Rapid Evolution during Habitat Change

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Outline

• What conditions enable Rapid Evolution?
• How do we detect Rapid Evolution?
  Comparative Common-Garden Experiments
  Selection Experiments
• Examples of Rapid Evolution in Copepods:
  Salinity Adaptation
  Response to Deep Horizon Gulf Oil Spill
  Temperature Adaptation
A fundamental problem in ecology and evolution regards limits to species distributions.
What constrains species distributions?
What mechanisms allow those distributions to shift?
Adaptation during Extraordinary Environmental Change

Over longer time scales, colonizations across habitat boundaries represent major events in the history of life, leading to the evolution of key innovations.

In general, transitions into more stressful environments have been accompanied by the evolution of increased physiological regulation.
Examples of extraordinary environmental change:

Transitions between

Saltwater $\rightarrow$ Freshwater

and

Freshwater $\rightarrow$ Land

Constitute Major Evolutionary Transitions in the History of Life
Life evolved in the sea, and freshwater and terrestrial habitats impose profound physiological challenges for most taxa (Hutchinson 1957; Lee & Bell 1999)

Of ~35 Animal Phyla, only 16 invaded fresh water, and only 7 invaded land

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<th>Partial List of Taxa</th>
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Over geological time scales, such major habitat transitions have been relatively rare...

However, some **invasive species are extraordinary** in their ability to breach such habitat boundaries (Lee and Bell 1999; Lee and Gelembiuk 2008; Lee 2010).

Hence, such **invasive species provide valuable models** for understanding **how organisms could adapt to radical environmental change**.
Many invasive species are undergoing fundamental niche expansions:

For example, many freshwater habitats are overrun with brackishwater invaders (Lee & Bell 1999; Ricciardi & MacIsaac 2000)
Many invasive species are undergoing fundamental niche expansions:

But, 5 PSU is a major biogeographic barrier for most aquatic invertebrates

(Khlebovich and Abramova 2000)

\[ \sim 70\% \text{ (1985-2000)} \]
Question

What accounts for the ability of some populations to invade novel habitats, when most cannot?
Increasingly, we are learning that a rapid evolutionary response is important
What do we need for an Evolutionary Response (in this case Adaptation) to occur?
Adaptation occurs through Natural Selection

- **Natural Selection occurs by culling genetic variation in a population**

  Frequency shift in a population, so that the average characteristics of a population is changed

  ***Need genetic variation upon which selection could act, at the relevant traits***
Adaptation occurs through Natural Selection

***Need genetic variation upon which selection could act

• **Sources of Genetic Variation:**
  - Smaller organisms (bacteria, viruses, phytoplankton): New Mutations can arise quickly after habitat change
  - Larger organisms: Generation times are too long and mutation rate is too slow → often must rely solely on *standing genetic variation* in a population
    - Need mechanisms for the maintenance of genetic variation ***at the relevant traits*** (not at neutral loci, not at microsatellites)
Freshwater Invading Copepod

A case of contemporary habitat shifts

Eurytemora affinis
Copepods are common as invaders

- Copepods form the largest biomass of metazoans in the world’s oceans (Hardy 1970; Verity & Smetacek 1996)
- Copepods also form the largest biomass in ship ballast water
- ~12 billion tonnes of ballast water transported annually
Saline to fresh invasion 55 years ago
Winkler, Dodson, Lee, 2008
Fluctuating Habitat

More Constant Habitat

15-25 PSU

5-40 PSU

Winkler, Dodson, Lee 2008 Molecular Ecology
Only the clade from the fluctuating habitat invaded fresh water
What is the pattern of physiological evolution?

Common-garden reaction norm experiment

A

B

Jane Remfert
Reaction norm evolution during invasions

Saline population has minimal survival in fresh water

Lee, Remfert, Chang, 2007

Lee, Remfert, Gelembiuk, 2003
Reaction norm evolution during invasions

%Survival to adulthood

Significant G x E in native range

Lee, Remfert, Chang, 2007
Lee, Remfert, Gelembiuk, 2003
Broad sense $H^2 = 0.66\sim0.87$

BUT, what’s interesting from an evolutionary point of view is the **variance**, more so than the mean response.

Because Natural Selection acts on the variance

*Lee, Remfert, Gelembiuk, 2003, 2007*
Much genetic variation in physiological tolerance in the native range

This genetic variation from the native range would allow Natural Selection to act during invasions


Broad sense $H^2 = 0.66~0.87$

%Survival

(A) St. Lawrence marsh (saline)

(B) Lake Michigan (fresh)

Salinity (PSU)
Significantly higher genetic variation in reaction norms in the invasive clade
(Shannon’s entropy, N = 15 clutches, P = 0.03)

Skelly, Chang, Winkler Lee, In Prep.
• Invasive clade: Nearshore and salt marsh
  Seasonal salinity fluctuations
  Select for different genotypes across seasons

• Noninvasive clade: Central portion of estuary
  Maintain more constant salinity
  No seasonal fluctuations

Winkler, Dodson, Lee 2008 Molecular Ecology
Noninvasive clades: More Constant Habitats (Restricted to central portions of estuaries and bays)

Invasive clades: Fluctuating Habitats (Nearshore, salt marsh ponds, marginal portions of estuaries)
Ponto-Caspian Basin: Hot-spot for invasive populations?

70% of invaders into the Great Lakes 1985-2000

Lee and Bell 1999
Ricciardi and MacIsaac 2000
Zebra Mussel *Dreissena polymorpha*
COI Haplotypes in the Endemic Range

Eurasia

May, Gelembiuk, Orlova Panov, Lee, 2006
Gelembiuk, May, Lee, 2006
Invasive populations likely arose from the Black-Caspian Sea

May, Gelembiuk, Orlova Panov, Lee, 2006
Gelembiuk, May, Lee, 2006
At least 6 invasive ant species in North America likely originated from the unstable floodplains of Argentina

- **Fire Ant *Solenopsis invicta*** (Caldera et al. 2008)

- **Argentine Ant *Linepithema humile*** (Tsutsui et al. 2001)
Invasive species often contain populations that are genetically and physiologically diverse → not all populations within a species are invasive (Lee, 1999; May et al, 2006; Gelembiuk et al. 2006; Caldera et al. 2008)

Invasive populations often originate from fluctuating habitats, with an evolutionary history of fluctuating selection (Lee & Gelembiuk 2008)

Fluctuating selection across generations would result in the creation and maintenance of standing (existing) genetic variation upon which Natural Selection could act during habitat change
Which particular traits are undergoing natural selection, allowing freshwater invasions to occur?
Problem: “Blood” must be Thicker than Water

Hemolymph Osmolality (mOsm/kg)

Surrounding water

Environmental Concentration (mOsm/kg)

Eurytemora affinis

Must maintain steep concentration gradient between hemolymph and dilute water

Lee, Posavi, Charmantier, 2012
Problem: “Blood” must be Thicker than Water

Saline Ancestral Populations

A Atlantic clade

B Gulf clade

Surrounding water

Lee, Posavi, Charmantier, 2012
For Freshwater Populations, “Blood” is even thicker

Evolution of increased body fluid regulation

Freshwater populations show higher hemolymph osmolalities at low salinities ($P < 0.001$)

Collaborator
Guy Charmantier

Lee, Posavi, Charmantier, 2012
For Freshwater Populations, “Blood” is even thicker

Evolution of increased body fluid regulation
Maintaining this higher hemolymph concentration in fresh water would require increases in ion uptake from the environment (and/or reduced ionic loss)
Gene Expression Analysis

Up in freshwater populations:

- Ion transport enzyme V-type H^+ ATPase
- some cuticle proteins
- Mitochondrial proteins (ADP/ATP carrier protein)
- Prohibitin
- Malate dehydrogenase
- S-adenosyl methionine synthase

Down in freshwater populations:

- Ion transport enzyme Na^+,K^+-ATPase
- some cuticle proteins
- Eukaryotic translation elongation factor 1 alpha
- Nicotinic acetylcholine receptor

Parallel shifts in expression of ion transport enzymes from saline to freshwater habitats

Lee et al. 2011 Evolution; Gelembiuk et al. In Prep.
• In salt water, Na\(^+\) transport into the cell is not a problem.

• But, Na\(^+\) transport from the cell to the hemolymph is more difficult; so, **Na\(^+\), K\(^+\)-ATPase** is the rate limiting step.

• In fresh water, **V-type H\(^+\) ATPase** creates a H\(^+\) gradient on apical side, enabling Na\(^+\) to enter cell against steep concentration gradient.

• But then, transport from the cell to the hemolymph is not a problem.
Does ion-transport ATPase function evolve during invasions?

Classic “common-garden” experiment, to remove effects of environmental acclimation

Enzyme Kinetics: V-type ATPase, Na,K-ATPase activity
Evolution of Ion Transport Activity in Larvae

- Enzyme activity in the ancestral saline populations

**Atlantic clade**

- **V-type ATPase**
  - St. Lawrence
  - Gulf

**Gulf clade**

- **Na,K-ATPase**
  - St. Lawrence
  - Gulf

*Lee et al., 2011 Evolution*
Evolution of Ion Transport Activity in Larvae

Atlantic clade

Gulf clade

V-type ATPase

Na,K-ATPase

Evolution of Ion Transport Activity in Larvae

Lee et al., 2011 Evolution
• Parallel evolution in ion transport activity during independent invasions

• Suggests common mechanisms during independent invasions

Lee et al., 2011 Evolution
• But is **salinity** really the factor causing the evolutionary change?

• Performed **selection experiments** to test whether the evolutionary change happens in response to salinity alone (and to establish a **causal link between salinity and V-ATPase activity**)

• Imposed laboratory selection on saline populations for freshwater tolerance over 12 generations
Selection in the Laboratory

A. Atlantic clade

B. Gulf clade

V-type H^+ ATPase activity of the saline populations

V-type ATPase activity x 10^3 (μmol Pi/hr/larva)

Salinity (PSU)
Selected lines show the same pattern of evolution as wild fresh populations.

Parallel Evolution across independent selection lines.

Varying salinity alone reproduced the same evolutionary shifts as in the wild, suggesting that salinity is indeed the factor imposing selection.

Lee et al., 2011 Evolution
Laboratory Selection Mimics Natural Invasions

Reproducibility of evolutionary pathways suggests that this is an evolutionary labile trait.

Lee et al., 2011 Evolution
Rapid Evolution during Habitat Change

- Parallel repeated mechanism of evolution
  - Parallel physiological mechanisms

- The capacity to evolve revealed by comparative reaction norm and selection experiments

- What is remarkable here is the high speed to which these evolutionary shifts could occur (~50 years in the wild, only 12 generations in the laboratory)
Mechanisms observed here might have relevance for different taxa crossing similar habitat clines and might yield powers of prediction on populations that are likely to invade.
Can zooplankton evolve in response to crude oil toxicity?

Compare pre-Oil Spill and post-Oil Spill populations to determine if adaptation has occurred.
Evolutionary Response to the Deep Horizon Oil Spill

The Post-Oil Spill population shows a significant increase in survival and reduction in development time in water soluble fraction of crude oil.
Evolutionary shift in response to crude oil

- Considerable genetic variation in oil tolerance exists in the pre-oil spill population

- Natural selection on standing genetic variation in the pre-Oil Spill population

Unpublished Data

Lee et al. In Prep.

Red = after oil spill
Blue = before oil spill
Temperature Adaptation?

Can Zooplankton populations evolve in response to temperature?
Fluctuating temperature across seasons, and across generations

That is, individuals do not experience the full temperature range of the environment

Generation time shorter than seasonal fluctuations:
~20 day generation time at 15°C

Seasonal change in temperature from 0 to 30°C

Example: Copepods in Chesapeake Bay
Prediction:

• Selection would favor different temperature tolerances at different generations (seasons), leading to the increase and maintenance of genetic variance in the population

• Fluctuating selection + the presence of an egg bank would help preserve genetic variation in the population
Difference in temperature tolerance across seasons

- Two populations from different seasons in the Chesapeake showed different temperature tolerance ($P < 0.01$) (Bradley 1975)

- March (colder) population experienced heat shock faster and took longer to recover

- Difference persisted for multiple generations in the lab (common-garden) revealing that the difference is heritable (genetically-based) and not due to phenotypic plasticity (acclimation)
Significant difference in temperature tolerance among selected lines ($P < 0.01$)

Selected lines reared at higher temperature for $\sim 25$ generations ($\sim 2$ years) had greater tolerance to heat shock ($32^\circ C$) (Ketzner & Bradley 1982)
Evolutionary Potential

• Genetic variance in temperature tolerance is present in the Chesapeake Bay *E. affinis* population due to seasonal fluctuations (not necessarily true of all *E. affinis* populations)

• **Common-garden Experiment ➔ Evolution Happened**: revealed significant heritable differences in temperature tolerance between two populations originating in different seasons

• **Selection Experiment ➔ Evolution can Happen**: revealed that populations of *E. affinis* can undergo natural selection in response to temperature
What about the Open Ocean?

• Do the populations contain genetic variance for the relevant traits?

• Is there a mechanism to maintain genetic variation in temperature tolerance?
  
  → For instance, is there fluctuating temperature across different generations?
  
  → The storage effect of an egg bank? Overlapping generations?
What about the Open Ocean?

**Data Needed:**

Amount of genetic variation for temperature **tolerance** ≠ genetic variation at neutral markers (e.g. microsatellites)

**Mechanism to maintain genetic variance?** Period of temperature fluctuations relative to generation time of organisms? Overlapping generations (egg bank)?

**Rate of Evolution?** Generation time (unit of evolution); mutation rate of smaller organisms (Bacteria, Archaea, Viruses)
Another issue to consider: The Host’s Microbiome

• Shift in the microbiome during habitat change
• The microbiome is a biogeochemical reactor
• Will affect host physiology
• Will affect the ecosystem
Dramatic Shift in Microbial Composition during Invasions

- Microbiome composition shifts radically with salinity change
- Significant Unifrac distances between saline vs. fresh microbial communities (P < 0.01)

Gelembiuk, Silva, Metzger, Lee, In Prep.
Microbial community diversity across latitudes

- Microbiome composition shifts with temperature

- Microbial community diversity increases at lower latitudes ($r = -0.95$, $P < 0.05$)

- This pattern has been found in free-living bacteria, and here it is found for host-associated microbiomes

Gelembiuk, Silva, Metzger, Lee, In Prep.
Potential roles in Biogeochemical Cycles

Enormous Copepod Biomass, Anaerobic Compartment in Guts

Affecting the fate of Nitrogen:

→ Zooplankton gut flora have been speculated to partly account for deficits in $N_2$ Fixation Budgets in some ocean basins: nitrogen loss exceeding the gain from dinitrogen fixation by approximately 200 Tg N yr$^{-1}$ (Mahaffey et al. 2005)

• **Denitrification** ($\text{NO}_3^- \rightarrow \text{N}_2$): Many species of denitrifying bacteria (e.g. Comamonadaceae, Pseudomonadaceae, Vibrionaceae, Rhodobacteraceae, Shewanellaceae)

• **Nitrogen fixation** ($\text{N}_2 \rightarrow \text{NH}_3$): All copepod microbiomes contained *Klebsiella* spp.
Summary

- Rapid Physiological Evolution can occur during Habitat Change
- Ample Genetic Variation for the relevant phenotype traits is essential for Natural Selection to occur
- Evolutionary history in the native range likely affects the levels of standing genetic variation and the potential to respond to habitat change (fluctuating habitats)
- Common-garden and selection experiments could provide insights into whether populations have evolved or could evolve
- When habitat changes, microbiome will also change; the Copepod Microbiome might play critical functional roles for the host, and might have profound ecosystem impacts given its huge size as a biogeochemical reactor
Mechanisms observed here might have relevance for different taxa crossing similar habitat clines…

…and might yield powers of prediction on how populations will respond to habitat change
Lee Lab Members
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