Origins and evolution of biological novelty

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ABSTRACT Understanding the origins and impacts of novel traits has been a perennial interest in many realms of ecology and evolutionary biology. Here, we build on previous evolutionary and philosophical treatments of the subject to encompass novelties across biological scales and eco-evolutionary perspectives. By defining novelties as new features at one biological scale that have emergent effects at other biological scales, we incorporate many forms of novelty that have previously been treated in isolation (such as novelty from genetic mutations, new developmental pathways, new morphological features, and new species). Our perspective is based on the fundamental idea that the emergence of a novelty, at any biological scale, is shaped by its environmental and genetic context. Through this lens, we outline a broad array of generative mechanisms underlying novelty and highlight how genomic tools are transforming our understanding of the origins of novelty. Lastly, we present several case studies to illustrate how novelties across biological scales and systems can be understood based on common mechanisms of change and their environmental and genetic contexts. Specifically, we highlight how gene duplication contributes to the evolution of new complex structures in the eye; how genetic exchange in symbiosis alters functions of both host and symbiont, resulting in a novel organism; and how hybridization between species can generate new species with new niches.

KEYWORDS evolution, gene duplication, gene loss, gene transfer, hybridization, innovation, introgression, mutation, novelty, symbiosis

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I. INTRODUCTION

At the heart of evolutionary biology is heritable variation. Heritable variation is often described as the raw material on which natural selection can act in ways that change organismal form and function over generations. The attention of biologists and the public alike has been drawn to the appearance and evolutionary success of novel characteristics, like new body plans seen during the Cambrian explosion (Chen 2009), the evolution of limbs from fins that is associated with a major life transition to land (Shubin *et al.* 2006), and the formation of mitochondria and chloroplasts through endosymbiosis (Margulis *et al.* 1991). Ultimately, biological novelty originates by mutations within single individuals—through single nucleotide changes in a gene, the duplication or deletion of genes or segments of chromosomes, or the duplication of whole genomes. These molecular changes precede the emergence of new alleles, new genes or protein functions, and new networks of gene interactions or the loss of a gene or its protein products (Hughes 1994; Long 2001; Krylov *et al.* 2003; Ratcliff *et al.* 2015).

Beyond their ecological and evolutionary significance, biological novelties have human health and economic impacts. For example, small mutations can increase viral spread to new tissues and species (Baranowski *et al.* 2003), mobile genetic elements enable pathogenic bacteria to resist antibiotics (Andersson and Hughes 2010; Stalder *et al.* 2017), and the establishment of a symbiotic association between legumes with rhizobia provides important agricultural ecosystem services for billions of people across the planet (Foyer *et al.* 2016). Unraveling the causes and consequences of biological novelty has broad real-world implications; however, this important task is complicated by the many discordant ways that novelty has been defined and the disconnect between sub-disciplines in the biological scale at which they study novelty.

We seek to bridge this divided literature and progress our understanding of biological novelty through a focus on common mechanisms. First, we discuss the contrasting ways that novelty has been defined in the literature. Second, we demonstrate how a diverse array of novelties can be understood through fundamental mechanisms of genetic and phenotypic change. This focus on common mechanisms enables prediction (e.g., of the spread of antibiotic resistance or lineage diversification) and helps unite research across biological scales by clarifying the connections between literature that explores mutations and novel proteins, for example, with literature investigating how those changes lead to novel functions, survival in new environments, or formation of new species. Finally, we develop several case studies that highlight how genomic data is advancing our understanding of novelty by unveiling the drivers underlying cascades of novelty in different evolutionary contexts.

(1) Defining novelty

(a) Previous perspectives

Unsurprisingly given the breadth of research on novelty, previous definitions of novel biological features vary dramatically in the scale and scope that constitutes novelty. For example, several published definitions of biological novelty suggest that new traits are those that arise from

differences in development (West-Eberhard 2003), enable a new function (Pigliucci 2008), permit an organism to climb a new adaptive peak on a fitness landscape (Hallgrímsson *et al.* 2012), and/or underlie adaptive radiation (Mayr 2013). Some authors indicate that "novelty" pertains to distinct structures (qualitative novelties; West-Eberhard 2003), rather than quantitative changes to existing structures (like variation in shape or size) or changes in the number of repeating units (as in the number of ray fins in fish; Müller and Wagner 1991). Müller and Wagner (1991) also indicate that novelty cannot originate from the loss of a gene or character. Many, however, disagree with the more restrictive definitions of novelty. For example, a focus on adaptive radiation omits numerous novel features that arose before the ecological opportunity for niche differentiation and radiation (Erwin 2015). Others posit that new combinations of existing traits are also novel (Pigliucci 2008), as are new phenotypes resulting from gene loss (see Ochman and Moran 2001) and quantitative changes to existing traits (e.g., as in hybrid offspring that are larger than either parent species; Dittrich-Reed and Fitzpatrick 2013). The most inclusive definition contends that any character or variation, no matter how small, can be a novelty (Arthur 2000).

Novelty is often described as traits that are non-homologous (i.e., are not similar to structures in ancestral lineages due to common descent). In this case, a trait can still be considered novel even if it has evolved in other lineages. Casquettes, which allow larval cave fish to adhere to surfaces, are novelties because of their absence in ancestral lineages, despite the presence of homologous structures in amphibians (Pottin *et al.* 2010; Hall and Kerney 2012). Conversely, a strict definition of novelties as non-homologous features would rule out tetrapod limbs (a classic example of novelty that enabled animal colonization of land), since homologous structures are present in earlier fossils (see Shubin *et al.* 2006; Hall and Kerney 2012). More generally, since 'new' structures are underlain by homologous tissues or gene expression, it remains challenging to pinpoint when novelty arises, at which biological scale, and how unique something needs to be in order to be considered novel when defining novelty as non-homology (discussed in Brigandt and Love 2012; Hall and Kerney 2012. As Brigandt and Love (2012) point out, focusing on the novelty of mechanisms (like new developmental pathways) rather than non-homologous traits does not resolve these challenges, for the same reasons. Moreover, there can be novelty (such as new combinations of traits) without new developmental pathways, for example (reviewed in Pigliucci 2008).

(b) Defining novelty through emergent impacts across biological scales

We define novelties as features at one biological scale that emerge through genetic, developmental, or environmental changes with effects across biological scales (Table 1). In our view, features that do not have impacts on other biological levels (e.g., mutations with no phenotypic effects, or new phenotypes with no effect on behaviours like hiding or mating) are natural variation, but not "biological novelties" of ecological and evolutionary interest. Our broad definition of novelty encompasses different scales, types, and origins: from small-scale novelties, larger-scale changes in organismal development, physiology, behaviour, and morphology can

emerge, with knock-on effects for the ecology and evolution of the organism. For example, in single-celled yeast (*Saccharomyces cerevisiae*) multicellularity can arise from a single mutation that disables a protein involved in cell separation after budding (Ratcliff *et al.* 2015). This transition to multicellularity changes the course of evolution for the yeast and adds a new level of selection, as larger, more spherical yeast clusters survive better than small, flat yeast clusters that remain suspended in solution (Ratcliff *et al.* 2015). As another example, in a group of legumes (*Medicago*), a slight alteration to a protein-coding sequence leads to a novel seed pod development and morphology, with likely impacts on plant fitness and seed dispersal strategy (Fourquin *et al.* 2013). Very famously, in cichlid fish, morphological novelty in their pharyngeal jaws (i.e., the second set of jaws in the throat) enabled dietary flexibility and ultimately specialization and adaptive radiation across a wide array of trophic roles and environments (Liem 1973). Although it may be counterintuitive to think of loss as novelty, well-known 'anomalous' creatures like large flightless birds and sight-less cave-dwelling fish owe their origins to losses of structures and functions (Jeffery 2005; Worthy *et al.* 2017; Clarke 2019). Lastly, while we focus on the emergent effects of novelties at higher biological scales, novelties could also impact lower biological scales (e.g., acquiring a new mobile genetic element by a host bacterium can lead to compensatory mutations on the bacterium's chromosome; Remigi *et al.* 2014; Hall *et al.* 2022). In these ways, understanding changes at one level of biological organization can provide insights into cascades of novelty at other scales (**Table 1**).

Our proposed definition includes many different types of novelty. For instance, new combinations of pre-existing traits, meristic changes, or the loss of particular traits generate new phenotypes and can be associated with the exploitation of new environments and formation of new species (e.g., Olson 1999; Rieseberg *et al.* 2003); hence, in our perspective, these mechanisms contribute to biological novelty. Some literature treats innovations (features that enable adaptive radiation into new environments) as distinct from novelties because of the macroevolutionary consequences of innovations and the important role that ecological opportunity plays (e.g., Erwin 2020). Here, we define all novelties as shaped by their environmental context, and having broader impacts across biological scales, so in our perspective, innovations are one extreme of a continuum of 'genetic changes and their emergent effects'. One persistent challenge in defining novelty is establishing coherent and predictive delimitation criteria (Brigandt and Love 2012). Martin and Wainwright (2013) tackled this challenge by gauging the novelty of a scale-eating behaviour in pupfish based on its frequency of occurrence in other clades. Another valuable approach may be to focus on quantifying how impactful a candidate novelty is instead. Generally, of the novel traits that arise in a population, those that are heritable when they arise should have the greatest evolutionary and ecological impact. Innovations are particularly impactful forms of novelty because of their far-reaching effects across biological scales (as in the cichlid adaptive radiation discussed above, for example). In contrast, other novelties have more local effects. For instance, the novel toxin resistance of some garter snake populations is important in the local coevolution with their toxic prey (McGlothlin *et al.* 2014), but broader impacts (e.g., in trophic

cascades or speciation events) are unknown. We believe that by focusing on the emergent effects of novelties, and the common mechanisms that underlie them, we will gain a bigger picture of the origins of novelty that cross-cuts biological scales and sub-disciplines.

The definition we propose includes not only adaptive novelty that evolves through natural selection, but also novelty brought about by processes that do not necessarily increase organismal fitness—that is, the forces of mutation, recombination, drift, and the relaxation of selection. For example, drift has been important in the evolution of complex, multicellular organisms (Lynch 2007). If different genetic architectures (e.g., modular vs integrated gene networks) produce the same phenotype, variation in developmental systems may be neutral, and novel features like genetic modularity can emerge, hidden from selection, in certain lineages through drift (Lynch 2007). In the context of metabolism, adaptation to one carbon source (like glucose) 'pre-adapts' organisms to metabolize a wide range of other carbon sources, setting the stage for novelties to emerge as selective environments change (Barve and Wagner 2013). Put another way, a pre-existing neutral mutation could have a new consequence when placed in a different genetic or environmental context, and therefore become a novelty. It follows that our definition of novelty is not restricted to new traits involved in adaptive radiations, since novelty can arise long before the ecological opportunity for diversification (Erwin 2015). To more fully understand biological novelty, we consider mechanisms of genetic change within the genomic and environmental context in which those changes occur.

(2) Mechanisms of novelty and their genetic and environmental context

The scale of genetic change can range from genetic mutations with small phenotypic effects through to genome duplications or acquisition of mobile genetic elements (MGEs) or symbionts that produce dramatic changes in organismal form or function (**Table 1**). For instance, novelty can arise from mutations to structural or regulatory genes (see Hoekstra and Coyne 2007), including mutations resulting in loss of function (Olson 1999). Gene loss, or loss of functions, can create new, ecologically impactful phenotypes. For example, loss of a particular gene in *Shigella* led to its virulence and ability to cause dysentery (Nakata *et al.* 1993). New genes, gene combinations, and protein functions can also be produced through recombination between non-homologous genes, gene duplication and divergence, and gene transfer between unrelated genomes through horizontal gene transfer or hybridization (Long 2001). Whole genome duplications, evident in the ancestors of many fish, amphibian, and flowering plant lineages, can yield new, reproductively isolated species with novel traits and distinct habitats (Van de Peer *et al.* 2009). More subtly, the enhanced genetic variation can permit new phenotypes, divergent patterns of gene loss among populations, and changes in function of gene copies (Van de Peer *et al.* 2009). Importantly, the ability of any of these kinds of genetic change to generate a novel phenotype is shaped by genetic constraints and interactions, and the ecological opportunities and selective forces imposed by the environment.

Table 1 Examples of novelties across biological scales highlighting their impacts on higher levels of biological organization.

Scale of feature	Feature	Examples	Emergent effect(s)
Molecular	SNPs (Single Nucleotide Polymorphisms)	Single mutation in yeast in ACE2 region	Reduced cell fission and gave rise to yeast multicellularity (Ratcliff *et al.* 2015)
	Gene loss	Loss of a protease gene (*omp*T) in *Shigella*	Increased ability of Shigella to spread, causing dysentery in its host (Nakata *et al.* 1993)
	Gene duplication	Duplication of the green opsin in old world monkeys and apes	The duplicated copy evolved the ability to detect red light, leading to trichromatic vision in primates (Hunt *et al.* 1998; Dulai *et al.* 1999), which may influence food and mate selection (Dominy and Lucas 2001)
	Gene acquisition	Acquisition of integrative and conjugative genetic elements by *Mesorhizobium*	Enabled plant host nodulation, nitrogen fixation, and symbiosis maintenance (Haskett *et al.* 2016)
	Plasmid acquisition	Horizontal gene transfer of plasmids to bacteria	Gave bacteria the ability to metabolize novel compounds (Dennis 2005)
	Structural variation in gene regulatory networks	Shuffling of gene modules in non-coding regions that control expression	Novel wing coloration patterns in butterflies (Wallbank *et al.* 2016)
	Protein structure	Alteration in a protein-coding sequence	Modified protein structure that led to novel seed pod development in a legume (Fourquin *et al.* 2013)
Functional	Development	Development of a novel morph in the presence of a food source (e.g., shimp)	Changes in diet, morphology, and behavior in the spadefoot toad (Levis *et al.* 2018)
	Symbiont acquisition	Horizontal acquisition of fungal endophytes	Grasses became more drought tolerant (Afkhami *et al.* 2014)
	Phenology (timing)	Shift in predator phenology	Changes in prey size and predator-prey dynamics between salamanders and frogs (Jara *et al.* 2019)
	Behaviour	Tail vibration	Led to the development of the rattle (Allf *et al.* 2016)
		A switch from fish-eating to scale-feeding	Associated with decreases in body size (Martin and Wainwright 2013; Kolmann *et al.* 2018)
	Morphology	Casquettes that facilitate adherence to surfaces	Allowed larval cave fish to inhabit a novel habitat (Pottin *et al.* 2010)
		Pharyngeal jaws of cichlids	Changes in dietary flexibility, specialization, and ultimately, adaptive radiation (Liem 1973)

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Scale of feature	Feature	Examples	Emergent effect(s)
	Hybridization / introgression	Diploid hybrid speciation resulting from niche separation	Allowed sunflowers to occupy novel, extreme habitats (Rieseberg *et al.* 2003)
Macroevolutionary	Genome duplication	Whole genome duplication event in the laurel family	Associated with changes in flower morphology and speciation (Chen *et al.* 2020)
	Symbiont acquisition	Strains of *Wolbachia* acquired by mosquitos	Resulted in cytoplasmic incompatibility leading to reproductive isolation and speciation (Shoemaker *et al.* 1999; Zabalou *et al.* 2004)

The broader impacts of a mutation depend on gene expression, regulation, the genetic networks in which the mutation is entangled, and how the environment modulates the selective regime (West-Eberhard 2003; Laubichler 2009; Visser *et al.* 2010). Plastic changes in behaviour—particularly behaviours that influence fitness components like survival, growth, or reproduction—can pave the way for morphological novelty (Zuk *et al.* 2014). Allf *et al.* (2016) suggest that the behaviour of snakes vibrating their tails when threatened could have paved the way for the evolution of the rattlesnake's rattle through the direct impact of the behaviour on tail morphology, or by changing the selective regime (i.e., rattles are only effective when coupled with tail vibrating behaviour). In spadefoot toads (*Spea*), plastic responses to food availability are thought to have led to the fixation of a novel carnivorous phenotype in certain lineages (Levis *et al.* 2018). Spadefoot toads are typically omnivorous, but populations that are fed shrimp develop a carnivorous morph that matures more quickly, is more active, is larger, and has modified mouthparts compared to the omnivore (Levis *et al.* 2018). These changes allowed carnivorous toads to colonize a new habitat: drying ponds with abundant shrimp and tadpole prey. In many cases, the interplay between morphology, physiology, and behaviour makes it difficult to discern which change first precipitated novelty (Galis 2001): much as new behaviours can influence the evolution of morphological traits, new structures can enable new behaviours (e.g., cranial novelty is associated with the evolution of frugivory in bats, Dumont *et al.* 2012).

An organism's environment determines the ecological impact and evolutionary fate of a novelty. Novelties may arise at one time point but only really increase in frequency or fuel diversification given the appropriate ecological opportunity (Erwin 2015). For example, the adaptive radiation of cichlid fishes with novel jaw morphologies into different ecological zones was made possible by the wide range of foods and environments available (Liem 1973). Additionally, rich environments

can render certain biosynthetic pathways unnecessary, leading to new phenotypes that lack these functions. This phenomenon is perhaps best characterized in parasitic and symbiotic systems, where gene loss (relative to ancestral free-living lineages) is common (Ochman and Moran 2001). The availability of host resources leads to gene loss in the symbiont or parasite as selection favours symbionts and parasites that conserve their own resources (metabolic complementarity; Morris *et al.* 2012), or as external resource availability relaxes selection to produce that resource, and genes are lost as mutations accumulate (Visser *et al.* 2010). For example, certain parasitic wasps have lost their ability to synthesize lipids as a result of their parasitic lifestyle (Visser *et al.* 2010).

Interweaving genetic, developmental, and environmental components of novelty should be a productive way to investigate the causal mechanisms and emergent ecological and evolutionary properties of novelties. The development of diseases like irritable bowel syndrome, for example, depends on mutations, gene interactions (epistasis), environmental risk factors, and gene-environment interactions (Ahmed 2006). Thus, in this paper, we take a holistic approach that seeks to bridge evolutionary development and genetic perspectives on novelty (e.g., Hoekstra and Coyne 2007; Laubichler 2009).

In the remainder of our synthesis, we present three case studies that provide insights into the origins of biological novelty across scales and systems and the importance of genomic data for understanding novelty. First, we describe gene duplication and the resolution of antagonistic pleiotropy as pathways to novelty in the evolution of complex structures. We use opsin duplication in the evolution of the complex vertebrate eye as an illustrative example. Second, we dissect how coevolutionary dynamics and genetic exchange among eukaryotic hosts and their prokaryotic symbionts generate novelty. We outline the new features that arise from a common plant-bacterial mutualism. Third, we discuss how hybridization within several plant and animal clades provides the genetic variation and novel features to form new species that inhabit new environments. Taken together, these case studies demonstrate how genetic modifications within species, and genetic exchange among species, generate novelty in microbial, animal, and plant systems.

II. CASE STUDIES: MECHANISMS UNDERLYING NOVELTY ACROSS BIOLOGICAL SCALES AND SYSTEMS

(1) Generating novel complex structures through gene duplication

An overarching model for the evolution of complexity is one in which simple structural units are duplicated and assembled into something larger, with properties that are greater than the simple sum of the individual units. Think of a house built using bricks. Each house is formed by the repeated duplication and assembly of bricks, yet the structure and complexity of different houses can vary tremendously depending on how the bricks are laid. In biology, each individual unit (the genes, which are our building blocks), once duplicated, can differentiate from the ancestral properties by mutation, resulting in an immense number of possible structures that can be built from the individual parts. When genes are duplicated, new opportunities for selection are created, and this new material may meet several different fates. Duplicated genes—new bricks to use—are typically functionally redundant and experience relaxed selection, but come ready-made with the function(s) of their parent. This relaxed selection introduces a unique opportunity for the gene copy to accumulate new mutations that allow it to perform a novel function distinct from its parents (**neofunctionalization**). Alternatively, duplicated copies (**paralogs**) can specialize on one particular function of a pleiotropic parent (in a process called **subfunctionalization**), or may be superfluous to organismal fitness, degenerate by mutation, and disappear (**pseudogenization**) (Ohno 1970; Zhang 2003; Roth *et al.* 2007). Understanding the ecological and evolutionary context in which the birth and death of genes occurs is critical in exploring the molecular basis of novelty.

Many dramatic examples of biological complexity owe their success to gene duplication (for an interesting exception in jellyfish, see Gold *et al.* 2018). Immune response (Peatman and Liu 2007; Sackton *et al.* 2017), morphological complexity (Lemons and McGinnis 2006; Galis and Metz 2007; Soshnikova *et al.* 2013), and sensory adaptations such as smell (Hughes *et al.* 2018) or vision (Collin *et al.* 2003; Bowmaker 2008; Feuda *et al.* 2012) are the culmination of successive rounds of single gene and whole genome duplication (thereby creating a gene family that distributes multiple functions across members). Even a single additional copy of a particular gene can result in the evolution of entirely new traits (e.g., key innovations such as the electric organ in electric fishes), allowing for explosive diversification of organisms into new environments (Arnegard *et al.* 2010). The diverse repertoire of building materials generated by gene duplication (and sometimes gene loss) paves the way for novelty and complexity to emerge as a response to ecological and

evolutionary demands.

(a) Gene duplication spurs novelty by resolving antagonistic pleiotropy

To fully grasp the complexity inherent in building an organism by the duplication and assembly of individual structural elements—namely genes and the products of genes—it is helpful to begin with the first gene. The first gene was likely pleiotropic (i.e., a single gene would have needed to perform several essential functions like replication, proofreading, and metabolism).

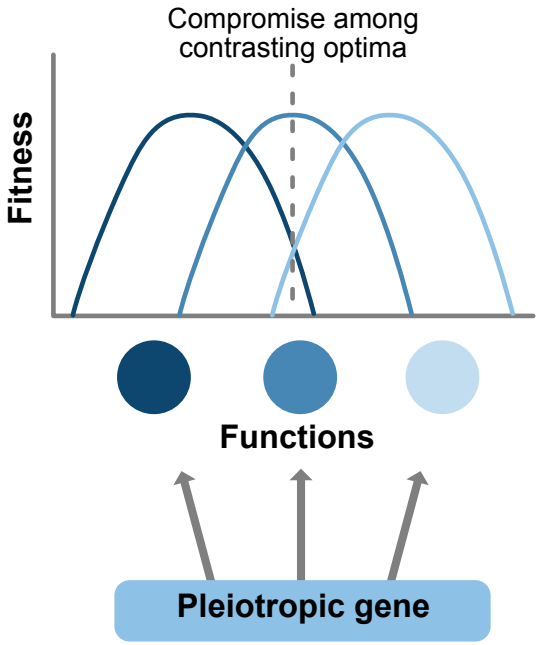

Figure 1 Antagonistic Pleiotropy in a multi-functional gene. When a gene performs multiple essential functions with different optima, it cannot tune the performance of each function. Gene duplication may resolve this antagonistic pleiotropy and allow a gene copy to specialize on maximizing one function.

The generation of novelty often stems from evolutionary and biochemical tradeoffs. While there is an economy to nature when there is pleiotropy, there is a fitness cost of pleiotropy that reflects the adage "a jack of all trades is a master of none." It is generally true that there is an optimum for any function. For a gene with multiple functions, it is likely that the optimum of one function is not the optimum for another function (**Fig. 1**; Bochdanovits and de Jong 2004; Maklakov *et al.* 2017). Functional trade-offs imply that as the number of functions a particular gene performs increases, the efficiency or effectiveness of any particular function declines. Given

performance trade-offs, pleiotropy is often antagonistic with respect to fitness (**Fig. 1**), and evolution should lead to a compromise that maximizes individual fitness by balancing different gene functions. Gene duplication can resolve this issue, freeing up gene copies to respond independently to selection (e.g., Des Marais and Rausher 2008). However, duplicated genes may also be lost to afford greater flexibility to the organism, highlighting that complexity can incur fitness costs. Elaborate organs or structures can be energetically demanding (Niven and Laughlin 2008; Moran *et al.* 2015), and multivariate genetic constraints may hamper the rate of adaptation (Orr 2010; Welch 2003). Perhaps counter-intuitively, gene losses can therefore also underlie novelty, just as removing a brick from a wall creates a window. For example, extensive gene loss likely facilitated adaptation to novel lifestyles in mammals: evolutionary transitions to aquatic and subterranean living, as well as flying, have resulted in the loss of dozens of genes involved in various metabolic, physiological, and morphological processes (Partha *et al.* 2017; Sharma *et al.* 2019; Huelsmann *et al.* 2019; Pyott *et al.* 2020).

(b) Opsin duplication and evolution of complexity in the visual system

The animal eye is a canonical example of the evolution of a novel complex structure (Nilsson 2013; Oakley and Speiser 2015). How can an organ that began as a simple cluster of light-detecting cells evolve to perform so many new, sophisticated functions and features, such as detecting light direction, color, movement, and producing images? Moreover, how can a sensory structure that is so essential to organismal fitness exhibit so much variation throughout the animal kingdom? The eye is the culmination of several novel tissues and structures that arose through duplication of a vast array of different genetic building blocks (e.g., Shimeld *et al.* 2005; Lagman *et al.* 2016; Lamb *et al.* 2016; McCulloch and Koenig 2020). Opsin genes code for the light-sensing proteins responsible for initiating the visual transduction cascade, which converts light into a neural signal. Molecular evolutionary and functional studies of these proteins have provided an immense contribution to our understanding of how gene duplication drives complexity, as early opsins faced a long list of ecological demands. Important information contained within light, such as spectral content, the timing of its availability, scattering, refraction, and simply the amount of light available for vision differ dramatically between terrestrial and aquatic environments and within those habitats (Warrant and Johnsen 2013). Opsin duplication therefore allowed for a division of labour, since tuning a single protein to detect these various properties would be biochemically impossible. Opsins are also a uniquely powerful system for exploring

the molecular basis of novelty, and the ecological contexts in which novelty emerges due to their ability to be readily examined via genomics, transcriptomics, and functional experiments (Hauser and Chang 2017). Exploring how duplication and diversification of the opsin gene family underlie major breakthroughs in animal vision has allowed us to understand the ecological and evolutionary forces driving complexity.

The evolutionary flexibility afforded by visual opsin duplication has optimized the eye for high performance vision while also allowing this organ to become specialized for impressively diverse and complex functions (Oakley and Speiser 2015). Duplication of visual opsins allowed some copies to specialize on detecting particular wavelengths of light (i.e., colours) at the expense of enhanced sensitivity, while other copies could instead optimize capturing miniscule levels of light (e.g., at night, or in the deep sea) at the expense of colour resolution. Visual opsin duplication precipitated a major breakthrough in animal evolution: colour vision. The ancestral vertebrate likely evolved in shallow-water habitats containing a broad spectrum of available light for colour vision. Accordingly, it possessed four different cone opsins encompassing sensitivity from the ultraviolet to red regions of the spectrum (Bowmaker 2008; Collin *et al.* 2003). From here, the opsin repertoire shrinks and expands extensively throughout vertebrate evolution in response to different selective pressures (Bowmaker 2008). Often, only one or two key duplications are sufficient to yield new visual system properties such as enhanced colour discrimination. For instance, the transition from dichromatic to trichromatic vision (i.e., colour vision mediated by three receptors) in old world monkeys and apes was made possible by the the duplication of a single green opsin gene followed by neofunctionalization (resulting in a red sensitive opsin) (Hunt *et al.* 1998; Dulai *et al.* 1999). This novel sensory trait is likely advantageous for detecting fruit among green foliage, and may have contributed to subsequent speciation and the transition to novel diets and habitats (Dominy and Lucas 2001; Carvalho *et al.* 2017; Dominy and Melin 2020). Certain selective environments in combination with genomic context (e.g., rounds of genome duplication in fishes) may also precipitate an explosion of opsin duplicates. For example, deep-sea spinyfin fishes possess a remarkable 38 dim light-detecting rod opsins. Each of these duplicates absorbs a slightly different wavelength of light, which may enhance detection of miniscule levels of sunlight penetrating the deep sea, and may also tune the visual system to the deep sea's bioluminescence spectrum (Musilova *et al.* 2019).

While this case study is centered on opsin genes, visual system adaptation cannot be accomplished solely through opsin duplication; rather, increasing complexity at several levels of

biological organization (duplications of genes, cells, tissues, structures, etc) works in tandem to accomplish various visual tasks and produce novel adaptations (**Fig. 2B,C**). Indeed, in animal groups with a wide variety of optical designs, genes involved in eye development and phototransduction are particularly likely to duplicate (Rivera *et al.* 2010). Following the evolution of opsins optimized for colour sensitivity, dim-light specializing opsins that are highly sensitive to light appeared. This opsin duplication combined with the duplication of the genetic machinery of the phototransduction cascade (i.e., duplication of a network of genes) enabled a substantial increase in vertebrate visual system complexity, as these interaction networks could be optimally tuned in dim light specializing photoreceptors (rods) and colour detecting photoreceptors (cones) respectively (Lagman *et al.* 2015, 2016; Lamb *et al.* 2016) (**Fig. 2B**). Duplex vision, the ability to sense low light levels as well as colour, resolved the trade-off between visual acuity and sensitivity and resulted in two physiological trajectories for photoreceptor cells in the retina: a subset that specialize in colour detection (cones) and a subset that specialize in low-light vision (rods; (Plachetzki and Oakley 2007; Hisatomi and Tokunaga 2002) (**Fig. 2C**). Occasionally, gene duplications and the corresponding complexity at higher biological levels can be extreme: the mantis shrimp retina has 33 different opsin transcripts (Porter *et al.* 2020), 16 photoreceptive structures in their retina, and numerous novel combinations of opsin expression within each photoreceptor (Porter *et al.* 2020). Studies of opsin duplication reveal a complex interplay of gene gain and loss driving the generation of novelty, and the cascading effects of such duplications through different levels of biological organization.

Opsin gene duplication can have emergent effects, impacting the outcomes of natural and sexual selection at the network, cell, tissue, and organismal levels. For example, butterflies have remarkably complex visual systems, with sexual dimorphism in ocular gene (including opsin) expression (Macias-Muñoz *et al.* 2016), and a diverse array of opsin expression patterns, gene loss, and newly evolved receptors that likely aid in mate recognition (McCulloch *et al.* 2017). Opsin duplications in insects have resulted in the evolution of highly labile visual systems Feuda *et al.* (2016), with recent studies of beetles (Lord *et al.* 2016), dragonflies (Futahashi *et al.* 2015), and fireflies (Sander and Hall 2015) linking opsin duplications to both mate selection and environmental adaptation. Colour vision facilitated by opsin evolution also gives rise to complex evolutionary phenomena like sensory drive, wherein sensory and signalling traits (e.g., colour) covary (Endler 1992; Price 2017; Cummings and Endler 2018) (**Fig. 2E**). In the classic example of cichlid adaptive radiation, reproductive isolation between closely related

species is achieved through colour-based assortative mating across a light gradient, where shallow and deep-dwelling females have an opsin tuned to blue and red colours, respectively. Over time, male colouration diverges across shallow and deep populations per these female preferences (Seehausen *et al.* 2008). Opsin subfunctionalization via differential gene expression is also associated with sexual selection (Bloch 2015), conspecific recognition and predator avoidance (Sandkam *et al.* 2015) and foraging (Stieb *et al.* 2016). Even in the most modest examples, where only one or two gene duplication events occur, opsin paralogs are highly flexible building blocks that ecological and evolutionary forces readily harness for the generation of novelty.

(2) Novel organisms evolve via symbiosis and horizontal gene transfer

Symbiosis, the prolonged physical intimacy among species (*sensu* de Bary 1879), is a major driver of biological novelty (Margulis *et al.* 1991). In some cases, symbiosis can generate novel organisms, as in the ancient symbiosis between archaea and bacteria that gave rise to eukaryotes, for example (Sagan 1967; Margulis *et al.* 1991). More commonly, the symbiosis between prokaryotic microbes and eukaryotic hosts gives rise to novel, emergent traits (Batstone 2021; Batstone *et al.* 2021), such as pathogen and herbivore resistance in agricultural crops (Van Wees *et al.* 2008), and improved digestion of lactose in human infants (Wall *et al.* 2009). Symbiotic interactions can vary from facultative to obligate for one or more partners, and outcomes range from pathogenic to beneficial, often depending on the environment and the genotypic identities of those interacting (Heath and Tiffin 2007; Batstone *et al.* 2018). Symbiosis leads to novelties, as we define them, when distinct species interact in particular genetic combinations or environmental contexts in such a way that produces emergent traits. For example, nodules located on the roots of leguminous plants are novelties given that they only form when the right combination of plant and nitrogen fixing bacteria (rhizobia) interact in the "right", often low-nitrogen, environment (**Fig. 3**). More generally, if we want to predict when and how novel traits evolve, we must take into account the multitude of symbiotic interactions producing such traits.

Mutualistic symbioses are those interactions wherein all partners gain net benefits. Often, mutualisms confer novel traits or the ability to withstand new environments or exploit new resources. For example, bobtail squid are able to hunt at night because of their symbiosis with light-producing bacteria (McFall-Ngai 2014). Certain fungi living within plant leaves can facilitate plant drought tolerance and enable plant populations to expand their geographic range (Afkhami *et al.* 2014). By acquiring a novel gut symbiont, *Regiella insecticola* vetch aphids can gain the

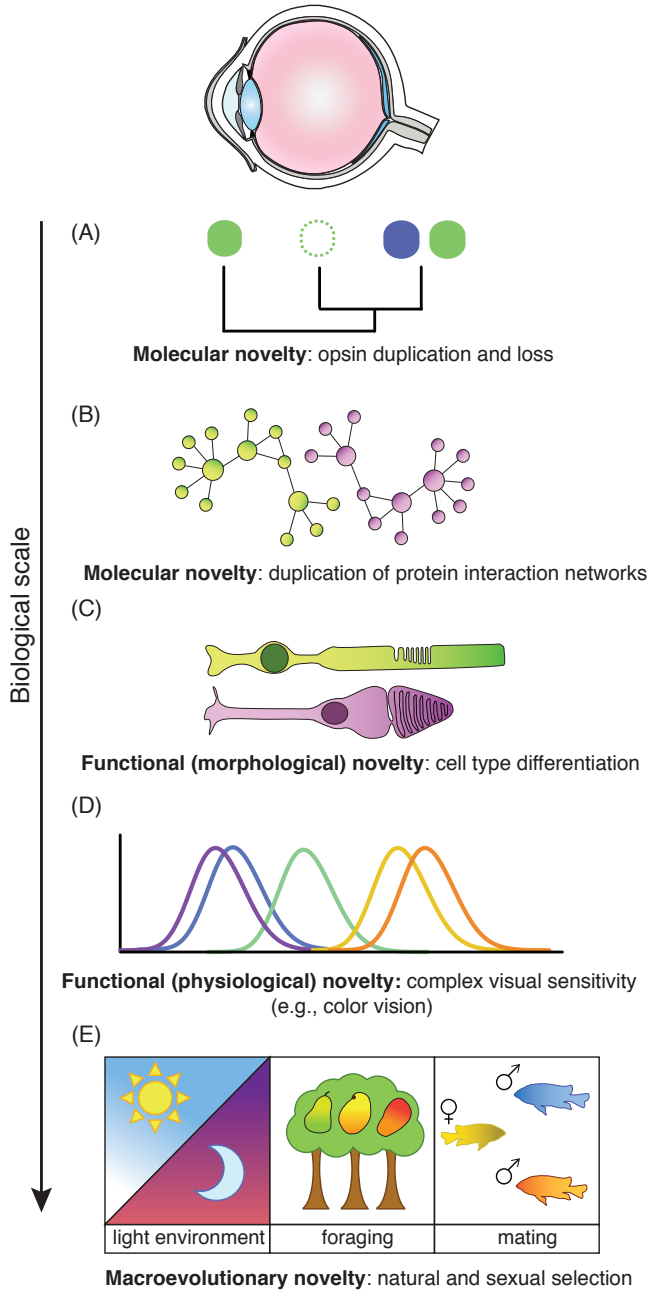

Figure 2 Gene duplication has had profound consequences for animal vision across different levels of biological organization. Opsin gene duplications have facilitated increasing complexity at the level of genes **(A)** gene networks **(B)**, cells, and **(D)** physiology. **(E)** Retention or expansion of these duplicates is influenced by both natural and sexual selection, and the physiological consequences of opsin duplication (e.g., colour vision) can also drive speciation.

ability to feed and reproduce on white clover (Tsuchida *et al.* 2011), potentially expanding the aphid's niche and making them more resilient to changes affecting their preferred host plant. Mutualistic symbioses thus produce novel, chimeric organisms, exhibiting properties not present in closely-related but non-mutualistic counterparts.

Antagonistic symbioses (e.g., pathogen-host interactions) have long been known to generate novelty through fluctuating selection and arms race dynamics (Common *et al.* 2019). Novelty in pathogen genes allows pathogens to evade host immunity, given that hosts use these genes to distinguish friend from foe, while novelty in host recognition systems allows hosts to defend themselves against a panel of pathogens (Hamilton 1980). For example, when prokaryotes are infected by phages, many prokaryotes acquire snippets of the phage genomes, to serve as a "vaccination record"; although the novel phage genomes are metabolically inactive once acquired by the host, they convey a new function by allowing hosts to recognize and render the same phage genotype inactive in the future ("CRISPR-Cas immunity"; Andersson and Banfield 2008). To counter host immunity, however, mutations at specific locations within the phage genome mask the phage from the host's CRISPR-Cas system, allowing the phage to go undetected (Laanto *et al.* 2017). In general, novelty is likely to emerge whenever rare genotypes are favored by selection (i.e., negative frequency-dependent selection; Hamilton 1980), and is especially apparent within antagonistically coevolving traits such as immunity and resistance.

Novelty that arises through the acquisition of viral-derived loci by a host is not limited to host-pathogen interactions as described above. Rather than functioning as cellular parasites, hijacking the victim cell's machinery for their own replication and transmission, many viruses instead integrate their genomes into the genomes of both prokaryotic and eukaryotic hosts. In fact, at least 8% of the human genome is viral in origin (Lancet *et al.* 2001; Roossinck 2011). These viral-derived loci encode important functions that have led to major evolutionary leaps, including the development of the placenta in the evolution of mammals (Harris 1991). Viruses can thus be thought of as vectors of genomic novelty, introducing loci that have the potential to take new and important functions once integrated into the host genome.

In addition to gene acquisition, gene loss can be an important source of novelty arising from adaptive, neutral, or deleterious processes. For example, when symbionts become obligately associated with their host, genes in the symbiont that are functionally redundant with those in the host may be prone to accumulating deleterious mutations, given a reduction in the efficacy of selection, and are eventually lost, such as genes involved in amino acid biosynthetic pathways in

obligate insect symbionts (Andersson and Kurland 1998; Ochman and Moran 2001). Gene loss can also be favoured by selection: the "Black Queen Hypothesis" (Morris *et al.* 2012) purports that mutualism among free-living organisms, particularly microbes, can result in the loss of genes encoding the production of costly metabolites, because such metabolites can be acquired from other microbes in the community instead. When genes encode environmentally-specific functions, such as antibiotic resistance genes in the presence of antibiotics, gene loss may be favoured in alternative environments, where their functions are no longer required and if maintaining such genes is costly (Andersson and Hughes 2010).

(a) Horizontal gene transfer as an important source of novelty

Genes move not only between generations from parent to offspring as we know, but also within generations among genomic backgrounds that do not necessarily share common ancestry. Such gene mobility, more commonly referred to as **horizontal gene transfer** (HGT), can be a more common source of genetic novelty than point mutations; for example, in order to colonize and adapt to the gut of their host, strains of *E. coli* relied on HGT mediated by a bacteriophage rather than point mutations (Frazão *et al.* 2019).

Horizontal gene transfer among prokaryotes, where it is especially rampant, has spurred entire fields of study (e.g., "mobilomics", "pangenomics"; Siefert 2009; Brockhurst *et al.* 2019. In bacteria, the overrepresentation of horizontally transmitted genes can be attributed to three main mechanisms: **transformation** (i.e., acquisition of genomic material from the environment), **transduction** (i.e., acquisition of genetic material via a virus or another MGE acting as a vector), and **conjugation** (i.e., the transfer of genetic material, often in the form of a plasmid, from one bacterial cell to another). HGT can have important phenotypic consequences on both the immediate recipient host, and often, the host of the recipient host. For example, genes carried by a transducing phage that infects *E. coli* transform this common gut-inhabitant into the infamous shiga toxin-producing pathogen causing severe foodborne diseases in humans and other mammals (O'Brien *et al.* 1984). Similarly, infection of the fungal symbiont *Curvularia protuberata* by a mycovirus is required for the fungal symbiont to confer thermotolerance of panic grass in geothermal soils (Márquez *et al.* 2007). Such **nested symbioses** among mobile genetic elements, prokaryotic microbes, and eukaryotic hosts are ubiquitous in nature and produce novel, chimeric organisms, whose emergent properties and phenotypes cannot be reduced to the sum of their parts (Batstone 2021). The origins and implications of biological novelty must therefore

take into account symbiosis among nested levels of biological organization and (co)evolution within the context of a "Tangled Tree" (Quammen 2018), whereby organisms, especially microbes, commonly exchange genes outside of parent-offspring relationships.

(b) Legume-rhizobium-MGE symbiosis: a model for understanding how nested symbioses give rise to novelty

More commonly known as the legume-rhizobium symbiosis, the interaction between leguminous plants and rhizobial bacteria depends on a third player —- **mobile genetic elements** (MGEs) such as plasmids and chromosomal islands that often carry the genes enabling rhizobia to both establish symbiosis with and fix nitrogen for their plant host (Remigi *et al.* 2016; Andrews *et al.* 2018; Wardell *et al.* 2022). Thus, this symbiosis represents multiple nested layers (i.e., legume-rhizobium-MGE), each interacting to produce novel emergent phenotypes (**Fig. 3**. Importantly, this nested symbiosis has given each interacting member a new role, resulting in novel functions and abilities at the collective-level. For example, symbiotic legumes are able to colonize new, nitrogen-poor environments, produce faster and larger growing phenotypes under low-nitrogen conditions, and modify their ecosystems by enriching the surrounding soil with fixed N. Below, we describe the implications of novelty across scales and describe how this nested symbiosis enables our understanding of the processes that generate novelty.

Novelty within the legume-rhizobium-MGE symbiosis can arise through different mechanisms at different biological scales: novel mutations, the acquisition of novel MGEs by rhizobium cells, and the acquisition of novel rhizobial strains by plant hosts (**Fig. 3**). Novel mutations can impact each downstream phase of the symbiotic interaction. For example, the acquisition of a MGE by a rhizobium cell can be accomplished by conjugation between free-living rhizobia in the soil (Peter *et al.* 1996; Remigi *et al.* 2016), and thus, novel mutations that arise within genes encoding the conjugative machinery could modulate the specificity and rate of conjugation (e.g., mutations in the *tra* genes of *Rhizobium etli*, Tun-Garrido *et al.* 2003). Although the factors determining rhizobium-MGE compatibility remain largely unknown, many prokaryotes possess restriction-modification systems that permit or destroy foreign DNA entering the cell (Thomas and Nielsen 2005; Oliveira *et al.* 2014); thus, a novel MGE may only be acquired if they contain the "correct" sequence motif or if the endonuclease that cuts foreign DNA does not recognize it as such. Once a compatible MGE has been acquired by a rhizobium cell, error-prone DNA polymerases present on the MGE can induce novel, "compensatory" mutations across the genome that mitigate the

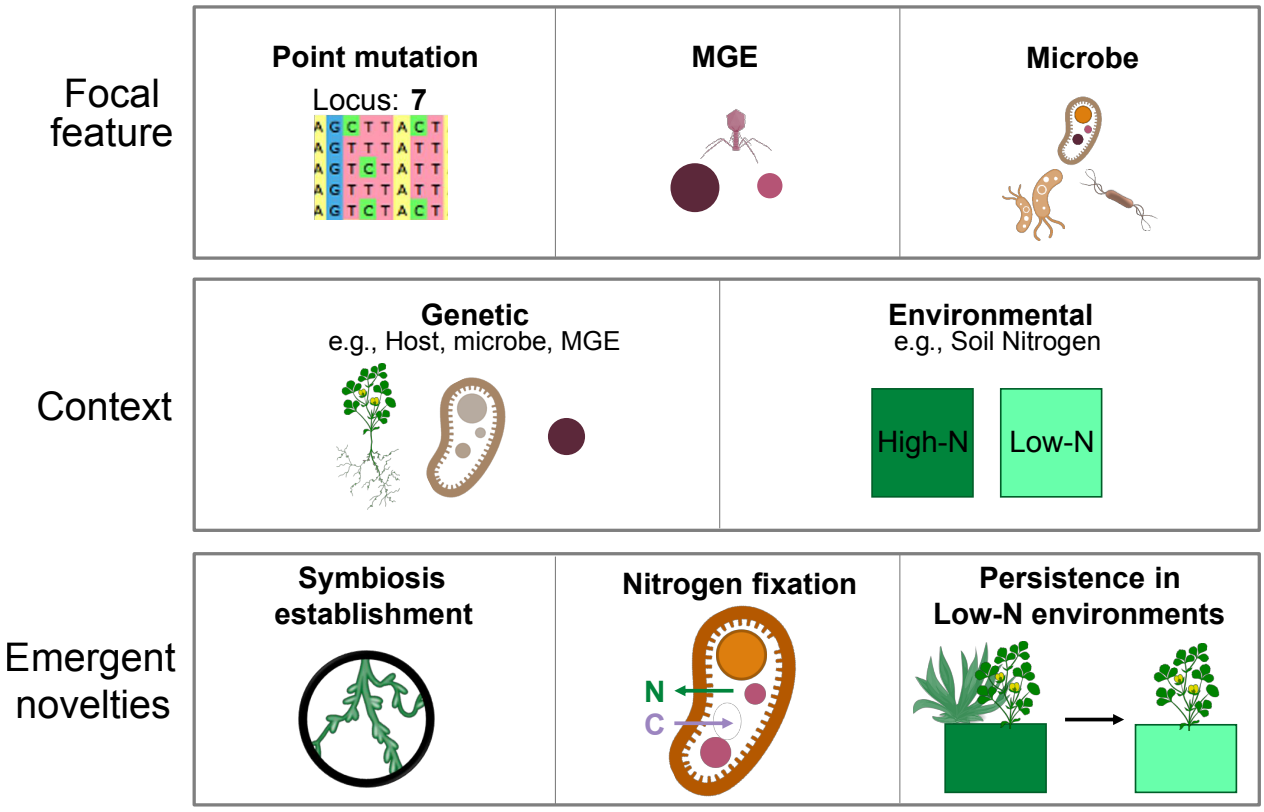

Figure 3 Nested symbioses as models for understanding the origins and impacts of novelty. Emergent novelties including symbiosis establishment, the ability to fix nitrogen (N) in exchange for carbon (C), or persisting in a particular environment arise when point mutations, mobile genetic elements (MGEs) or entire microbes interact with either (or both) a genetic (e.g., host, microbe, or MGE) or environmental (e.g., high- or low-N) background.

cost of acquisition, and permit the rhizobium to adapt to a novel host plant (Remigi *et al.* 2014; San Millan and Maclean 2017). Acquisition of a novel rhizobia strain by the host plant relies on a complex cascade of signaling and recognition molecules being exchanged by both the plant and rhizobium, and thus, novel mutations present in genes controlling these pre-infection pathways, as well as both MGE-rhizobium and rhizobium-plant compatibility, largely determine whether symbiosis will be established.

The expression of novel emergent traits is likely contingent on the history of coevolution among nested levels. That is, novelty at any scale can manifest as intraspecific genetic variation in traits central to the nested symbiosis, such as number of nodules formed, plant growth, and leaf nitrogen content. From a quantitative genetics perspective, such intraspecific genetic variation is largely generated by epistatic interactions that occur between rather than within genomes (i.e., G x G interactions, or **intergenomic epistasis**; *sensu* Wade 2007). For example, intergenomic epistasis could mean that one host genotype's beneficial partner is another host genotype's poor quality partner, even when environmental conditions are held constant (Heath and Tiffin 2007; Heath 2010). Importantly, intergenomic epistasis generates heritable variation, and thus, is a prerequisite for coevolution (Heath 2010). Although untested, intergenomic epistasis is likely to arise from interactions between MGEs and rhizobial chromosomal backgrounds, and thus, may be a hidden source of variation in symbiotic traits. In other words, traits expressed at the host level might differ when hosts associate with rhizobial strains that recently acquired a MGE, versus strains that have coevolved with the MGE over a longer period of time. Whenever symbiotic interactions involve multiple nested layers, coevolution may be similarly multi-scaled, emerging between the host and the bacterial chromosome, the bacterial chromosome and the MGE, and/or the host and the MGE. Thus, we must take into account each interacting layer in order to fully understand and predict the emergence of evolutionary novelty.

(3) Hybrid origins of extreme traits, novel niches, and new species

The most commonly used species concept for eukaryotes (the biological species concept) is based on the idea that species only breed with others of their same species (e.g., see Dobzhansky 1935), so each species is a distinct branch in the tree of life. However, it is increasingly apparent that relationships among species are often better described as a web or network, given the frequency of genetic exchange across species boundaries (Mallet *et al.* 2016). Among eukaryotes, genetic exchange can take several forms, including **hybridization** (interbreeding between species,

yielding offspring), **introgression** (transmission of genes or alleles from one parent species to another by back-crossing of hybrids with parents), and **horizontal gene transfer** (without reproduction, by means of a vector that transfers DNA). As we will describe, these means of combining genomes of different species have generated new combinations of traits, allowed species to move into new environments, and formed new species.

(a) Hybridization, introgression, and horizontal gene transfer as pathways to novelty

By definition, hybridization involves recombining genes of different parent species and can generate an explosion of phenotypic variation and novel traits. Recombination between parent genomes generates new allele combinations on which selection can act. These new combinations can result in similar phenotypes to the parent species, novel intermediate phenotypes, or novel extreme phenotypes (**transgressive segregation**) relative to the parent species. Transgressive segregation leading to novel phenotypes and niches is seen in sunflowers, for example: a hybrid species (*Helianthus deserticola*) has smaller leaves and develops more rapidly (flowers earlier) than either parent species (**Fig. 4A**; Rieseberg *et al.* 2003). These new features likely enabled its colonization of extreme desert environments from the more mesic parental habitats (**Fig. 4A**; Rieseberg *et al.* 2003). In *Heliconius* butterflies, experimental hybrids had novel wing colour patterns and shapes, distinct from those seen in parent species (Mérot *et al.* 2020). Wing colouration patterns warn predators that these butterflies are unpalatable, so this trait has direct fitness consequences: certain hybrid colour patterns suffered greater predation (Merrill *et al.* 2012) because they were unfamiliar to predators. Nonetheless, hybridization has contributed to the high diversity of warning colouration patterns maintained among these butterfly species (Merrill *et al.* 2012). More generally, numerous taxa show signatures of ancestral hybridization, and hybridization is increasingly recognized as a source of novelty underlying adaptive radiations and speciation (Taylor and Larson 2019).

Several hurdles must be overcome for hybrids to be formed and persist as distinct lineages. First, individuals from different species must be able to mate (i.e., overcome pre-zygotic barriers; Coyne *et al.* 2004) – for example, angiosperms that flower at the same time (Lamont *et al.* 2003) or birds that recognize each other's songs (Willis *et al.* 2014). Then, the species must be genetically compatible enough that a viable hybrid offspring can be produced (i.e., overcome post-zygotic barriers; Coyne *et al.* 2004). Horses and donkeys, for example, have different numbers of chromosomes, so their hybrid offspring (mules) are sterile. When viable hybrid offspring are produced,

they can contribute to novelty by having unique phenotypes (see transgressive segregation, above), forming a new reproductively isolated species (hybrid speciation), or transferring new alleles into a parent species (introgression).

Hybrid speciation involves cascades of novelty across biological scales. **Polyploid hybrids** (i.e., that retain full copies of both parental genomes) are immediately reproductively isolated from parent species due to their increased chromosome count. In contrast, **homoploid hybrids** (that have the same chromosome number as their parent species) are generally more compatible with their parent species. Homoploid hybrids that share parental environments would be at risk of genetic swamping from parent populations and less likely to endure as a separate species; hence, the homoploid hybrids that persist as distinct species are almost always those that have novel ecologies compared to their parents (e.g., Gross and Rieseberg 2005; Mao and Wang 2011). Although more rare, novel hybrid traits may also directly contribute to their reproductive isolation from parent species, within a shared environment. For instance, hybridization can disrupt the phenotypes used in mate recognition and assortative mating, leading parents and hybrids to preferentially mate within their own taxon (Mavárez *et al.* 2006). Alternatively, hybridization and repeated "back-crossing" of hybrids with parent populations over time can transfer genomic segments, containing new genes or alleles, from one parent species into the genome of the other (introgression) without forming a separate hybrid species.

Introgression can allow species to rapidly adapt to new environments by providing novel genes or alleles that have already been tested by selection in the donor species. While introgression can generate new genetic combinations with negative impacts on organismal phenotypes and fitness, these are more likely to be eliminated by selection and contribute to reproductive isolation between the interbreeding species ("**reinforcement**", e.g., Lemmon and Juenger 2017). Similarly, introgressed alleles with neutral effects are less likely to rise to high frequency unless by drift, hitchhiking with more favourable alleles, or a change in the selective regime that renders them adaptive. Hence, we focus on the novelty created by adaptive introgression, here. Recent work in snowshoe hares (*Lepus americanus*) demonstrates that introgression of an allele from black-tailed jackrabbit (*Lepus californicus*) has enabled certain snowshoe hare populations to molt to a brown coat in the winter (rather than their usual white winter coat; **Fig. 4B**; Jones *et al.* 2018). The snowshoe hare populations that remain brown in winter are found in mild habitats that do not maintain snow cover, where a brown coat would provide better camouflage from predators (**Fig. 4B**; Jones *et al.* 2018). Therefore, by creating a new combination of pre-existing characteristics in

the *Lepus* genus, introgression has led to a new snowshoe hare phenotype that directly increased its fitness in milder climates. As another example, in Atlantic killifish (*Fundulus heteroclitus*), a deletion mutation that led to loss of function of a hydrocarbon receptor gene has allowed them to tolerate high concentrations of pollutants (Oziolor *et al.* 2019). This locus has introgressed into Gulf killifish (*Fundulus grandis*), where it has permitted populations to adapt to sudden increases in pollution (Oziolor *et al.* 2019).

More broadly, gene flow has been documented between wild and domesticated species in 12 of 13 staple crops around the globe (Ellstrand *et al.* 1999). Natural introgression from wild to domesticated species is thought to have helped domesticated species adapt to new environments (reviewed in Burgarella *et al.* 2019), including conditions that would ordinarily lower productivity, like poor soils or reduced watering (Warschefsky *et al.* 2014). This idea has also been experimentally applied to improve crops; for example, a gene conveying resistance to leaf rust has been deliberately introgressed into barley (*Hordeum vulgare*) from a related species (Yu *et al.* 2018). Similarly, gene flow between different domesticated species has created new combinations of desirable traits for cultivation (Burgarella *et al.* 2019). In contrast, introgression from domesticated species into wild species could create unwanted novelty, such as the production of weeds with new phenotypes that resemble crops and are hard to mechanically distinguish and remove, or lead to the genetic swamping and extinction of wild species (Ellstrand *et al.* 1999).

In some cases, genes or alleles may be introduced from one eukaryotic species to another without interbreeding, via a vector (like a virus or pathogen; Gilbert and Cordaux 2017) or exchange during symbiosis between eukaryotes (like fungi and plants; Zhang *et al.* 2020). Such horizontal gene transfer (HGT) can occur between eukaryotes that could never reproduce with each other. For example, the novel photoreceptor thought to underlie the fitness and diversification of ferns in low-light habitats originated through HGT from hornworts (Li *et al.* 2014). In fungi, certain species have gained the ability to take up new types of nutrients from their environment, thanks to the transfer of transporter genes between different fungal phyla (Milner *et al.* 2019). Although not "hybridization", because it does not involve creating hybrid offspring through reproduction, HGT is another way genomes from two different eukaryotic species can combine and create novel features.

Several factors influence whether genetic exchange between species, new mutations, or standing genetic variation is the more important source of adaptive potential and novelty. New variants that start at a higher frequency, convey a larger selective benefit, and are not involved in

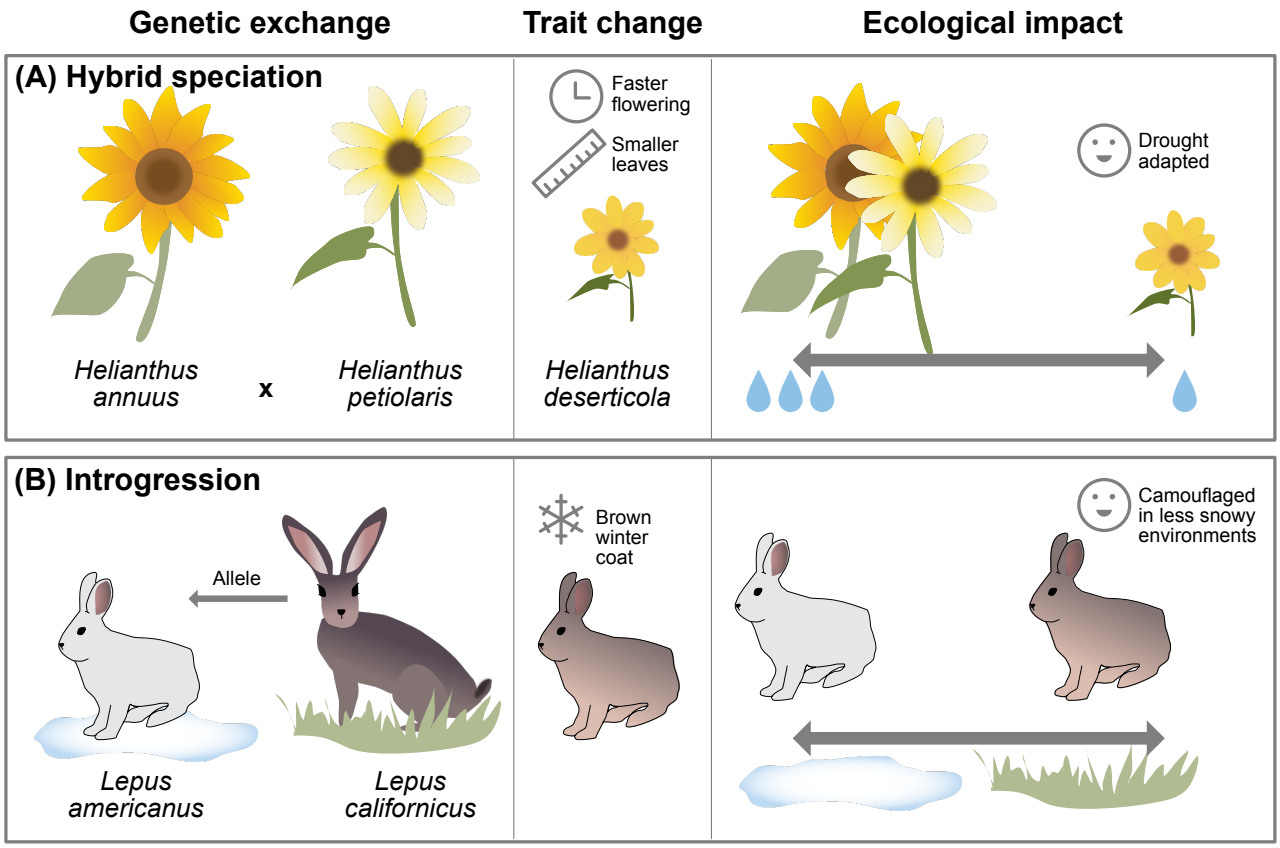

Figure 4 Hybridization and introgression contribute to novelty across biological scales. In these examples, new genes lead to new traits (or trait combinations) and the ability to exploit different or extreme environments (Based on studies: (Rieseberg *et al.* 2003; Jones *et al.* 2018)).

antagonistic pleiotropy or linked with maladaptive alleles are likely to contribute the most to adaptation. High rates of genetic exchange (hybridization, introgression, or HGT) may increase the starting frequency of a new allele over what mutation alone could accomplish, and accelerate the spread of a beneficial novelty. Unlike mutations, frequent genetic exchange generates a suite of recombinant genotypes that may be able to interbreed and propagate the novelty (Dittrich-Reed and Fitzpatrick 2013). Of course, if genetic exchange is infrequent, the recombinant lineages are maladaptive due to genetic incompatibilities, or the mutation rate is high, mutation or standing genetic variation may be more important sources of adaptive novelty. In terms of the selective benefit, alleles gained through genetic exchange may be more likely to be adaptive, compared to an average mutation that arises. Transferred genes or alleles have already survived the sieve of selection and recombination (in the donor species), so are primed to contribute to rapid adaptation to new environments by providing new functions to the recipient genome (Hedrick 2013; Dunning and Christin 2020). Recall the killifish example above, where Gulf species were able to take advantage of a mutation that arose and underpinned pollution tolerance in the Atlantic species (Oziolor *et al.* 2019). Although there are several good examples of single introgressed alleles providing adaptive benefits like this, these adaptations occur through genetic exchange that affects multiple loci simultaneously, unlike single mutations (Dittrich-Reed and Fitzpatrick 2013; Hedrick 2013). Hence, genetic exchange may provide more raw material on which selection can act, within the recipient genome, and therefore more opportunities for novelty (Olofsson *et al.* 2019). The fitness effects of the genes or alleles being transferred, and their interactions with other genes (pleiotropy, dominance, linkage), influence whether an instance of genetic exchange produces adaptive novelty (reviewed in Hedrick 2013; Connallon and Hall 2018).

(b) Context-dependence of novelty from genetic exchange

Environmental and genetic contexts of hybridization shape the occurrence, persistence, and impacts of novelty that arises through genetic exchange. First, the environmental context determines whether hybridization even occurs (see Grabenstein and Taylor 2018). For example, eutrophication of Lake Victoria reduced the ability of cichlids to select mates based on colour, relaxing reproductive isolation and resulting in hybridization (Seehausen *et al.* 1997). Second, the environment also shapes the opportunities for hybrids to find their own non-parental niche and survive. Hybrid fitness, relative to parents, often varies with environmental conditions (Lexer *et al.* 2003), with certain hybrid genotypes outperforming parents in new environments

that are intermediate to parent habitats or even more extreme (**Fig. 4A**; Arnold 1997). As in the snowshoe hare example above, the selective environment determines the adaptive value of introgressed genes or alleles, and their contribution to novelty. Similarly, the new genetic background of a transferred gene affects both the gene's expression and impact on hybrid phenotypes. For instance, new gene combinations can produce negative epistatic interactions, such that the fitness effects of a set of genes is worse than the sum of its parts (reviewed in Hedrick 2013). The likelihood of a hybrid having more extreme (novel) traits than the parents depends on the genetic make-up of the hybrid. That is, different hybrid ancestries (e.g., first-generation, later generation, back-cross to parent species) and genotypes will likely result in a wide range of different phenotypes, some of which closely resemble parental types (Arnold 1997; Lexer *et al.* 2003).

III. METHODS FOR INVESTIGATING THE ORIGINS OF NOVELTY

Because biological novelty encompasses a wide array of biological scales, mechanisms, and systems, many scientific approaches have been brought to bear to understand how biological novelty originates. The breadth of definitions of novelty (see section entitled **Defining novelty**) is a reflection of the diversity of methods and perspectives used to study origins (Table 1, Brigandt and Love 2012). In recent years, the advent of genomic tools such as Next Generation Sequencing has revolutionized our ability to study the origins of novelty (e.g., Moran and Jarvik 2010; Renfree *et al.* 2011; Taylor and Larson 2019). Genomic data enables detection of genetic novelties and the molecular drivers of higher-level novelty. In our case study of opsins, genomic approaches allowed researchers to identify and annotate paralogs and examine the genomic arrangement of duplicates. These genomic tools unveiled the history of gene duplication and loss that underlies novel features during the evolution of the eye across a diverse array of organisms (e.g.,Musilová *et al.* 2019; Macias-Muñoz *et al.* 2019; Porter *et al.* 2020). Additionally, genomic sequencing has revealed symbiosis as a key vehicle for novelty by showing that symbioses between macrobes (like eukaryotes) and microbes are ubiquitous and that genes and genomic elements are surprisingly mobile among species.

Genomic data can also be used to test whether the relative composition of nucleotides differs from expectations and pinpoint genes in the focal species that are more closely related to genes of distant relatives, clues that a particular biological novelty arose from horizontal rather than vertical transmission (Gogarten and Townsend 2005; Keeling and Palmer 2008). In this same way,

genomic tools have made it possible to detect the transfer of alleles between species and unravel the hybrid origins of many modern taxa (Smith and Kronforst 2013; Taylor and Larson 2019). Genomic data are particularly useful for understanding the origins of novelty in morphologically complex and non-model taxa whose history of hybridization may be difficult to discern using alternative methods (Twyford and Ennos 2012). Importantly, genomic data can differentiate different hybrid classes (e.g., back-crossed to parent, late-generation hybrid; Arnold 1997) and therefore discern whether hybridization generates an interbreeding swarm (with potentially novel characteristics) or creates new, reproductively isolated hybrid taxa (Gompert and Buerkle 2016). Beyond its importance in the case studies we emphasize in the previous section, genomic data has also identified (for example) gene and genome duplications that contribute to new phenotypes and adaptive evolution in plants (Flagel and Wendel 2009) and the new genes and patterns of gene expression that differentiate worker from queen ants, shedding light on the origins of eusociality (Feldmeyer *et al.* 2014; Sumner 2014). Simply stated, genomic approaches allow us to understand the "molecular building blocks of natural variation" and test whether new features share a common origin across clades (Sumner 2014).

Genomic approaches to understanding novelty are especially powerful in conjunction with empirical techniques that probe the downstream consequences of molecular novelty. For example, testing the expression of a gene and the function of its protein product can clarify the adaptive relevance of novel gene copies (as in the example of opsin duplication, e.g. Musilová *et al.* 2019) or determine the impacts of gene transfer (i.e., has it been transcribed? Does it provide a new capability? Dunning *et al.* 2019). Experimental evolution of microbes with different host genotypes, when combined with genomic sequencing before and after the experiment, can detect novel variants that arise as microbes adapt to their host (e.g., Batstone *et al.* 2020). Studies that experimentally create hybrids and look for novel phenotypes can target those phenotypes with functional tests Mérot *et al.* 2020; Selz and Seehausen 2019). Taken together, these examples emphasize how coupling genomic data with empirical studies and functional assays can improve our understanding of the mechanisms underpinning species' novel traits and niches, and the formation of new species.

IV. CONCLUSIONS

Biological novelty is a central interest in evolutionary biology, but novelty has often been defined in narrow and contrasting ways. These divisions reduce our ability to build a robust and

cohesive body of literature that would spur further advances by identifying common mechanisms underlying different types of novelty.

Disparate kinds of novelty are generated by common processes: gene duplication, gene loss, genetic exchange, and interactions of genes with their genetic and environmental context. Therefore, we view these formerly different camps of novelty through the lens of shared mechanisms of change and their impacts across biological scales. Under this perspective, novelty includes but is not limited to innovations involved in adaptive radiations.

Many important novelties, such as the vertebrate eye, novel symbiotic organisms, and the ability of hybrids to exploit new environments, are typically explored in very different bodies of literature and using seemingly dissimilar approaches. However, we aim to illustrate how common mechanisms of genetic change and emergent effects across biological scales unite even the most unique and important of novelties.

Genomic data gives us unprecedented insight into the origins of novelty. Combining genomic data with experiments and functional tests of molecular changes is a powerful way to study how novelties originate, impact other biological scales, and evolve.

V. AUTHOR CONTRIBUTIONS

All authors contributed substantial original text and figures, participated in revisions, and approved the manuscript for submission. K.A. Carscadden outlined the paper and led the writing.

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VII. GLOSSARY

- **Novelty**: when a focal feature (e.g., point mutation, gene, mobile genetic element) interacts with a genomic (i.e., host) and/or environmental background to produce one or more emergent traits (i.e., molecular, physiological, morphological, behavioural) not present in the ancestral population.
- **Variation**: when a focal feature varies among individuals within a population in the same generation (i.e., standing variation) or was absent in the ancestral population (i.e., novel variation), but does not lead to emergent traits across biological scales in the current context (e.g., silent point mutation).
- **Symbiosis**: the intimate interaction among two or more species – *sensu* de Bary (1879). Can range in outcomes from harmful to beneficial.
- **Horizontal gene transfer (HGT)**: movement of genes from one individual to another within the same generation. Can be among unrelated individuals (i.e., inter-species, inter-domain).
- **Horizontal transmission**: the acquisition of a symbiont by a host from the environment or another host within the same generation. Conceptually similar to HGT, but typically used when cellular organisms (prokaryotes, eukaryotes) are being transferred rather than genes.
- **Transformation**: the acquisition of DNA by a host cell from the environment.
- **Transduction**: the transfer of DNA from one host cell to another via a bacteriophage.
- **Conjugation**: the transfer of DNA from one host cell to another via the temporary union between two cells facilitated by a bridge-like connection known as the pilus.
- **Mobile genetic elements (MGEs)**: "entities that have evolved to persist and replicate through adaptations that move DNA" – Hall *et al.* (2022). e.g., plasmids, transposons ("jumping genes"), bacteriophages, integrative and conjugative elements (ICEs).
- **Intergenomic epistasis**: non-additive interactions among the genomes of two or more species that leads to significant variation in a trait of interest. In other words, trait variation that depends on the genotypic identities of each interacting species.
- **Nested symbioses**: symbiotic interactions among mobile genetic elements, prokaryotic microbes, and eukaryotic hosts.
- **Neofunctionalization**: Following a gene duplication event, a duplicated gene acquires a novel function.
- **Subfunctionalization**: Following a gene duplication event, gene duplicates retain part of

the ancestral function of their parent protein. e.g., if the parent gene performs function AB, gene duplicate 1 performs function A, and gene duplicate 2 performs function B.

- **Pseudogenization**: Inactivation of a gene due to the accumulation of mutations, typically following relaxation of selective constraint.
- **Gene family**: a set of similar genes that arose as a result of duplication of an original parent gene.
- **Paralog**: a gene copy that arose as a result of duplication (e.g., alpha and beta globin genes are paralogs).
- **Pleiotropy**: one gene may mediate one or more phenotypic traits.
- **Hybridization**: interbreeding of different species, yielding viable offspring.
- **Introgression**: incorporation of genes or alleles from one species into another, through hybridization and back-crossing of hybrids with individuals of the parent species.
- **Transgressive segregation**: hybrids with phenotypes more extreme than those observed in the parent species.

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