reclaimed mine site. In the jargon of rangeland management, two very different pasture conditions. You can tell from the density of dark pixels on the left map that there is a lot more juniper trees at Fossil Rim than at The Wilds. So we compared the social cohesion of sable on these two sites. On these maps, the brown circles represent the sable. At FR, the white circles represent addax and at TW white circle is oryx. Notice that the sable used the habitat differently than the other two species at both range sites. Any ideas what influenced the overlap at TW? You got it, food! That is where they were fed supplemental hay.

Group Cohesion Index



We predicted the sable females would be more cohesive at FR where they had a breeding bull, compared to TW where the social group was only females. However, the evidence led us to reject our hypothesis. The two sable herds did not differ significantly, even though the social context and the ecological context were really different at the two sites. | By the way, the three species were significantly different. So this is a practical application to illustrate how hypotheses can be tested in behavioral ecology by comparing individuals, or as in this case, groups of individuals.

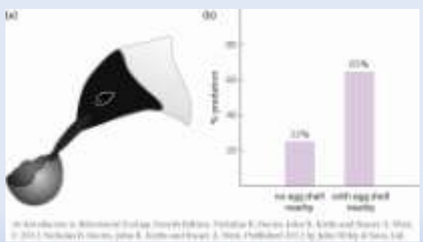
1.4 Poll- lets see if you understand

Comparing individuals, when behavioral tactics vary across groups (habitat fragments) what does this suggest about the genotype?

a) Fixed, non-plastic phenotype and genotype
b) Plasticity in tracking environmental variables
c) Mixed strategies in the gene pool
d) Each tactic is controlled by multiple genes

Now lets see if you understand the implications of comparing individuals to test hypotheses about behavioral ecology. Which is the correct answer? Why? Lets discuss this on elearning.

Davies et al. 2012, Fig 2.15



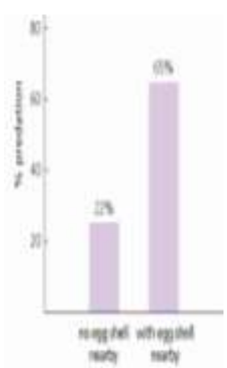
Compare "treatment" vs. "controls"

2. EXPERIMENTS (LAB & FIELD)

The second major approach to testing hypotheses in behavioral ecology is comparing "treatments" vs. "controls". You have probably learned about this in your science classes, as the experimental method. Initially, many folks in this class find it odd to think about experiments in the field, not only in the lab. However, Niko Tinbergen was a pioneer in promoting rigorous methods of doing field experiments. To illustrate this idea, lets look in more detail at his studies of the egg-shell removal of black headed gulls.

2.1 Application (Davies et al. 2012:47, Fig. 2:15)

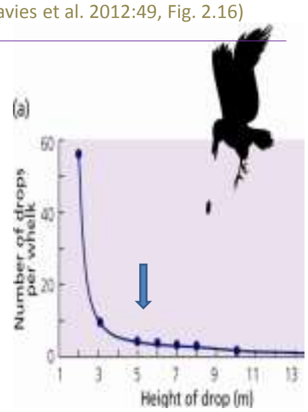
- Black headed gulls pick up eggshells after hatching
- H1: increased nest predation due to conspicuousness
- Experiment
  - Treatment: egg shell
  - Control: no egg shell
- Accepted hypothesis (Tinbergen 1963)



As you recall from your reading, the gulls nest on open flats near the shore. Their eggs are camouflaged, but after hatching the white interior of the empty shells attracts crows and other gulls to prey on the newly hatched fledglings. | Tinbergen hypothesized that egg shell removal was instinctive and the function was an anti-predator defense. | He devised a simple experiment, dividing nests into treatment and control groups. The treatment nests received an empty egg shell, the controls did not. | Results were clear: predation was higher on treatment than control nests | Therefore Tinbergen accepted his hypothesis about the function of this behavior based on comparison of treatment and control groups.

2.2 Crows & whelks (Davies et al. 2012:49, Fig. 2.16)

- Crows drop & eat whelk
- H1: Crows fly to an optimal height to break shell, minimizing effort
- Experiment:
  - Predicted: 5 m high
  - Observed: 5.2 m
- Accepted hypothesis (Zach 1979)



Another classic study that you read about is about crows that forage on shellfish along the Pacific northwest coast. | Zach studied the behavior of crows that feed on whelks, which are a "snail like" organism that can be picked off the rocks and tide pools. Their shells are an effective anti-predator adaptation. | Crows open small whelks by dropping them from a couple meters. However, the larger, more preferred whelks do not break open unless dropped many times from a short height, or a few times from very high. | So Zach's hypothesis was that crows had learned to fly to an optimum height, determined by the trade-offs between energy flying high and energy making repeated drops. This was a proximate hypothesis about causal mechanisms. | Zach made his prediction about the optimal height by standing on a ladder, dropping shells from different heights and counting how many drops it took to break the shell. His prediction was 5 meters. Then he made observations and found the average height at which crows dropped shells was 5.2 m. | Pretty close match! Zach accepted his hypothesis based on comparison of the predicted and observed values.

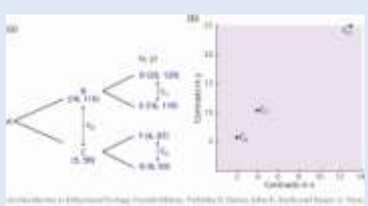
2.5 Poll- lets see if you understand

Why is an experimental design so widely accepted for testing hypotheses in behavioral ecology?

a) It only applies to field conditions
b) All variables can be controlled in the lab
c) Comparison of "treatment" and "control" groups allows specific hypotheses to be tested

Now lets see if you understand the implications of applying the experimental approach to comparing treatment and control groups of individuals. Which is the correct answer? Why?

Davies et al. 2012:39



Phylogenetic history: genetic variation

3. COMPARE SPECIES

Remember what we covered in Unit 1 about the importance of time scale? The "comparative method" is traditionally associated with formulating and testing hypotheses based on phylogenetic history (very long time scale). However, this only applies to variants of behavioral traits that are highly heritable. BIG distinction, from what we just covered in parts 1 & 2 of this ppt.

In the past, the use of phylogenetic analyses to test hypotheses has been criticized and the techniques have been improved. I am going to try to convince you that the old style tables comparing traits across species (e.g. Tables 2.1, 2.2, 2.3) are better for "formulating" than for "testing" hypotheses.

Remember how we talked earlier about comparing the antelope species at Fossil Rim

3.1 Application (Davies et al. 2012:30, Fig. 2.3)

- Mating system varies among African ungulates *(74 species; Jarman 1974)*
- Small species
  - high quality diet
  - small groups
- Large species
  - low quality diet
  - large groups

3.2 Compare species (Davies et al. 2012:32)

Body Size	Habitat	Diet	Mating system
Small	Forest	Browse: fruit/buds	Pair *(single male)*
Medium	Riverine woodland	Browse/ Graze	Males defend harem/resource
Large	Grassland	Graze: grasses	Multi-male *(age-graded hierarchy)*

3.3 Critique (Davies et al. 2012:31)

1. **Alternative hypotheses**: due to confounding variables, test alternatives
2. **Quantification of ecological variables**: how to measure “dispersed” and “patchy”
3. **Cause and effect:** is diet consequence or cause for grouping?
4. **Alternative adaptive peaks**: ecological conditions shift between alternative steady states
5. **Statistics:** “points” are not independent due to phylogenetic history

Not all ungulate species behave the same! This may seem really obvious to you, but it was not obvious to my colleagues from animal science. They were used to thinking of a pasture as a homogeneous stand of grass, and expected all the species in that pasture to use it in the same way. Remember the differences we talked about earlier about how the sable did not use the pastures in the same way as the addax and oryx in the same pastures? How do we begin to make sense of this variation?

The comparative method has been very useful. In Chapt 2, you learned that there are patterns among the 74 species of African antelope that have been studied. | The small forest duikers require a high quality diet and live in small monogamous groups | In contrast, the large species like the wildebeest are adapted physiologically to a low quality diet. They aggregate in large multi-male groups as the females move with the rainfall as the grass greens up on the Serengeti plains.
In Table 2.3, Jarman described some of the general patterns in ungulates and how traits seemed to be linked. He compared species across a range of body sizes. There was a pattern, small species that required highly nutritious items lived in the forest, females were solitary and males defended those solitary females in monogamous relationships. On the other end of the continuum, he proposed were the large grassland species. In between, were the species that lived at the forest/grassland edge. The species that are plastic
The radiation of bovid species started with forest species that became progressively better adapted to grassland habitat, then arid habitats. (within the last 5.3 mya) associated with cycles of dry and wet periods. For more details, read deMenocal (2004).

However, correlation is not necessarily a test of causation!

In Chapt 2, Davies et al. outline some of the major critiques of the comparative method. | These are mostly cautions against “weak science”, for “strong science” we recommend using the descriptive comparison of species to formulate hypotheses, not to test hypotheses. Lets move on to an example of what is considered “strong science” in applying the comparative method.

3.4 Sexual swellings in female primates

(Davies et al. 2012: 42, Fig. 2.11; credit: K. Wedemeyer , edited by J. Packard)

- Many primate species live in groups, some with multiple males and others with a single male
- In some old world monkey and ape species, females show their reproductive receptiveness with conspicuous sexual swelling
  - 0/29 species living in single-male groups have sexual swelling
  - 29/41 (71%) multi-male group species have sexual swelling
- H1: multiple male groups=> swelling

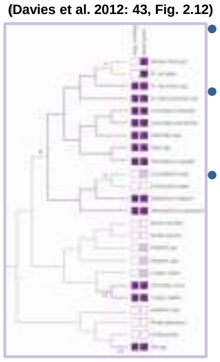
→ *Which came first? Did living in multiple male groups put selection pressure on females to exhibit sexual swelling, or did sexual swelling put selection pressure on groups to adapt the multiple male structure?*

Sexual swellings were noted by Darwin (1876), an intuitive hypothesis was formulated by Clutton-Brock & Harvey (1976) , then tested by Purvis (1995) and Nunn (1999). How would you test this hypothesis?

3.5 Phylogenetic analysis

(credit: K. Wedemeyer edited by J. Packard)

(Davies et al. 2012: 43, Fig. 2.12)



- There is an association between swelling and multi-male mating system
- Swelling has evolved independently 3 times, each time associated with the evolution from a single-male to a multi- male group (Nunn 1999)
- Further phylogenetic analysis (see Fig. 2.13, p. 44) and statistical methods suggest that sexual swelling followed the evolution of multi-male mating systems (Pagel & Meade 2006)

→ Selection pressure from multi-male groups led to the evolution of swellings, not vice-versa. Why?

The answer for how to test hypotheses about phylogenetic history is provided in Figures 2.12 and 2.13. In this phylogenetic tree from Fig 2.12, the boxes on the left column code for variation in the trait "swelling" (white is "no" and purple is "yes"). The boxes in the right column code for the type of "mating system" (white is "single-male", lavender is "switcher" and purple is "multi-male"; "switcher" means groups switch back and forth between single- and multi-male). For further info, Davies et al. (2012:44-45) elaborate on the cost/benefit predictions to explain why the genotype for "swelling" might have been selected in multi-male groups over evolutionary time scales.

3.6 Discrete variables and the order of change

(Davies et al. 2012, Fig 2.13; credit: K. Wedemeyer, edited by J. Packard)

- **If** a taxonomic group (e.g. old world monkeys) has distinct behavioral traits, which seem to have arisen from separate evolutionary changes,
- **then** biologists can use transition analysis to discover which evolutionary change came first.

# species	Single-male	mixed	Multi-male
Swelling	0	0	12
No-swelling	6	4	2

Key Words:

Continuous variable: factors that can be measured across a complete range. *(e.g. weight, home-range-size, testes-size, dimorphism, sex-ratio)*

Discrete variables: distinct characters, that cannot be measured across a continuous range. *(e.g. blood-type, sexual-swellings, mating-system)*

If swellings evolved first, then we would expect to see species with swellings associated with all 3 forms of mating systems. The data do not support this hypothesis, so we reject it.

If "no swelling" is the ancestral form of the trait, then we would expect to see species with all three forms of mating systems associated with this variant of the trait. The data support this hypothesis, so we accept it.

If there was "selective pressure for swellings" within multi-male systems, we would expect to see more multi-male species with swellings than without. The data support this hypothesis so we accept it.

CHAT Q: Why is it important to understand the difference between continuous and discrete variables in analysis of phylogenetic history?

3.7 Who cares?

C2S2 CONSERVATION CENTERS FOR SPECIES SURVIVAL

Sustainable Herds Project Benchmarks

- Herd Health
- Demographics
- Genetic Diversity
- Economics
- Ecosystem
- Behavioral Resilience

Manage 64 species of ungulates (44 bovid)

SOURCE: (Sawyer et al. 2011)

We have just covered a couple examples of how the comparison of species has been used to formulate and test hypotheses in a manner that would be considered "strong science" in Behavioral Ecology.

Now lets turn to the question "so what"! Why is this comparative approach useful, is it only for academic scientists. One group that cares a lot about practical applications is the Conservation Centers for Species Survival. Fossil Rim is a member of this consortium, which collectively manages 64 species of ungulates, of which 44 are bovids.

The five institutions within this consortium are dedicated to managing sustainable herds of these species for the next 200 years! No small challenge! Behavioral resilience is one of six benchmarks for success that the practitioners and scientists within this consortium have identified. Comparisons between species are extremely important, because not all species respond to the same management tool in the same manner. You will be helping us with this ongoing research during the Fossil Rim field trip.
Lets compare the three species that are the focus of our studies, starting with the most highly endangered, the Scimitar-horned oryx. The main thing to remember here is that they adapted to a desert environment.

3.8 Scimitar oryx- almost extinct

- We know
  - IUCN: extinct in wild
  - Formerly N Africa - arid desert
  - Mixed-sex breeding groups
  - Age-graded male hierarchy
- Need to know
  - Lethal combat - new bull
  - Mate rejection - young bull
  - Infanticide - by bull new to calves

3.9 Addax- ecological restoration

- We know
  - IUCN: critically endangered
  - Sahara desert
  - Mixed-sex breeding groups
  - Age-graded male hierarchy
- Need to know
  - Individual plasticity related to social density
  - Resiliency when moved from rangeland to restoration site

Compare the oryx with the addax and we find similarities. Both are desert adapted and both live in mixed-sex breeding groups with an age-graded male hierarchy (the older guys keep the younger ones from mating).

We need to know how resilient are the individuals of these two species when they are moved from one social context to another (think of moving from a zoo to the pastures at FR and TW). How resilient will they be when moved from ranches like FR, to restoration sites in Africa?

3.10 Sable- suitable surrogate?

- We know
  - IUCN: least concern
  - S & E Africa, seasonally productive lowlands
  - Matriarchal groups
  - Harem-defense
- Need to know
  - More ancestral traits?
  - How does resource distribution influence females?
  - How does female clumping influence male defense of females?

Since sable are not endangered, our question is whether we can use them as surrogates for our studies. However, look at how different is their habitat. They live in seasonally flooded lowlands. Females clump together in tight groups, which can be defended by one male. The 500 acre pasture at FR is only big enough for one male. Males will fight to the death over access to a group of females.

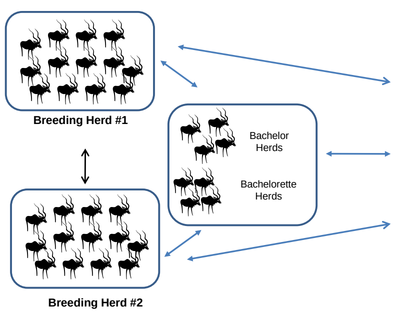
So our practical reasons to compare species relate to several questions in the “need to know” category.

3.11 Testing hypotheses- compare species

Herd Variation (social groups)
- Breeding
- Bachelor (with nanny)
- Bachelorette
- Loners

Individual Variation
- temperament (genetics)
- learned coping styles
- social experience
- health/soundness
- age-related development

Decisions about movements
- remove pre-breeding males
- turn-over breeding bulls
- remove breeding females
- bull-switching (vasectomized)



SOURCE: www.conservationcenters.org

As we move forward with this ambitious project, we will be testing hypotheses based on comparing species. There are practical decisions that managers are now making about management of these herds. | Using the comparative method, we can make predictions about the results of these decisions. We can test these hypotheses and built up from what is largely an intuitive management system now, to one that is based on sound science.

3.12 Poll- lets see if you understand

Which is recommended from the perspective of “strong science”?

a) Test hypotheses using descriptive correlations that emerge from comparing species
b) Formulate hypotheses based on descriptive correlations and test hypotheses based on trait contrasts
c) Only use trait contrasts because descriptions based on species comparisons are flawed

Now lets see if you understand the implications of comparing species to formulate and test hypotheses based on phylogenetic history. Which is the correct answer? Why?

Summary

(Davies et al. 2012:21)

Three ways to formulate and test hypotheses:

1. **Comparison between individuals <u>within a species</u>**: e.g. compare tactics within groups, compare across diverse groups, compare across diverse populations
2. **Experiments (lab & field)**: e.g. manipulate one factor ; compare behavior of "controls" vs. "treatment"
3. **Comparisons <u>among species</u>**: e.g. analyze the results of "natural experiments" over evolutionary time

In summary, there are three major approaches to testing hypotheses in Behavioral Ecology. | The first two are more in the proximate sense, short-term approaches that you can use to test phenotypic variation in the field in your own studies. | The last is more on an evolutionary time scale (ultimate). It is useful for predicting why different species may respond very differently to the same environmental conditions (like the FR pastures). The underlying model is that species differences are due to genotypes, not phenotypes. However, you need to be really cautious about avoiding the pitfalls of "weak science" to be really clear that descriptive correlations based on the comparative method are more appropriate for developing proximate hypotheses than for testing ultimate hypotheses. This insight should reinforce the reason we made such a big distinction between proximate and ultimate perspectives in Unit 1.