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Experimental evolution of innovation and novelty

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Highlights

- Microbial systems provide a unique opportunity to dissect both the genetic mechanisms and ecological conditions that lead to the evolution of novel traits and functions.
- All novel functions are derived from pre-existing ones but the major obstacle to their evolution is accessing novel kinds of genetic variation under ecological conditions that allow this variation to spread.

- A range of genetic mechanisms can promote the generation of novel genetic variation and bias the kind of variation produced.
- Ecological factors that influence population size impact the likelihood that novelty will spread through a population.
- Selection – sometimes driven by adaptation to conditions not obviously connected to the novel function that eventually evolves – can move lineages into regions of mutational space that allow novel variation to be accessed.

Keywords: experimental evolution, epistasis, gene amplification, ecological opportunity

Abstract

How does novelty, a new, genetically based function, evolve? A compelling answer has been elusive because there are few model systems where both the genetic mechanisms generating novel functions and the ecological conditions that govern their origin and spread can be studied in detail. Here, I review what we have learned about the evolution of novelty from microbial selection experiments. This work reveals that the genetic routes to novelty can be more highly variable than standard models have led us to believe and underscores the importance of considering both genetics and ecology in this process.

The paradox of novelty

The evolution of novelty – the origin of a new function – involves a paradox. How does something new come about if all that natural selection has to work with is something old? The answer, first articulated by Francois Jacob [1], is that new functions are not produced from scratch. Evolution, Jacob said, is more like a tinkerer that uses old materials in new ways.

Appealing as this metaphor is, more precise statements about how tinkering happens – the genetic mechanisms that generate a novel function and the ecological conditions that promote its origin and spread – remain elusive.

The leading explanation, also known as the exaptation-amplification-diversification (EAD) model [1-3], attributes the origins of novelty to exaptation and amplification: some pre-existing function is co-opted for growth and reproduction under novel conditions, even if it only barely allows an organism to get by, and increases in fitness are caused by increases in production of a limiting enzyme, usually through gene amplification. Better to make more of what you already do, even if you do it poorly. The additional genetic material from gene amplification means that selection is free to modify one copy and not others, leading ultimately to functional divergence. While there are other means of generating novel gene function – horizontal gene transfer, reverse transcription of RNA back into DNA, exon shuffling, mobile element transposition, genome rearrangement, and even *de novo* selection from previously noncoding DNA [5-7] have all been suggested to play a role –the EAD model is thought to be among the most important and best supported [8].

Evaluating this model has proven challenging for three reasons. The first is disagreement and confusion over just what, precisely, a novelty – or its near synonym innovation – actually is (see Box 1). Does novelty refer to a trait, like wings for flight, or an ecological function, like the ability to occupy a new environment? The answers depend to a large extent on to whom one is talking: developmental biologists tend to focus on traits and their genetic basis, because that is

the data they have available to them. Evolutionary ecologists tend to focus on, not surprisingly, ecology, as the ultimate driver of novel trait evolution and lineage diversification. Both are, obviously, important but few systems exist where the two processes can be studied in detail together.

The second, which is closely linked to the first, is that different disciplines have interpreted the problem of innovation and novelty through very different conceptual lenses. Developmental biology and protein biochemistry tend to see the trajectory of evolution being shaped by what genetic variation is available to selection [14-17]. Evolutionary ecology has assumed, by contrast, that genetic variation is unlimited in the long-run, with novelty being the result of conventional natural selection operating in unconventional ecological settings [13, 18]. The central argument comes down to whether the rate at which novel traits and functions evolve is governed more by access to genetic variation or to novel ecological opportunities.

The third is that the model itself does not directly account for the striking variation in the time required for novelty to evolve. The evolution of aerobic citrate metabolism in *Escherichia coli* – a trait whose absence is actually diagnostic for this strain – took ~31,500 generations, or approximately 15 years of daily sub-culturing the populations to evolve and, even then, it occurred in only one out of 12 replicate populations [19]. On the other hand, there are many examples (reviewed below) of far more rapid adaptation, often on the order of tens to hundreds of generations, to novel environmental conditions such as the degradation of toxic

compounds, the use of a novel substrate, or infection of a novel host. Why should one kind of novelty take so much longer than others?

Here I draw on the literature from experimental evolution with microbes to address these gaps in our understanding of the factors driving the evolution of novelty. Microbial selection experiments have the advantage of being performed under defined conditions where the genetic changes can be uncovered through whole genome sequencing and their impact on traits such as fitness, population size, and the degree of novelty in ecological function measured directly. It is thus possible to watch the evolution of novelty happening in real time and to dissect the genetic and ecological mechanisms responsible. My aim here is to point the way towards a more general theory of novelty that accounts more readily for the variety of genetic routes to novelty and the ecological conditions that lead to its evolution.

Box 1. Novelty versus innovation

Novelty is a familiar word but difficult to define, in part because it is often used synonymously with *innovation*. I think it is helpful to follow the Organization for Economic Cooperation and Development (OECD), arguably the standard setters for global economic policy in research, who define innovation as a new or significantly improved product, service, or process [9] and interpret novelty as the extreme end of the innovation spectrum.

A more biological interpretation might be that a novel trait is one that confers a new (to a lineage) ecological function underlain by a qualitatively distinct (relative to the ancestral form)

genetic architecture. A loss-of-function mutation that leads to constitutive expression of an otherwise inducible system, leading to the over-production of a pre-existing enzyme important for growth, would be counted as an innovation. The enzyme itself hasn't changed, nor has the underlying genetic architecture governing how it is produced, though the ecological conditions have. The evolution of an enzyme capable of degrading a compound that the ancestral lineage could not otherwise use would be considered a novelty, especially if this new ability is underlain by genetic rearrangements and changes to enzyme activity [10]. Put another way, innovation is doing something better, novelty is doing something new.

Note that when an innovation or novelty also contributes to evolutionary diversification we say the trait is a *key innovation* [11. 12]. Because diversification requires a lineage first gain access to a range of ecological opportunities [4], key innovations must evolve before diversification. Interestingly, there is evidence that key innovations can evolve well before diversification happens [13], suggesting that the ecological conditions promoting innovation and novelty may be very different from those that promote diversification.

Genetics of innovation and novelty

The origins of innovation and novelty lie in exapted enzymes that perform both a native or canonical role but also possess a number of often fortuitous side functions that allow them to 'moonlight' in different roles if and when necessary. The nature and evolution of such enzyme promiscuity has been reviewed previously and interested readers can consult Copley [20, 21], Bergthorsson *et al*. [3], and Kheronsky *et al*. [22] for further details. Microbial studies of

adaptation to novel resources [10, 23-25] or toxins such as antibiotics [26, 27] provide many examples of the importance of exaptation as a first step in ecological innovation.

The second step involves population expansion, typically through gene amplification. There is good evidence that amplifications have contributed to the emergence of many different novel phenotypes, from proteins [15, 28] to morphologies and body plans [7, 29, 30] in many taxonomic groups [31-33]. Amplifications occur frequently, especially in microbes, but they are usually unstable and costly so can be lost quickly [34]. The EAD model solves this problem by invoking selection on the amplification itself through increased enzyme production leading to population expansion [8], and there is good evidence for this mechanism from microbial experiments [35-44]. Yet, amplifications are not the only route to population expansion. Toll-Riera *et al.* [45], for example, found amplifications in only 4% of *Pseudomonas aeruginosa* lineages that had evolved the ability to metabolize a novel resource not previously used by the ancestral strain, with mutations in transcription factors leading to the de-regulation of alternative metabolic pathways being far more common. The environmental context within which selection happens can also play a role: selection on substrate mixtures composed of a readily-used native resource and a novel resource that does not support growth can support sufficiently large population sizes for long enough to access rare beneficial mutations allowing improved growth on the novel resource [46-48].

The final step involves divergence of genes or genetic interactions that result in a novel function. A novel function requiring multiple mutations can be built because each mutation on

its own confers a fitness advantage at every step. It has also been suggested that multiple mutations accumulate through neutral processes for a time until some final mutation 'discovers' a new phenotype and the whole lot – driver and neutral mutations together – are driven to fixation by positive selection [49]. Microbial experiments, perhaps unsurprisingly given how effective selection can be in large populations like those usually studied in the laboratory, overwhelmingly come down on the side of selection as the driver of divergence, although the genetic and ecological routes taken can be variable. Three examples illustrate this point.

The first, by design, closely recapitulates the EAD model. Nasvall *et al*. [41] evolved populations of *Salmonella enterica* containing a modified *hisA*, which codes for an enzyme required for histidine synthesis as well as some rudimentary ability to synthesize tryptophan, on a plasmid prone to amplification in the absence of both histidine and tryptophan. Prolonged selection over ~3000 generations resulted in increased fitness driven by duplication to *hisA* and subsequent modification of one or both copies leading to either distinct enzymes specializing on either histidine and tryptophan synthesis, respectively, or generalist enzymes performing both functions. The other two examples involve more idiosyncratic pathways. Aerobic citrate metabolism (Cit+) in *E. coli*, for example, resulted from specialization on acetate (via a citrate synthase gene, *gltA*, also important for assimilating acetate), an overflow by-product of glucose metabolism, and then the fortuitous capture of a citrate transporter (*citT*) that is normally silent under aerobic conditions by an aerobically active promoter (*rnk*) following duplication and genomic rearrangement [50, 51]. Meyer *et al*. [52] documented the role of coevolution

between bacteriophage λ and its *E. coli* host leading to the fixation of at least four mutations all of which improve adsorption on the host [53], before access to a final key mutation allows the lineage to switch binding receptors from the ancestral LamB to the novel OmpF.

Genetics versus ecology in the evolution of novelty

Evolutionary developmental biologists have long argued that trait evolution cannot be understood independently of the developmental system that produces them; it is the spectrum of genetic variation that governs the evolution of novelty. Evolutionary ecologists, on the other hand, assume genetic variation is essentially unlimited and so view the range of ecological opportunities and interactions among species as the major driver of novelty and lineage diversification. Which view is more often correct?

A survey of the microbial evolution literature reveals there is merit to both. Ecological opportunity, or vacant niche space, is clearly a major driver of evolutionary innovation and novelty in these experiments. The citrate added to minimal glucose medium, for example, is an untapped ecological opportunity for *E. coli* that, eventually, a lineage evolved to exploit. We have observed that our laboratory strain of *Pseudomonas fluorescens*, SBW25, which lacks a key gene (*xylB*) for xylose metabolism, evolves the ability to grow rapidly on xylose within 100-200 generations when xylose is provided in abundance [48] through mutations to *gntR*, a transcriptional regulator. The literature is replete with similar examples [4, 20]. Ecological interactions can also drive novelty, as the co-evolution of bacteriophage λ with its *E. coli* host

demonstrates [52, 54]. Resource competition can also be important in acquiring novel bacteriophage hosts [55] or resources [56-58].

There is also growing evidence that the spectrum of genetic variation available to selection can be biased in ways that make it more likely that some genomic sites contribute to innovation and novelty than others [59]. We have found, for example, that resistance to the fluoroquinolone antibiotic, ciprofloxacin in the opportunistic pathogen *Pseudomonas aeruginosa* occurs repeatedly through single base pair deletions in *orfN* in either poly-T or poly-G repeats, genomic regions that are prone to mutation [60]. More generally, Bailey *et al*. [61] have shown, using a modelling approach, that mutational heterogeneity could account for between 9-45% of the variation in parallelism in evolve-and-resequence studies in bacteria and yeast, depending on the study. Clearly, mutational heterogeneity along a genome biases the spectrum of genetic variation available to selection.

Variation in time to the emergence of new functions

The ability to aerobically grow on citrate in *E. coli* and the ability to grow rapidly on xylose in *P. fluorescens* are both examples of the evolution of novel substrate use. The examples are compelling because, in both cases, the absence of the ability to use each respective substrate was diagnostic for the strain. Why did the former take over 31,000 generations to evolve whereas the latter took only ~ 150?

One answer is 'potentiation', the evolution of a genetic background that affords a lineage access to genetic variation that would otherwise be inaccessible. The immediate ancestor to

the *E. coli* lineage that evolved the ability to aerobically utilize citrate, for example, was far more likely to give rise to other Cit+ phenotypes than strain that founded the experiment [19]. By contrast, rapid adaptation to a novel resource, like in the case of xylose utilization in *P. fluorescens*, typically involves far fewer mutations, sometimes only one [45, 62, 63]. We have found, for example, that ciprofloxacin-resistance mutations resulting from knocking out the small molecule efflux pump regulator *nfxB* almost always evolve in under 100 generations in *P. aeruginosa* [64]. Similar results likely underlie many cases of rapid evolution of innovation. The ability of a strain to access relevant genetic variation can thus contribute to the time required for innovation or novelty to evolve.

Potentiation may be a common phenomenon that could explain apparent all-or-none epistasis in the evolution of novel function. It has been seen in bacteriophage λ experiments by Meyer *et al.* [52] and may also be occurring in other gain-of-function experiments in viruses. The ability of avian influenza virus, for example, to be transmitted through the air to mammals requires multiple mutations, often on the order of at least 5 and possibly more [65]. It has been suggested that many proteins seem to be able to tolerate the introduction of mutations without severely compromising function [15], implying that potentiating mutations might fix through neutral processes that allow a gene to explore more mutational space before hitting on the 'right' combination of mutations that permit novelty to evolve under positive selection [49]. It is hard to see how this could happen in the experiments reviewed here. In bacteriophage λ, for example, the ability to infect via the novel OmpF receptor involved the fixation of at least four potentiating mutations within 9-17 days. Since neutral mutations fix at a rate that is equal

to the mutation rate, which for most viruses is on the order of 10^{-6} per nucleotide per generation [66], this result that is hard to reconcile with the time required to fix the equivalent number of neutral mutations. However, selection, as explained earlier, is likely to be important in these kinds of experiments by design because population sizes are so large, so this result must be interpreted with caution.

Ecological constraints that prevent the spread of novel genotypes is a second possibility. Patches containing novel substrates will, by definition, support fewer individuals than those containing preferred resources. For novelty to evolve the population must overcome drift and survive the swamping effect of immigrants arriving from more productive patches [67-69]. Competitors [57, 58, 70], parasites [71], and predators [72] can also reduce population size of a focal lineage, making it harder for it to access the relevant beneficial mutations leading to novelty, or by occupying ecological opportunities that effectively eliminate the opportunity for selection to do its work.

Rethinking the theory for the evolution of novelty

Because evolution is a process of descent with modification, novel phenotypes *must* originate from the re-tooling existing gene functions in new ways. The EAD model spells out more formally how, and in what order, this re-tooling is expected to happen. However, the model has remained for the most part untested simply because there are few systems where each step of the process can be rigorously and empirically evaluated.

Microbial selection experiments are especially valuable, then, because they provide an opportunity to confront the EAD model directly with data. The work reviewed here tells us that, while the EAD model can be an accurate description of how innovation and novelty evolve in some situations, reality can be more complex in at least two ways.

First, gene amplifications are not the only way for a lineage to increase fitness in a new environment. Other mechanisms including regulatory changes or the availability of alternative resources that can support growth can also be important in increasing population size and allowing a lineage to persist under novel conditions. Second, genotypes vary in their ability to access novel phenotypes through mutation, a feature that likely underlies both the distinction, as I have described it, between innovation and novelty and the time to the emergence of novel phenotypes. A genetic background that has ready access to novel phenotypic variation, for example through a loss-of-function mutation that results in deregulation of an otherwise inducible pathway, is an innovation that can evolve very quickly. On the other hand, the fixation of multiple mutations arising from adaptation to one function, like acetate metabolism, that fortuitously provides access to mutations that allow another, novel function to evolve, like the ability to aerobically utilize citrate, is more likely to take a much longer time and be counted as a genuine novelty.

Taken together, it may be time to abandon the strict form of the EAD model. If so, it could be replaced, provisionally, with one that recognizes the importance of genetic factors like potentiation, alternative routes to increasing gene dosage beyond just gene amplification, and

integrates key elements of ecology, like ecological opportunity and ecological interactions, as drivers of the evolution of novelty. A new acronym might help – call it the 'PEAD' model, where 'P' here stands for potentiation, and 'A' represents amplification of enzyme products. We can leave the last 'D' for divergence, but we need to be ready to expand on it dramatically and integrate ecology more directly into our thinking about how novelty evolves. We will need to first answer a number of key questions on the relative contribution of genetics and ecology in driving the emergence and spread of novel traits (Box 2). Doing so, I suspect, will take us a long way towards understanding when and why novelty evolves, or not.

Box 2 – Outstanding questions

- To what extent is the evolution of novelty limited primarily by access to genetic variation versus access to novel environmental conditions?
- What is the relative importance of internal genetic changes like gene amplifications and rearrangements versus those coming externally through horizontal gene transfer in driving the emergence of novel traits?
- Is selection or drift more often responsible for the emergence of potentiated lineages in gaining access to novel variation?
- How does the distribution of fitness effects among mutations – and especially the ability to access novel phenotypes – change with genetic background?

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