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, perhaps best 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 [1–4], 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 acquiring 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 very general, applying equally to both prokaryotes and eukaryotes, and among the most commonly cited explanations for the origin, as opposed to the transfer among lineages, of new genes and genetic functions [2,3,8].

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 [9–12]. 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 [13,14]. 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 the species – 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 [15]. On the other hand, there are many examples (reviewed below) of 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 (Table 1) 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

88 the genetic changes can be uncovered through whole genome sequencing and the impact of
89 these changes on traits such as fitness, population size, and the degree of novelty in ecological
90 function measured directly. It is thus possible to watch the evolution of novelty happening in
91 real time and to dissect the genetic and ecological mechanisms responsible. Importantly, these
92 experiments are performed in a region of evolutionary parameter space – large population sizes
93 (on the order of 10^5 – 10^9 individuals in most cases), genetic variation introduced solely via
94 mutation, often (though not exclusively) haploid asexuals – where natural selection can be very
95 effective at generating adaptation. The generality of the inferences made must therefore be
96 evaluated in other systems where conditions differ. My aim here is to point the way towards a
97 theory of novelty that accounts more readily for the variety of genetic routes to novelty and the
98 ecological conditions that lead to its evolution.
99

100 **Box 1. Novelty versus innovation**

101 *Novelty* is a familiar word but difficult to define, in part because it is often used synonymously
102 with *innovation*. I think it is helpful to follow the Organisation for Economic Cooperation and
103 Development (OECD), arguably the standard setters for global economic policy in research, who
104 define innovation as a new or significantly improved product, service, or process [16] and
105 interpret novelty as the extreme end of the innovation spectrum.
106

107 A more biological interpretation might be that a novel trait is one that confers a new (to a
108 lineage) ecological function underlain by a qualitatively distinct (relative to the ancestral form)
109 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 [17]. 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* [18,19]. Because ecological 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 leading to the evolution of innovation and novelty could be distinct from the ecological opportunities 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 [17,23–25] 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 [9,28] to morphologies and body plans [6,29,30] in many taxonomic groups [31–33]. While they can be as small as a few base pairs or as large as entire chromosomes (aneuploidy) or genomes (polyploidy), the amplifications thought to most often underlie novel gene functions are of intermediate size and caused by homologous recombination between sites on sister chromatids resulting in tandem duplications of kilo- or mega-base regions [8,34]. Amplifications occur frequently, especially in microbes, but they are usually unstable and costly so can be lost quickly [35]. 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 [36–46] Yet, amplifications are not the only route to population expansion. Toll-Riera *et al.* [47], 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. Similar results have been observed for the recovery of glucose metabolism [48,49] and growth on novel substrates [50,51] in *E. coli* as well as biofilm formation in *P. fluorescens* [52–54]. The environmental context within which selection happens can also play a role: growth on a readily-used, native substrate together with

154 a novel resource, stressor, or toxin can support sufficiently large population sizes for long
155 enough to access rare beneficial mutations allowing improved growth in the novel condition
156 [15,51,55–57]. Indeed, this principle – ensuring population viability in the presence of a novel
157 substrate by supplementing the growth medium with a native substrate – is common practice
158 in microbiology, biochemistry, and bioengineering where the aim is to isolate novel metabolic
159 or toxin-degrading variants [58,59].
160
161 The final step involves divergence of genes or genetic interactions involved in the novel
162 function. Improvements to the novel function requiring multiple mutations can be built
163 because each mutation on its own confers a fitness advantage at every step. It has also been
164 suggested that multiple mutations accumulate through neutral processes for a time until some
165 final mutation 'discovers' a new phenotype and the whole lot – driver and neutral mutations
166 together – are driven to fixation by positive selection [60]. Microbial experiments, perhaps
167 unsurprisingly given how effective selection can be in large populations like those usually
168 studied in the laboratory, overwhelmingly come down on the side of selection as the driver of
169 divergence, although the genetic and ecological routes taken can be variable. Three examples
170 illustrate this point.
171
172 The first, by design, closely recapitulates the EAD model. Nasvall *et al*. [42] evolved populations
173 of *Salmonella enterica* containing a modified *hisA*, which codes for an enzyme required for
174 histidine synthesis as well as some rudimentary ability to synthesize tryptophan, on a plasmid
175 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 [15,61]. Meyer *et al*. [62] 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 [63], 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, do not see genetic variation as a major constraint on adaptive evolution over very long time scales, 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. Ecological opportunity, or vacant niche space, creates the conditions for innovation and novelty to spread, once they have evolved. The citrate added to minimal glucose medium, for example, is an untapped ecological opportunity for *E. coli* that, eventually, a lineage evolved to exploit. When ecological opportunities themselves generate strong selection for novelty, the rate at which a novel trait evolves and spreads can be very rapid, provided genetic variation is not limiting (as it rarely is in microbial experiments). Selection for access to oxygen, which becomes rapidly limiting in liquid culture but is abundant at the air-broth interface, leads to the emergence of biofilm-forming genotyopes in static (unshaken) microcosms of *Pseudomonas fluorescens* within tens of generations, for example [52,54,64]. Moreover, we found that the same founder strain of *Pseudomonas fluorescens*, 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 through mutations to *gntR*, a transcriptional regulator [55,65]. The literature is replete with similar examples [4,20]. Ecological interactions can also drive the rapid spread of novelty, as the co-evolution of bacteriophage λ with its *E. coli* host demonstrates [62,63,66]. Resource competition can also be important in acquiring novel bacteriophage hosts [67] or resources [68–70].

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 adaptation than others [71,72]. Local nucleotide context, repeats and homopolymer runs, and proximity to the replication terminus can be hotspots mutations in microbes [73,74] that could contribute

disproportionately to adaptation associated with innovation and novelty. 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 [75]. More generally, Bailey *et al*. [76] 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, at least in microbial selection experiments. It remains to be seen whether similar biases exist when selection for innovation and novelty occurs from standing genetic variation as well.

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 [15].

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 [47,52,77]. 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* [27]. 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 what appears to be all-or-none epistasis, where multiple mutations that are neutral on their own appear to become beneficial in the presence of another, critical mutation. It has been seen in bacteriophage λ experiments by Meyer *et al.* [62,63] and may also be occurring in other gain-of-function experiments. 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 [78]. It has been suggested that many proteins seem to be able to tolerate the introduction of mutations without severely compromising function [9], 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 [60]. 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 [79], 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 [80–83]. Competitors [69,70,83,84], parasites [85], and predators [86] 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 of existing genes and genetic sequences 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. If these regulatory changes also result in increased expression of downstream genetic regions, they could lead to the transcription and translation of non-coding sequences and so provide a substrate for the creation and selection of genes *de novo* [8]. 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.

Concluding Remarks

306 Taken together, it may be time to abandon the strict form of the EAD model. If so, it could be
307 replaced, provisionally, with one that recognizes the importance of genetic factors like
308 potentiation, alternative routes to increasing gene dosage beyond just gene amplification, and
309 integrates key elements of ecology, like ecological opportunity and ecological interactions, as
310 drivers of the evolution of novelty (see Fig. 1). A new acronym might help – call it the 'PEAD'
311 model, where 'P' here stands for potentiation, 'E' stands for exaptation, and 'A' represents
312 amplification of enzyme products either through conventional gene duplication or other
313 mechanisms that increase population size of the exapted lineage. We can leave the last 'D' for
314 divergence, but we need to be ready to expand on it dramatically and integrate ecology more
315 directly into our thinking about how novelty evolves. We will need to first answer a number of
316 key questions on the relative contribution of genetics and ecology in driving the emergence and
317 spread of novel traits (see Outstanding Questions). We will also need to consider seriously how
318 often, and under what conditions, novelty evolves through alternative mechanisms like *de novo*
319 selection from noncoding sequences. Doing so, I suspect, will take us a long way towards
320 understanding when and why novelty evolves, or not.
321

322 **Box 2 – Outstanding questions**

323 • To what extent is the evolution of novelty limited primarily by access to genetic
324 variation versus access to novel environmental conditions?

325 • What is the relative importance of internal genetic changes like gene
326 amplifications and rearrangements versus those coming externally through horizontal
327 gene transfer in driving the emergence of novel traits?

- What causes potentiation and how does it allow a lineage to gain 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?
- How often, and under what conditions, does novelty evolve through alternative mechanisms involving, for example, the *de novo* origins of genes from non-coding sequence?

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Literature cited

1 Jacob, F. (1977) Evolution and tinkering. *Science* 196, 1161–1166
2 Francino, M.P. (2005) An adaptive radiation model for the origin of new gene functions. *Nature Genetics* 37, 573–578
3 Bergthorsson, U. *et al.* (2007) Ohno's dilemma: Evolution of new genes under continuous selection. *Proceedings of the National Academy of Sciences* 104, 17004–17009
4 Kassen, R. (2014) *Experimental evolution and the nature of biodiversity*, Roberts and Company.
5 Ochman, H. *et al.* (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* 405, 299–304

6 Chen, S. *et al.* (2013) New genes as drivers of phenotypic evolution. *Nature Reviews Genetics* 14, 645–660
7 Hall, J.P.J. *et al.* (2017) Sampling the mobile gene pool: innovation via horizontal gene transfer in bacteria. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372, 20160424
8 Andersson, D.I. *et al.* (2015) Evolution of new functions de novo and from preexisting genes. *Cold Spring Harbor Perspectives in Biology* 7, a017996
9 Soskine, M. and Tawfik, D.S. (2010) Mutational effects and the evolution of new protein functions. *Nature Reviews Genetics* 11, 572–582
10 Wagner, G.P. and Lynch, V.J. (2010) Evolutionary novelties. *Current Biology* 20, R48–R52
11 Hallgrímsson, B. *et al.* (2012) The generation of variation and the developmental basis for evolutionary novelty: variation and developmental basis for evolutionary novelty. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 318, 501–517
12 Harms, M.J. and Thornton, J.W. (2013) Evolutionary biochemistry: revealing the historical and physical causes of protein properties. *Nature Reviews Genetics* 14, 559–571
13 Erwin, D.H. (2015) Novelty and innovation in the history of life. *Current Biology* 25, R930–R940
14 Schluter, D. (2000) *The ecology of adaptive radiation*, Oxford University Press.
15 Blount, Z.D. *et al.* (2008) Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proceedings of the National Academy of Sciences* 105, 7899–7906
16 Organisation for Economic Co-operation and Development and Statistical Office of the European Communities, eds. (2005) *Oslo manual: guidelines for collecting and interpreting innovation data*, 3rd ed.Organisation for Economic Co-operation and Development : Statistical Office of the European Communities.
17 Clarke, P.H. and Drew, R. (1988) An experiment in enzyme evolution studies withPseudomonas aeruginosa amidase. *Bioscience Reports* 8, 103–120
18 Heard, S.B. and Hauser, D.L. (1995) Key evolutionary innovations and their ecological mechanisms. *Historical Biology* 10, 151–173
19 Hunter, J.P. (1998) Key innovations and the ecology of macroevolution. *Trends in Ecology & Evolution* 13, 31–36
20 Copley, S.D. (2009) Evolution of efficient pathways for degradation of anthropogenic chemicals. *Nature Chemical Biology* 5, 559–566
21 Copley, S.D. (2014) An evolutionary perspective on protein moonlighting. *Biochemical Society Transactions* 42, 1684–1691
22 Khersonsky, O. *et al.* (2006) Enzyme promiscuity: evolutionary and mechanistic aspects. *Current Opinion in Chemical Biology* 10, 498–508
23 Lin, E.C.C. *et al.* (1976) Experimental models of acquisitive evolution. *BioScience* 26, 548–555
24 Hall, B.G. (1989) Selection, adaptation, and bacterial operons. *Genome* 31, 265–271
25 Mortlock, R.P. (1983) Experiments in evolution using microorganisms. *BioScience* 33, 308–313
26 Weinreich, D.M. (2006) Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* 312, 111–114

27 Melnyk, A.H. *et al.* (2017) Evolution of cost-free resistance under fluctuating drug selection in *Pseudomonas aeruginosa*. *mSphere* 2, e00158-17

28 Conant, G.C. and Wolfe, K.H. (2008) Turning a hobby into a job: How duplicated genes find new functions. *Nature Reviews Genetics* 9, 938–950

29 Zhang, J. (2003) Evolution by gene duplication: an update. *Trends in Ecology & Evolution* 18, 292–298

30 Carroll, S.B. (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134, 25–36

31 Lynch, M. and Conery, J.S. The evolutionary demography of duplicate genes. *J. Struct. Funct. Genomics* 3, 35–44

32 Andersson, D.I. and Hughes, D. (2009) Gene amplification and adaptive evolution in bacteria. *Annual Review of Genetics* 43, 167–195

33 Katju, V. and Bergthorsson, U. (2013) Copy-number changes in evolution: rates, fitness effects and adaptive significance. *Frontiers in Genetics* 4, 273

34 Reams, A.B. and Roth, J.R. (2015) Mechanisms of gene duplication and amplification. *Cold Spring Harbor Perspectives in Biology* 7, a016592

35 Taylor, J.S. and Raes, J. (2004) Duplication and divergence: the evolution of new genes and old ideas. *Annual Review of Genetics* 38, 615–643

36 Sonti, R.V. and Roth, J.R. (1989) Role of gene duplications in the adaptation of *Salmonella typhimurium* to growth on limiting carbon sources. *Genetics* 123, 19–28

37 Brown, C.J. *et al.* (1998) Multiple duplications of yeast hexose transport genes in response to selection in a glucose-limited environment. *Molecular Biology and Evolution* 15, 931–942

38 Kugelberg, E. *et al.* (2006) Multiple pathways of selected gene amplification during adaptive mutation. *Proceedings of the National Academy of Sciences* 103, 17319–17324

39 Devers, M. *et al.* (2008) Fitness drift of an atrazine-degrading population under atrazine selection pressure. *Environmental Microbiology* 10, 676–684

40 Sun, S. *et al.* (2009) Contribution of gene amplification to evolution of increased antibiotic resistance in *Salmonella typhimurium*. *Genetics* 182, 1183–1195

41 Pränting, M. and Andersson, D.I. (2011) Escape from growth restriction in small colony variants of Salmonella typhimurium by gene amplification and mutation. *Molecular Microbiology* 79, 305–315

42 Nasvall, J. *et al.* (2012) Real-time evolution of new genes by innovation, amplification, and divergence. *Science* 338, 384–387

43 Maharjan, R.P. *et al.* (2013) A case of adaptation through a mutation in a tandem duplication during experimental evolution in Escherichia coli. *BMC Genomics* 14, 441

44 Dhar, R. *et al.* (2014) Increased gene dosage plays a predominant role in the initial stages of evolution of duplicate tem-1 beta lactamase genes: experimental evolution of duplicate genes. *Evolution* 68, 1775–1791

45 Farslow, J.C. *et al.* (2015) Rapid Increase in frequency of gene copy-number variants during experimental evolution in *Caenorhabditis elegans*. *BMC Genomics* 16,

46 Elde, N.C. *et al.* (2012) Poxviruses deploy genomic accordions to adapt rapidly against host antiviral defenses. *Cell* 150, 831–841

47 Toll-Riera, M. *et al.* (2016) The genomic basis of evolutionary innovation in pseudomonas aeruginosa. *PLOS Genetics* 12, e1006005

48 Blank, D. *et al.* (2014) The predictability of molecular evolution during functional innovation. *PNAS* 111, 3044–3049
49 McLoughlin, S.Y. and Copley, S.D. (2008) A compromise required by gene sharing enables survival: Implications for evolution of new enzyme activities. *Proceedings of the National Academy of Sciences* 105, 13497–13502
50 Szappanos, B. *et al.* (2016) Adaptive evolution of complex innovations through stepwise metabolic niche expansion. *Nature Communications* 7,
51 Lee, D.-H. and Palsson, B.O. (2010) Adaptive evolution of escherichia coli k-12 mg1655 during growth on a nonnative carbon source, l-1,2-propanediol. *Applied and Environmental Microbiology* 76, 4158–4168
52 Bantinaki, E. *et al.* (2007) Adaptive divergence in experimental populations of *pseudomonas fluorescens* . Iii. Mutational origins of wrinkly spreader diversity. *Genetics* 176, 441–453
53 McDonald, M.J. *et al.* (2009) Adaptive Divergence in Experimental Populations of *Pseudomonas fluorescens* . IV. Genetic Constraints Guide Evolutionary Trajectories in a Parallel Adaptive Radiation. *Genetics* 183, 1041–1053
54 Lind, P.A. *et al.* (2015) Experimental evolution reveals hidden diversity in evolutionary pathways. *eLife* 4,
55 Bailey, S.F. and Kassen, R. (2012) Spatial structure of ecological opportunity drives adaptation in a bacterium. *The American Naturalist* 180, 270–283
56 Spencer, C.C. *et al.* (2007) Adaptive diversification in genes that regulate resource use in *Escherichia coli*. *PLoS Genetics* 3, e15
57 Zhang, Q. *et al.* (2011) Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* 333, 1764–1767
58 Hegeman, G.D. and Rosenberg, S.L. (1970) The evolution of bacterial Eenzyme systems. *Annual Review of Microbiology* 24, 429–462
59 Winkler, J.D. and Kao, K.C. (2014) Recent advances in the evolutionary engineering of industrial biocatalysts. *Genomics* 104, 406–411
60 Wagner, A. (2008) Neutralism and selectionism: a network-based reconciliation. *Nature Reviews Genetics* 9, 965–974
61 Quandt, E.M. *et al.* (2015) Fine-tuning citrate synthase flux potentiates and refines metabolic innovation in the Lenski evolution experiment. *eLife* 4,
62 Meyer, J.R. *et al.* (2012) Repeatability and contingency in the evolution of a key innovation in phage lambda. *Science* 335, 428–432
63 Burmeister, A.R. *et al.* (2016) Host coevolution alters the adaptive landscape of a virus. *Proceedings of the Royal Society B: Biological Sciences* 283, 20161528
64 Rainey, P.B. and Travisano, M. (1998) Adaptive radiation in a heterogeneous environment. *Nature* 394, 69
65 Bailey, S.F. *et al.* (2015) The Effect of Selection Environment on the Probability of Parallel Evolution. *Molecular Biology and Evolution* 32, 1436–1448
66 Hall, A.R. *et al.* (2011) Bacteria-phage coevolution and the emergence of generalist pathogens. *The American Naturalist* 177, 44–53
67 Bono, L.M. *et al.* (2012) Competition and the origins of novelty: experimental evolution of niche-width expansion in a virus. *Biology Letters* 9, 20120616–20120616

68 Tyerman, J.G. *et al.* (2008) Experimental demonstration of ecological character displacement. *BMC Evolutionary Biology* 8, 34
69 Zhang, Q.-G. *et al.* (2012) The effect of a competitor on a model adaptive radiation. *Evolution* 66, 1985–1990
70 Bailey, S.F. *et al.* (2013) Competition both drives and impedes diversification in a model adaptive radiation. *Proceedings of the Royal Society B: Biological Sciences* 280, 20131253–20131253
71 Stoltzfus, A. and Yampolsky, L.Y. (2009) Climbing mount probable: mutation as a cause of nonrandomness in evolution. *Journal of Heredity* 100, 637–647
72 Blount, Z.D. (2016) A case study in evolutionary contingency. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences* 58, 82–92
73 Lee, H. *et al.* (2012) Rate and molecular spectrum of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing. *Proceedings of the National Academy of Sciences* 109, E2774–E2783
74 Dettman, J.R. *et al.* (2016) The properties of spontaneous mutations in the opportunistic pathogen *Pseudomonas aeruginosa*. *BMC Genomics* 17,
75 Wong, A. *et al.* (2012) Genomics of adaptation during experimental evolution of the opportunistic pathogen *Pseudomonas aeruginosa*. *PLoS Genetics* 8, e1002928
76 Bailey, S.F. *et al.* (2017) What drives parallel evolution?: How population size and mutational variation contribute to repeated evolution. *BioEssays* 39, e201600176
77 Schick, A. *et al.* (2015) Evolution of fitness trade-offs in locally adapted populations of *pseudomonas fluorescens*. *The American Naturalist* 186, S48–S59
78 Herfst, S. *et al.* (2012) Airborne transmission of Influenza A/H5N1 virus between ferrets. *Science* 336, 1534–1541
79 Sanjuán, R. *et al.* (2010) Viral mutation rates. *Journal of Virology* 84, 9733–9748
80 Holt, R.D. and Gomulkiewicz, R. (1997) How does immigration influence local adaptation? A reexamination of a familiar paradigm. *American Naturalist* 149, 563–572
81 Jasmin, J.-N. and Kassen, R. (2007) On the experimental evolution of specialization and diversity in heterogeneous environments. *Ecology Letters* 10, 272–281
82 Hall, A.R. and Colegrave, N. (2007) How does resource supply affect evolutionary diversification? *Proceedings of the Royal Society B: Biological Sciences* 274, 73–78
83 Leon, D. *et al.* (2018) Innovation in an E. coli evolution experiment is contingent on maintaining adaptive potential until competition subsides. *PLOS Genetics* 14, e1007348
84 Fukami, T. *et al.* (2007) Immigration history controls diversification in experimental adaptive radiation. *Nature* 446, 436–439
85 Buckling, A. and Rainey, P.B. (2002) The role of parasites in sympatric and allopatric host diversification. *Nature* 420, 496–499
86 Meyer, J.R. and Kassen, R. (2007) The effects of competition and predation on diversification in a model adaptive radiation. *Nature* 446, 432–435
87 Blount, Z.D. *et al.* (2012) Genomic analysis of a key innovation in an experimental Escherichia coli population. *Nature* 489, 513–518

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531 **Table 1.** Microbial selection experiments on the evolution of innovation and novelty.

Organism	Ecological novelty	Genetic mechanism	Generations	Comments	Citation
Salmonella typhymurium	Growth-limiting carbon sources	Amplification of genes associated with carbon source transport	180	Selection on each of arabinose, malate, and sorbitol leads to duplication of chromosomal regions containing permease genes	[36]
	Cephalosporin resistance	Amplification of *bla*-TEM1 followed by second site point mutations	Not reported	Point mutations, which occurred only in strains with strains with amplified *bla*-TEM1, confer resistance by reducing porin expression	[40]
	Growth recovery from a costly mutation in *hemC*	Amplification of *hemC* followed by point mutations in amplified copies	Not reported	Non-mutated *hemC* copies were eventually lost, leaving only the mutated versions	[41]
Salmonella enterica	Tryptophan synthesis in medium lacking tryptophan and histidine	Amplification and subsequent point mutations in *hisA*	3000	Ancestral strain lacks a key gene (*trpF*) for tryptophan biosynthesis	[42]
	Lactose limitation	Amplification via tandem duplication of *lac*	Not reported	*lac* is located on F'_{128} plasmid	[38]
Escherichia coli	Limited glucose	Amplification via tandem duplication and subsequent deletion of *orgK yegSR* from one duplicated copy	100	Genetic target of selection unclear	[43]
	Cefotaxime, a novel antibiotic	Amplification of *bla*-TEM1	80	A designed experiment where	[44]

				duplications were present at the beginning and their evolution tracked	
	Growth on L-1,2-propanediol	IS5 insertion leading to constitutive activation of *fucAO* operon	700	Glycerol added to growth medium as the ancestral strain cannot grow on L-1,2-propanediol alone	[51]
	Growth on glucose of a Δ*argC* strain	Structural and promoter mutations to *proAB*	Not reported	Mutations restore some arginine biosynthesis capacity at the cost of proline biosynthesis	[49]
	Limiting glucose in the presence of excess citrate	Duplication and rearrangement of *citT* downstream of aerobically active promoter *rnk*	35,000	Potentiation involved specialization on acetate, a by-product of glucose metabolism, in part via mutations in a gene (*gltA*) that codes for citrate synthase	[15,61,83,87]
	Rapid switching from glucose to acetate metabolism	Transposon-mediated mutation causing constitutive expression if *aceB* (malate synthase) allowing acetate metabolism	1000	Resource competition drives the evolution of the rapid switcher and helps support diversity	[56,68]
	Metabolism of propylene glycol (PG) and ethylene glycol (EG)	Overexpression of *fucO* allows growth on PG; *fucO* overexpression and amplification of *aldA* required for growth on EG	Not reported	Stewpise acquisition of metabolic activities, with metabolism of PG preceding that of EG.	[50]

	Growth on minimal glucose medium	1-6 structural or regulatory mutations most common, amplifications were just 4% of all genomic changes	145	Starting strains each had one of 87 genes knocked out that prevented growth on minimal glucose; 22/87 strains showed evidence of recovery	[48]
***Pseudomonas* sp. ADP**	Atrazine as a sole source of nitrogen	Amplification via tandem duplication of *atzB*	320	*atzB*, a gene involved in atrazine degradation, is located on a low-copy number, stable plasmid (pADP1)	[39]
Pseudomonas aeruginosa	Novel carbon sources	Mutations to regulatory genes associated with metabolism predominate; *de novo* gene duplication rare	140	Carbon sources are those found on commercially available Biolog plates	[47]
	Fluorquinolone (ciprofloxacin) resistance	Exaptation resulting from single mutations	80-100	Mutations are often loss-of-function to efflux pump regulators (*nfxB*) or protein conformation changes to DNA gyrases (*gyrA, gyrB*) or topisomerases (*parC, parE*)	[27,75]
Pseudomonas fluorescens	Biofilm formation at the air-broth interface	Loss-of-function mutation, usually in *wspR*, resulting in constitutive expression of *wss* operon	50	Resource competition, especially for oxygen, promotes the evolution of biofilm-forming genotypes	[52,54,64]
	Growth on xylose	Unclear but mutations in transcriptional regulator	100-200	Ancestral strain grows very poorly on xylose	[55,65]

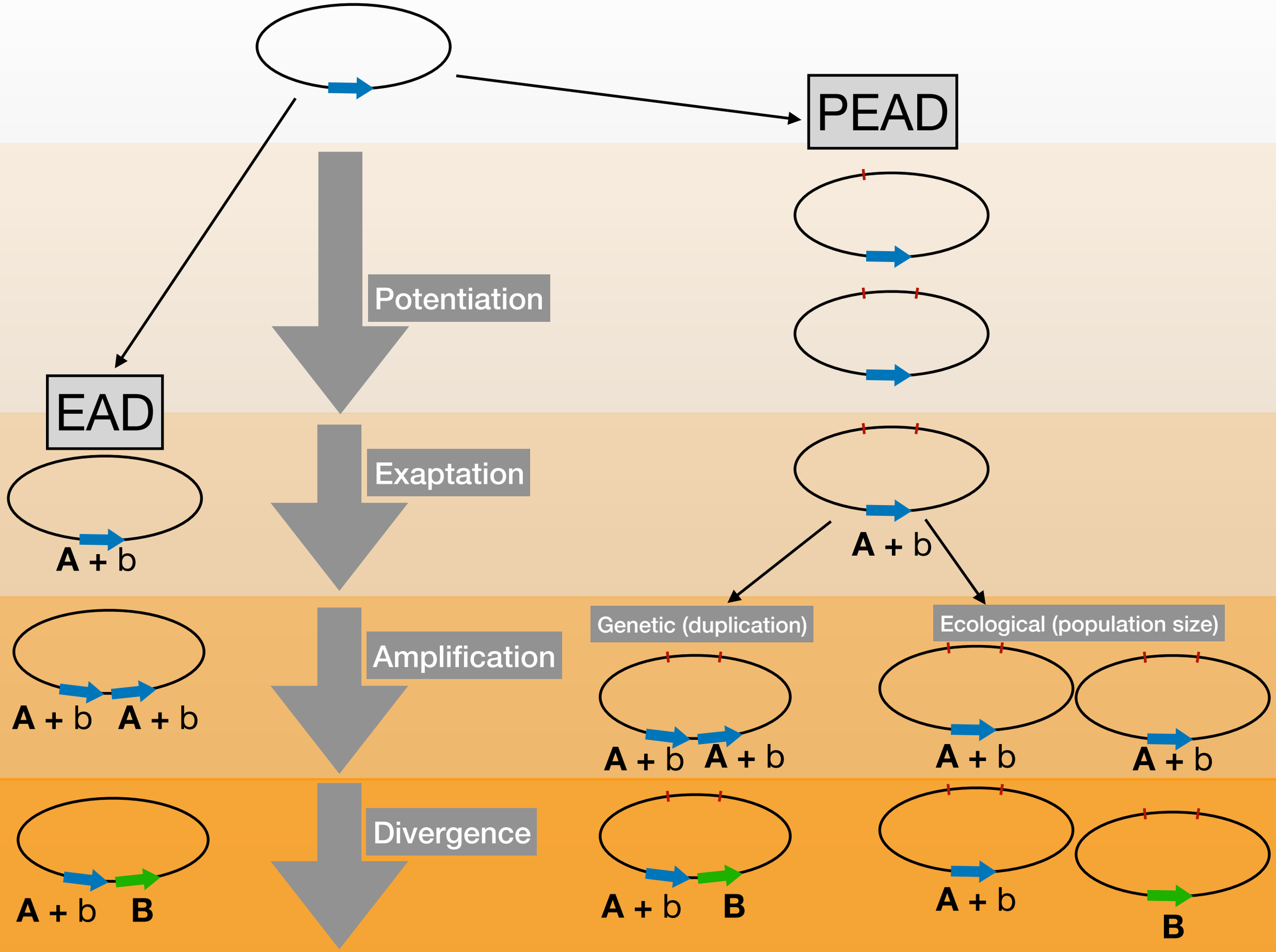
		(*gntR*) likely responsible		because it lacks *xylB*	
Saccharomyces cerevisiae	Glucose limitation	Amplification via tandem duplication leading to chimeric *HTX7/6*	450	*HTX7/6*, a hexose transport chimera, is comprised of the upstream promoter of *HTX7* and coding sequence of *HTX6* presumed to result from unequal crossing over	[37]
Caenorhabditis elegans	Growth recovery in a strain containing a costly mutation	Amplification via duplication	200	Genomic targets of selection unclear but duplications were often highly parallel across independently evolved lines, implying shared sites under strong selection	[45]
Bacteriophage λ	Infection of *E. coli* host via novel receptor	Coevolution causes multiple mutations in phage protein J required for entry via novel receptor	Not reported	Potentiating mutations initially improved fitness via native receptor, LamB	[62,63]
Bacteriophage SBW25ϕ2	Infection of novel *P. fluorescens* variants	Coevolution leading to multiple mutations in phage genes associated with infection	Not reported	Infection of novel hosts evolves only through coevolution with bacteria	[66]
Bacteriophage ϕ6	Infection of novel *Pseudomonas* spp.	Not reported but previous work suggests single point mutations required for infection of each novel host	168-254	Strong competition among phage for access to hosts promotes emergence of novel host range mutants	[67]
Influenza A/H5N1	Airborne transmission	5-9 mutations required	10 serial passages in ferrets	Ancestral strain for the serial passage	[78]

				experiments had three mutations introduced, two in HA and one in PB2; remaining mutations accumulated during serial passage	
Poxvirus	Ability to infect human cells (HeLa) in the absence of host range gene E3L	Amplification of related host range gene, K3L	10 passages	Amplifications faciltate rapid evolution in spite of low genome-wide mutation rates	[46]

Figure legend

Figure 1. Schematic illustration of the differences between the standard (exaptation-amplification-diversification; EAD) and revised (potentiation-exaptation-amplification-diversification; PEAD) models for the evolution of innovation and novelty. Each oval represents the genome of an individual. Under the EAD model (left), the exaptation is afforded because a gene (blue arrow) that produces a primary product with function A can also perform side activity b that has become important in a new environment. Amplification occurs through gene duplication, leading to increased production of b, and divergence occurs due to positive

545 selection for improved B. The PEAD model (right) differs because of an additional potentiation
546 step preceding exaptation, where mutations accumulate elsewhere in the genome (red marks)
547 that allow the side function of the focal gene (b) to become important. Amplification can then
548 proceed as it does in the EAD model, through gene duplication, or through other mechanisms
549 allowing population expansion without gene duplication. Divergence proceeds as before,
550 through positive selection for improved B.


PEAD
EAD
Potentiation
Exaptation
Amplification
Divergence
A + b
A + b
A + b
A + b
Genetic (duplication)
A + b
A + b
Ecological (population size)
A + b
A + b
A + b
B
A + b
B