

MEETING REVIEW

Ecological genomics—changing perspectives on Darwin's basic concerns

SUZY C. P. RENN*† and DAVID H. SIEMENS*†

**Department of Biology, Reed College, Portland, OR 97202, USA, †Integrative Genomics Program, Department of Biology, Black Hills State University, Spearfish, SD 57799, USA*

Abstract

Ecological Genomics is an interdisciplinary field that seeks to understand the genetic and physiological basis of species interactions for evolutionary inferences. At the 7th annual Ecological Genomics Symposium, November 13–15, 2009, members of the Ecological Genomics program at Kansas State University invited 13 speakers and 56 poster presentations.

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Introduction

Ecological Genomics seeks a more thorough understanding of species interactions by integrating genetics, physiology, ecology, and evolution. At the core of this integrative approach is the genomics-enabled search and study of genes that function in species interactions. Inception of the field was formalized in a publication by Feder & Mitchell-Olds (2003) with the descriptive title 'Evolutionary and Ecological Functional Genomics'. Recently Ungerer *et al.* (2008) described Ecological Genomics as 'an integrative field of study that seeks to understand the genetic mechanisms underlying responses of organisms to their natural environment'. Here we review recent work presented at the 7th annual Ecological Genomics Symposium (November 13–15). This conference was organized by members of the Ecological Genomics Institute at Kansas State University (<http://ecogen.ksu.edu/>) and sponsored by KSU and NSF.

We feel that the plenary speaker, Jonathan Wendel (Iowa State University), identified the field's basic concerns by drawing on Darwin's original questions about the nature of variation, the process of adaptation, the origin of species and the importance of species interactions. We borrow this loose and overlapping framework and use the four topics as the vertices to define a space of inquiry that guides our meeting review (Fig. 1). We

place species interactions at the apical position to reflect its overarching guidance. While this scheme was not laid out in the symposium program, it appropriately captures the broad goals and results of the prominent work in the field that are otherwise diverse.

What is the nature of the variation upon which natural selection acts?

The genomics era alters our perspective on patterns of genetic variation and inheritance at levels ranging from individuals, through populations, to species. The available techniques now reveal new dimensions of investigation and expose genomic architecture as a dynamic source of variation through processes such as gene duplication, ploidy, mutation, co-option and accommodation. While classic genetics is always meaningful, understanding the mechanism and influence of variation on a genome-wide scale reinvigorates Darwin's initial question on the nature of variation.

Wendel's own work is focused on the consequences of genetic variation due to polyploidy in *Gossypium* (cotton) species of hybrid origin (allopolyploids). He has found that modulation of homeolog expression depends on the time since hybridization, the developmental stage and also the parent genome of origin. These findings raise new questions about whether this epigenetic variation represents latent variation for later evaluation by natural selection, the degree to which

Correspondence: Suzy C. P. Renn, E-mail: renns@reed.edu

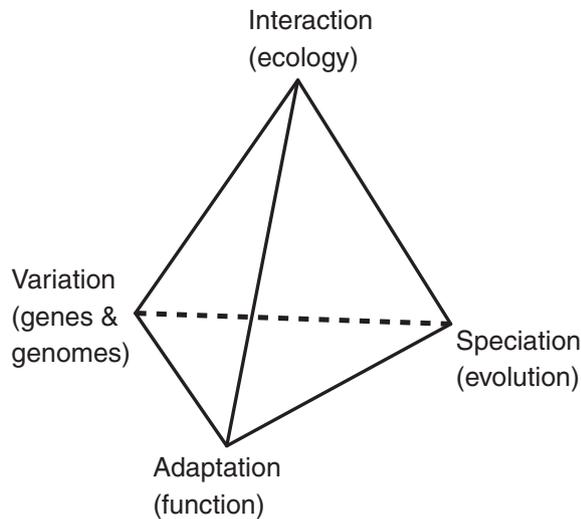


Fig. 1 Tetrahedron model showing inquiry space defined by four topics. Studies in Ecological Genomics fall along the various continua in this space.

these findings can be generalized to other species and the degree to which polyploidy models more modest instances of gene duplication. Because proliferation of transposons can produce duplication, Kawakami *et al.* (KSU) surveyed such events in hybrid zones for three hybrid sunflower species. The lack of large-scale transposon proliferation associated with the origins of these hybrid plant species suggests that, unlike hybrid cotton species, at least this source of gene duplication is not an important factor in hybrid sunflower species (Ungerer *et al.* 2009).

While these surveys of genomic variation took a forward genomics approach, ecological genomics can also employ a reverse genomics approach and draw on past studies of ecological genetics. One example of this approach was demonstrated with cytochrome P450 monooxygenases (P450), a large gene family that is diversified for substrate specificity. This diversity provides novel detoxification and biosynthetic pathways and is known to underlie coevolution among insects and plants (e.g. Wen *et al.* 2006). May Berenbaum (University of Illinois) is now taking this research to a genomic level through a phylogenetic analysis of ~7000 P450s.

Some modern studies now move beyond simple description of standing genetic variation, and even beyond variation of gene product function, in order to actually quantify potentially functional variation of gene regulation, particularly in an ecological context. As such, the field has great potential to weigh in on our understanding of the importance of gene regulation in adaptive evolution (Hoekstra & Coyne 2007; Chan *et al.* 2010). Toward this goal, using a cross between lab and

Hawaiian ecotypes of the model nematode *Caenorhabditis elegans*, Matthew Rockman (New York University) examined the magnitude of heritable variation in gene regulation. While the traditional explanation of mutation-selection balance accounted for some of the heritable variation in transcript abundance, selection on linked sites proved to have a much greater effect. This result emphasizes an important role for random events mediated by genome architecture.

Genetic variation, upon which selection acts, may also involve the somewhat controversial mechanism of genetic accommodation (Braendle & Flatt 2006; McGuigan & Sgro 2009) where an environmentally induced (phenotypically plastic) trait is transformed into a stably expressed trait without the eliciting environmental stimulus. Ecological genomic studies may at least help to understand the occurrence and consequences of genetic accommodation. For example, the life cycle of the pea aphid (*Acyrtosiphon pisum*) includes both environmental (in females) and genetic (in males) winged morphs. As a molecular basis of the polyphenism, Jennifer Brisson (University of Nebraska) and colleagues identified *apterous 1* as a candidate gene in addition to the known X-linked locus *aphicarus (api)* (Brisson *et al.* 2010). Brisson speculated that the genetic male polymorphism is the result of genetic accommodation having evolved by co-option of ancestral pathways for environmental female polyphenism. Similarly, Siemens *et al.* (Black Hills State University), working on a close wild relative of *Arabidopsis*, and Tonia Schwartz and Anne Bronikowski (Iowa State University), working on garter-snake, are investigating whether the maintenance of genetic variation by balancing selection may be a consequence of the genetic accommodation of multiple signal transduction pathways that negatively interact.

These examples demonstrate the limitations and tradeoffs that exist in describing variation when using traditional genetic-model vs. less well developed model organisms. With *C. elegans*, *Arabidopsis*, etc., researchers can address a greater genomic scope while research with salmonids, midges, garter-snake, etc., offers a broader ecological scope and tempered genomic scale. The innovative combination of these approaches such as the use of close wild relatives of models and greater sequencing capabilities continues to push the field forward.

How do evolutionary processes lead to adaptation?

Having a genome-wide description of the underlying variation is a potentially powerful tool for addressing Darwin's concern about the process of adaptation.

Many studies in ecological genomics are now moving beyond the important process of gene discovery to address the genomic basis of adaptation; however, the successful studies thus far have primarily focused on a small number of gene interactions or pathways.

The transition away from major gene explanations may be aided by the application of genomic tools to long-standing non-genetic model systems for which a substantial theoretical framework exists regarding the nature of adaptation. Complementary studies at the symposium that used altitudinal and latitudinal clines to model the evolutionary process of adaptation reveal the nature of this challenge. Jay Storz (University of Nebraska) addressed concerted evolution among paralogous genes that encode the α and β subunits of haemoglobin in deer mice (*Peromyscus maniculatus*) across altitudes. Mechanistically, these polymorphisms make the molecule less sensitive to inhibition by 2,3-diphosphoglycerate (DPG), suggesting an evolutionary co-option of the mechanism that serves increased oxygen transport in fetal haemoglobin. On a broader genomic-scale, adaptation to altitude was addressed by Julian Mensch (University of Buenos Aires) using the western Argentinean populations of *Drosophila melanogaster*. By examining genomic architecture in relation to developmental, physiological and behavioural clines, a complex dynamic relationship was described. Similarly, using heterologous hybridization to the zebra finch microarrays, Cheviron *et al.* (2008) (UCLA) tied elevation gradient to differential gene expression patterns in the rufous-collared sparrows (*Zonotrichia capensis*) across the Andes. Together these three studies illustrate the tradeoff between the understanding of mechanistic details possible with major gene explanations and the challenge to describe broad patterns across a natural range. The genetic architecture underlying evolutionary change across environmental gradients is likely to be subtle and also involve many systems and genes (Kroymann & Mitchell-Olds 2005). It is crucial to continue efforts to balance these tradeoffs and capitalize maximally on each approach as the field struggles to move beyond gene discovery.

In order to overcome some of the challenges associated with non-genetic model organisms, two complementary approaches that draw on the resources from genetic model systems have emerged. The first employs the use of genetic model organisms in creative ecological contexts. For example, Chris Toomajian (KSU) and colleagues took advantage of the 250 000 SNPs available through the Arabidopsis 2010 project to perform association mapping to identify signatures of selection for flowering time among natural isolates of this genetic model organism. Consistent with a hypothesis of recent adaptation, they found high levels of haplotype sharing

for genes key to the vernalization response (e.g. FRI). Similarly, using the genetic model organism *C. elegans*, the Herman lab (KSU) has addressed the adaptation of nematodes to a changing prairie soil microbial environment. By exposing several *C. elegans* innate-immunity mutants to different bacteria, Kelsey Hixon-Bowles *et al.* found strain-specific and bacterium-specific differences that identified new pathways. Further microarrays and functional analyses by Vinod *et al.* identified candidate defense and metabolism genes responsible for these differences. Meanwhile, Ziyi Wang *et al.* used a QTL approach to refine the position of identified candidates.

The second approach employs the use of non-model organisms that are closely related to the genetic models, and can take many forms. Stephen Keller (University of Minnesota) and colleagues took advantage of genomic sequence information from the black cottonwood, *P. trichocarpa*, genome (Yang *et al.* 2009) in order to identify signatures of selection in balsam poplar (*Populus balsamifera*) across its range. They found evidence of recent adaptive evolution of candidate genes for phenology in the flowering time network. With respect to genomic tools, similar benefit can be derived by their application to study closely related species. Accordingly, Riston Haugen *et al.* in the Siemens lab (Black Hills State University) have studied plant pseudoflower-forming fungal infections (Roy 1993) using *Boechera holboellii*, a wild relative of *Arabidopsis*. Heterologous hybridization to the *Arabidopsis* microarray allowed them to describe the parasitic manipulation of host gene regulation with regard to immune responses and for flower development. Interestingly, when members of the Herman lab attempted to employ RNAi techniques to *Caenorhabditis* species that are closely related to the *C. elegans* genetic model, their failed attempts with RNAi (Dave Wheeler *et al.*) suggested that the robust RNAi response of *C. elegans* may not be wide-spread. These studies of adaptation demonstrate the need for integrative genomic experiments involving both model and non-model organisms. Integration of these tools and approaches requires creative experimental design and rigorous use of controls.

How does speciation occur?

Many studies that fall under the umbrella of Ecological Genomics contribute critical information to the speciation process because they are part of the continuum represented by patterns of variation, mechanisms of adaptation and speciation. Again, the genomic perspectives are being applied to long-standing models for which theoretical frameworks exist. Similar to the use of altitudinal gradients as a model for adaptation on

evolutionary timescales, adaptive radiations provide a model with which to study speciation. The numerous, closely related species that result from accelerated rates of speciation provide a model for more modest ongoing processes. For example, analysis of expressed sequence tags from representative species in the rockfish (*Sebastes*) radiation have allowed Andres Aguilar (University of California, Merced) to identify genes subject to adaptive evolution and reproductive isolation, while analysis of neutral markers revealed recent selective sweep processes and suggested cryptic species. Renn and Machado (Reed College) used cross-hybridization of genomic DNA to cDNA microarrays to describe gene duplication rate across lineages the African cichlid radiation and also to control for sequence divergence in their study of species-specific neural gene expression profiles among species (Machado *et al.* 2009). Using a similar approach, Brian Darby (Herman lab KSU) found that the confounding effects of GC content and other factors hindered his ability to identify orthologs for cloning. Such novel applications of standard genomic techniques must be developed in order to maximize the information gleaned from these studies.

Host shifts provide another powerful model for the study of speciation. Ever since Guy Bush's original work on sympatric host races of *Rhagoletis* fruit flies, the number of genes required for such parasite-host shifts has been of great interest. Now, Jeffrey Feder (University of Notre Dame) takes a genome-wide approach to describe widespread sequence divergence dispersed throughout the genome associated with incipient speciation of the apple maggot fly *Rhagoletis pomonella*. Another study (Cristian Corio *et al.*, University of Buenos Aires) emphasized the importance of gene expression divergence in host shift of *Drosophila koepferae* between cacti.

While these genome-wide studies represent the integrated goals of Ecological Genomics it remains a challenge to move beyond gene discovery in order to understand the evolutionary processes at work in other exciting systems. Erica Rosenblum (University of Idaho) described simultaneous parallel divergence within different species such that the novel phenotypes, driven by ecological conditions, are convergent. The events involve different mutations of the same Melanocortin-1-receptor (Mc1r) gene in three different lizard species that cause cryptic coloration on the dunes of White Sands, New Mexico. Interestingly the mutation in one species, which causes poor membrane integration, is dominant (*Sceloporus undulatus*) while that in another species, which compromises signal transduction, is recessive (*Aspidoscelis inornata*). This genetic analysis sets the stage for future genomic work to address different processes of convergent evolution driven by ecological conditions.

How do interactions moderate evolutionary processes?

Species interactions such as social systems, coevolved multi-species symbioses and indirect interactions, are inherently complex. As such, it is not only necessary to model or study the species independently or in parallel but to actually make the interactions *per se* the focus of ecological genomic studies. For example, the focus on relatively simple cooperative behaviours in the social amoeba *Dictyostelium discoideum* has revealed the importance of *lag* genes that encode transmembrane proteins that participate in cell adhesion and signalling for kin recognition (Benabentos *et al.* 2009). Using cheater mutants of this gene as selective agents, David Queller (Rice University) and colleagues recently observed the evolution of cheater-resistant lines (Khare *et al.* 2009) suggesting reciprocal adaptive evolution between cheater and cheater-resistant traits. Patterns of natural population genetic structure are now bringing new insights to the evolution of these inherently complex traits and processes (Gilbert *et al.* 2009).

Other coevolved systems are even more complex and involve multi-species symbiotic relationships. For example, members of Cameron Currie's lab (University of Wisconsin-Madison) combined genomics, chemistry and bioassays to address complex symbiotic associations between animals and microbes. Micheal Poulsen *et al.* identified novel antibiotics by characterizing Actinobacteria-derived compounds that mediate fungus-insect symbioses. Garret Suen *et al.* combined sugar composition analysis, 16S rDNA sequencing, community metagenomics and whole-genome sequencing to show that lignocelluloses, cellulose and hemicellulose decomposition involves a community of microbes in leaf-cutter ant fungal gardens. Lignocelluloses are also a major component of organic matter of boreal forest soils, but appear to be degraded mainly by soil fungi, as Robert Burgess in Lee Taylor's lab (University of Alaska, Fairbanks) determined by stable isotope probing (SIP). Microbial mediators of species interactions are no doubt common and important for understanding ecology and evolution, and represent opportunities for the discovery of new species, pathways and compounds that may also have important commercial applications.

The species interactions mentioned above do not occur in isolation and therefore a future challenge in ecological genomics is to fully embrace the network of interactions and indirect effects involved. Towards this goal Clayton Coffman in the Appel/Schultz lab (University of Missouri) found that experimental light conditions (red:far-red ratio) that mimic competitive shade environments altered herbivore-induced plant chemical defense response and suggested a role for ethylene signalling.

Similarly, Sue Hum-Musser and Richard Musser (Western Illinois University) found that simultaneous exposure of tomato to ozone and caterpillar herbivory attenuates the level of expression for many defense genes demonstrating environment-specific species interactions. For evolutionary insights, much more work is needed to understand the ecological consequences of genomic variation in the regulatory pathways involved.

Summary and conclusions

Ecological genomics, as presented at the conference, embodies Darwin's four main questions but restates them in a modern context that spans multiple research levels. The diverse field is in part defined by the tools and approaches that are used. The complementary approaches currently being employed by researchers in Ecological Genomics presents a great strength that is pushing the field forward. However, the tradeoff between these complementary approaches and the gaps remaining between alternate solutions still present a considerable challenge. Regardless of whether the research employs genetic models in creative contexts, applies genomic resources to species closely related to these genetic model, relies on forward or reverse genomics, or develops genomic tools *de novo* for long standing evolutionary model systems, the studies encompassed by Ecological Genomics are unified by their broad research goals.

Having identified DNA sequence as a primary source of variation, we are now poised to identify the relative importance of different sequence characteristics such as single nucleotide polymorphisms, gene clustering, chromosomal rearrangements, gene copy number and co-opted and repetitive elements. Furthermore, the field aims to incorporate functional information and address variation in protein function, gene regulation and epigenetic effects.

Identification of the variation that selection may act upon lays the groundwork for understanding the relationship between genotype and phenotype as it relates to ecological adaptation. Here the field struggles to maintain a genome-wide perspective while establishing mechanistic understanding. Progress is being made on this front through innovative combinations of forward and reverse approaches.

These efforts dovetail with research concerning the process of speciation. In both areas, progress is aided by the integration of information derived from studies in traditional genetic model organisms with information derived from the application of modern techniques to ecological and evolutionary model systems such as adaptive radiations and insipient speciation situations. These efforts also lead to the establishment of new 'model'

organism with which to address these questions that fall within the research space of ecological genomics.

The entangled bank that Darwin reflected on epitomizes the complexity of many species interactions. For integrative genomic studies that embrace this complexity, microbes and plants appear to be best suited thus far for their relative simplicity, sessile nature and central importance in many ecosystems. We predict that as more genomes are sequenced, a reverse genomics approach that seeks to understand the ecological consequences of naturally occurring sequence variation will connect genomes, phenotypes, ecosystems and the evolutionary forces that have shaped them.

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Associate Professor David Siemens is working on the ecological consequences of genetic variation in signalling pathways for evolutionary inferences. Associate Professor Suzy Renn studies the evolution of behaviour using analysis of gene expression and genome content among African cichlid fishes.
