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NEWS AND VIEWS

Meeting Review

WILEY MOLECULAR ECOLOGY

UNVEILing connections between genotype, phenotype, and fitness in natural populations

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Abstract

Understanding the links between genetic variation and fitness in natural populations is a central goal of evolutionary genetics. This monumental task spans the fields of classical and molecular genetics, population genetics, biochemistry, physiology, developmental biology, and ecology. Advances to our molecular and developmental toolkits are facilitating integrative approaches across these traditionally separate fields, providing a more complete picture of the genotype-phenotype map in natural and non-model systems. Here, we summarize research presented at the first annual symposium of the UNVEIL Network, an NSF-funded collaboration between the University of Montana and the University of Nebraska, Lincoln, which took place from the 1st to the 3rd of June, 2018. We discuss how this body of work advances basic evolutionary science, what it implies for our ability to predict evolutionary change, and how it might inform novel conservation strategies.

KEYWORDS

adaptation, biotechnology, conservation genetics, molecular evolution, phenotypic plasticity, population ecology

1 | INTRODUCTION

How does genetic variation translate into fitness variation in the wild? Answering this question is a central goal of evolutionary genetics (Endler, 1986; Fisher, 1930; Lande & Arnold, 1983; Lewontin, 1974; Schluter & Conte, 2009; Wright, 1932), but a comprehensive understanding of the genetics of adaptation requires a complete description of the links between genotype, phenotype, and fitness (Barrett & Hoekstra, 2011; but see Rausher & Delph, 2015): What are the genetic loci important to adaptation? How does genetic variation affect cellular function and developmental processes to influence phenotypic variation? And how does natural selection shape variation within and among populations in their natural environment? Answering these questions is a monumental task and one that spans the domains of multiple fields, from classical and molecular genetics, to biochemistry, physiology, and development, to population genetics, evolutionary genetics, and ecology. Now, with advances to our molecular and developmental toolkits, integrated approaches are providing a more detailed picture of the connections between genotype, phenotype, and fitness in many, even traditionally "non-model", systems. But what have we learned so far?

From the 1st to the 3rd of June, 2018, evolutionary geneticists, ecologists, and conservationists met at Flathead Lake Biological Station for the first annual symposium of the UNVEIL Network. Funded through the US National Science Foundation's Established Program to Stimulate Competitive Research (EPSCoR), the UNVEIL Network (***U**sing **N**atural **V**ariation to **E**ducate, **I**nnovate, and **L**ead*; www.unveilnetwork.org) is a collaborative effort between the University of Montana and the University of Nebraska, Lincoln, to understand the links between genetic, phenotypic, and fitness variation in nature and to use these research programs to train scientists and inform new conservation strategies. The integrative nature of the collaboration led to a diversity of ideas over the two-day symposium. The 55 attendees came from 17

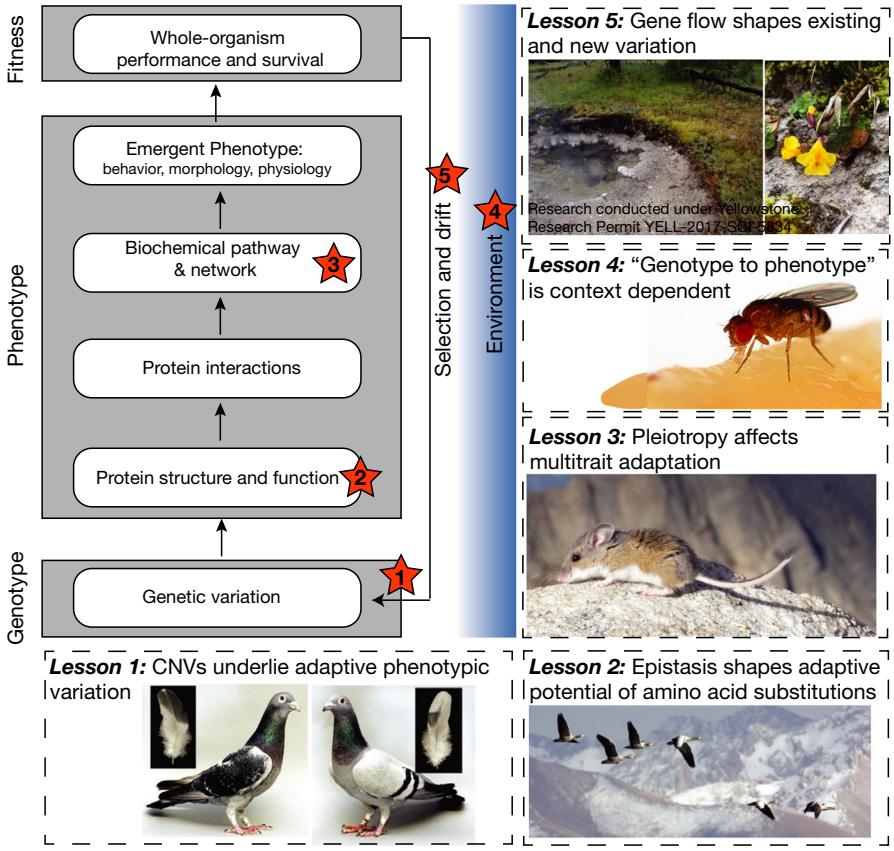

FIGURE 1 UNVEILing connections between genotype, phenotype, and fitness in wild populations. Five lessons learned from the first annual UNVEIL meeting are placed within the context of a genotype to phenotype to fitness schematic (redrawn from Dalziel et al., 2009). Lessons (indicated with orange numbered stars) are as follows: (1) Copy number variation commonly underlies adaptive phenotypic variation, such as in rock pigeons, (2) Epistasis shapes the paths available to adaptive protein evolution, as in haemoglobin adaptations of bar-headed geese, (3) Pleiotropy constrains and facilitates multitrait adaptation, as seen in selection on pleiotropic transcription factors in high-altitude deer mice, (4) The translation of genotype to phenotype is context dependent, as seen in fruit fly populations experimentally evolved in constant or temporally variable thermal environments, and (5) Gene flow acts as both a sieve and source of large-effect adaptive variation, as in local adaptation to thermal and nonthermal environments in the yellow monkeyflower. See text for full discussion of each lesson. Image credits: pigeons, Figure 1 of Vickrey et al. (2018)/Creative Commons (CC); bar-headed geese, Coke Smith (www.cokesmithphototravel.com); deer mouse, C. Wolf; Drosophila, Sanjay Acharya/CC; thermal pool, K. Kolis; Mimulus, T. Nelson

institutions across 12 states and three countries, and as far away as New Zealand. By design, those presenting at the UNVEIL symposium were in nearly equal part established scientists and young investigators.

In this meeting review, we summarize research presented at the UNVEIL Symposium with the goals of informing readers of recent advances in the field and stimulating new research aimed at making deeper genome-to-phenome connections. Research presented at the symposium at once highlighted how far we have come in our understanding of the genetics of adaptation (e.g., the molecular functions and evolutionary histories of loci under natural selection) and how far we have yet to go (e.g., the genetic basis of complex traits in nature). We identify five major lessons from the symposium that illuminate different components of the genotype-phenotype-fitness map (Figure 1). We discuss how these lessons advance basic evolutionary science, what they imply for our ability to predict evolutionary change, and how they might inform novel conservation strategies.

2 | FIVE LESSONS ON THE GENETICS OF ADAPTATION

2.1 | Lesson 1: Copy number variation commonly underlies adaptive phenotypic variation

Characterizing the kinds of mutations that contribute to adaptive phenotypic variation is foundationally important to the genetics of adaptation. Most studies in the genomics age focus on SNPs and small insertion-deletion (indel) polymorphisms because they are easily identifiable, abundant, and no doubt contribute greatly to phenotypic diversity and divergence (Davey et al., 2011; Field et al., 2016; Schlotterer, Tobler, Kofler, & Nolte, 2014). Large chromosomal mutations, particularly inversions, are also well known as targets of selection, principally due to their effects on linkage among multiple adaptive loci (Kirkpatrick & Barton, 2006; Lee et al., 2017; Rako, Anderson, Sgrò, Stocker, & Hoffmann, 2006). An emergent theme from UNVEIL is that copy number variants (CNVs) of entire genes

or genomic regions may be an underappreciated source of adaptive variation in the wild (Figure 1).

In his keynote address, Mike Shapiro (University of Utah), highlighted work led by his graduate student, Anna Vickrey (Vickrey et al., 2018), on the discovery of a single locus associated with four discrete colour pattern morphs in domesticated rock pigeons. They used whole-genome sequencing to identify a single differentiation (pF_{ST}) outlier between pigeons with barred and checkered wing pattern morphs, which are likely under selection in natural and urban environments. By examining sequence read depth across the outlier region, they found that differentiated SNPs were in fact markers for a 15 kb intergenic CNV with at least three different copies. Both copy number and wing pattern morph were associated with altered expression of a nearby gene, *NDP*, in developing feathers. This research provides a promising functional link between a large CNV and phenotypic variation under selection.

In another bird species, the common murre (*Uria aalge*), Anna Tigano (Cornell University) investigated the genetic basis of bridling, a plumage pattern polymorphism that shows latitudinal clinal variation in the Atlantic Ocean (Tigano, Reiertsen, Walters, & Friesen, 2018). She used a genome-wide association study (GWAS) approach to find a single outlier region. In this case, Tigano noticed that the genomic region associated with bridling was extremely polymorphic and showed positive Tajima's D and elevated nucleotide diversity among unbridled birds, indicating high-frequency, divergent haplotypes. By including read depth analysis, Tigano showed that these unusual patterns of sequence variation were in fact due to sequence reads from multiple copies of a CNV mapping to the same region of the single-copy reference genome. Her work demonstrates how CNVs can create aberrant signals in genomic data but also the ability to deconvolute those signals.

Copy number variation also made its mark in plants. Thom Nelson (University of Montana) used pooled sequencing of multiple cohorts to identify the genetic basis of life history and fitness variation within a single population of the yellow monkeyflower (*Mimulus guttatus*). Nelson and colleagues identified a single-gene outlier associated with variation in germination time, flower size, and year-to-year survival and female fitness (seed set). Patterns of SNP variation across pools were not always informative, but read depth analysis and genomic quantitative PCR of individual plants revealed a CNV with at least three copy number haplotypes of a gene putatively involved in stress response pathways. Two of the CNV haplotypes were nearly identical in sequence, suggesting a very recent gene copy expansion. Even more surprisingly, the genomes of some plants contained over 300 tandemly arrayed copies (Nelson et al., 2019). This extreme copy number expansion, the largest known in plants, demonstrates how strong selection can quickly affect genome structure and maintain large-scale genomic variation.

These three talks demonstrated the complications inherent in identifying CNVs using methods designed to identify SNP variation and will help guide future work seeking to understand CNVs in wild populations. In pigeons and murres, SNP variants served as reliable markers for CNV haplotypes, creating patterns of differentiation that could initially be mistaken for single-copy variants. In murres, though, a presumably ancient CNV created aberrant patterns of polymorphism not easily explained by sequence variation in a single-copy genomic region. In contrast, the monkeyflower CNV, despite its size, contained no diagnostic SNPs, likely because it arose very recently; methods only able to detect SNP variation (e.g., SNP chips) would therefore have missed this genomic variant entirely. The initial PoolSeq strategy of Nelson et al. (2019) also removed genotype information, resulting in underestimation of gene copy number until qPCR and whole-genome sequencing revealed the extent of the copy number expansion.

To effectively include CNVs as an important class of genomic variant, future studies should rely on sequencing methods that provide read depth information, retain individual genotypes, and allow breakpoint detection (e.g., paired-end Illumina sequencing, see Layer, Chiang, Quinlan, & Hall, 2014). Combining traditional genome scan approaches with long-read sequencing (e.g., Pacific Biosciences single-molecule real-time [SMRT] or Oxford Nanopore sequencing) or long-range haplotyping (e.g., BioNano optical mapping) will help resolve CNV genomic architectures and infer their evolutionary histories. Furthermore, generation of multiple reference genomes and access to multiple fully sequenced individuals in a study taxon will provide a clearer view, not only of ecologically relevant CNVs but of the genomic distribution and general abundance of CNVs in natural populations.

2.2 | Lesson 2: Epistasis shapes the paths available to adaptive protein evolution

The genotype-phenotype map is fundamentally a description of the actions and interactions of biomolecules. Perhaps nowhere is this more evident than in the study of amino acid polymorphisms and substitutions in proteins (Bridgham, Ortlund, & Thornton, 2009; Harms & Thornton, 2013; Natarajan et al., 2018; Storz, Natarajan, Cheviron, Hoffmann, & Kelly, 2012). Changes to protein sequence impact structure and function in complex and often unpredictable ways, and epistasis among substitutions has become a central theme of protein evolution. Understanding the effect of an observed substitution requires knowledge of the genetic background and evolutionary history under which that substitution arose. For this reason, techniques such as ancestral sequence resurrection (Thornton, 2004), which uses phylogenetic inference to determine the likely amino acid sequence at each node of a phylogenetic tree, have become key advances in studies of biochemical adaptation. Inferred sequences can be synthesized, mutagenized, structurally modelled, and cloned into vectors, allowing direct comparisons between extant, ancestral, and intermediate protein products. Speakers at the UNVEIL Symposium applied these tools to studies of adaptation in the wild, demonstrating that the functional effects of mutation depends upon the genetic background and thus the evolutionary context under which they arose (Figure 1).

Jay Storz (University of Nebraska, Lincoln) used ancestral protein resurrection to understand the mutational pathways of biochemical

adaptation in haemoglobin of bar-headed geese that routinely fly over the Qinghai-Tibetan plateau at over 5,000 m above sea level (Natarajan et al., 2018). First, Storz found that bar-headed geese have a higher haemoglobin-O_2 affinity than their strictly lowland relative, the greylag goose, and identified five amino acid substitutions between the two taxa, three in the α-globin chain and two in the β-globin chain. Combining ancestral state reconstruction with protein engineering, Storz then functionally tested recombinant haemoglobin (Hb) proteins and found that the increased Hb-O_2 affinity in bar-headed geese could be largely attributed to the three α-chain substitutions. Using site-directed mutagenesis, they then synthesized all possible evolutionary intermediates between bar-headed and greylag goose α-globin and found that the effect sizes of the substitutions depended on the genetic background in which they were expressed. These experiments demonstrate that, in the presence of epistasis, mutational effects can be highly dependent on the sequential order of amino acid substitutions.

In another study, Anthony Signore (University of Nebraska, Lincoln) aimed to find the genetic basis of the exceptional hypoxia tolerance of Tibetan mastiffs, a dog bred as a flock guardian at high altitudes in the Himalayas. The adult haemoglobin of Tibetan mastiffs has a higher O_2 binding affinity than the haemoglobin variant present in grey wolves and domestic dog breeds and differs by two amino acid replacements in the β-chain subunit of haemoglobin. Three-dimensional structural modelling suggested that these substitutions together result in the formation of two additional intrahelical H-bonds that are predicted to increase the reactivity of β-haeme in the deoxy state. Signore synthesized and experimentally tested each possible combination of ancestral/derived amino acids at the two positions and found that indeed both substitutions are necessary to confer the increased O_2 affinity. This result suggests that the adaptive potential of each individual amino acid substitution in increasing haemoglobin-O_2 is not realised in isolation but only in the presence of the other substitution. Not only do the approaches used by Storz and Signore functionally characterize the effects of specific mutations, they also help emphasize the role of historical contingency in shaping mutational pathways through which evolution proceeds.

The mutations discussed here all occurred within a single gene of relatively large effect. Understanding more complex interactions among mutations in different genes represents an even more challenging objective for future research. For example, how do the mutational effects of molecular systems as a whole (such as regulatory networks) differ from the distributions of their constituent parts? Approaches that leverage genome-wide data sets to identify genome-wide patterns of epistasis (Skwark et al., 2017) may prove fruitful for understanding the role of epistasis in adaptation in increasingly complex scenarios.

2.3 | Lesson 3: Pleiotropy constrains and facilitates multi-trait adaptation

An integrated understanding of adaptation should incorporate how individual adaptive traits and loci are nested within trait and gene networks that interact with one another. Indeed, a fundamental question in evolutionary biology, dating back at least to RA Fisher, concerns the degree to which pleiotropy shapes adaptation (Fisher, 1930; Orr, 2005). Pleiotropy is often thought to constrain adaptation because multiple phenotypic effects of a single allele are unlikely to align in the direction of selection (Agrawal & Stinchcombe, 2009; Mitchell-Olds, 1996), although putative cases of adaptive pleiotropy have been described in nature (Lovell et al., 2013). As our understanding of gene networks and interactions grows, it is also critical that we identify the molecular mechanisms underlying pleiotropic allelic effects. Do pleiotropic mutations in transcription factors and other genes that form network "hubs" constrain or otherwise alter evolutionary trajectories? How do new variants alter network interactions, and is compensatory evolution necessary and/or common at other loci in the network?

High-altitude adaptation often involves a suite of evolutionary responses across a hierarchy of phenotypes. Thus, understanding how this adaptation proceeds at the genetic level may provide important insights into how pleiotropy shapes adaptation. Rena Schweizer (University of Montana) presented work on the functional consequences of selection on *Epas1*, a gene encoding a transcription factor with well-studied pleiotropic effects (Semenza, 2001, 2012). *Epas1* is the hub of a transcriptional network regulating myriad responses to hypoxia. Defying the theoretical predictions outlined above, genome-wide scans for selection have repeatedly shown *Epas1* to be the target of natural selection in several high-altitude species, including high-altitude human populations, defying the theoretical predictions outlined above (Beall et al., 2010; Huerta-Sánchez et al., 2014; Li et al., 2014; Newman et al., 2014; Xu et al., 2011; Yi et al., 2010; Zhang et al., 2014). However, the phenotypic effects of genetic variation at *Epas1* are not well-characterized, though they have been linked to putatively adaptive changes in red blood cell production and haemoglobin concentration in humans (Simonson et al., 2010). In their research in high-altitude deer mice (*Peromyscus maniculatus*), Schweizer and colleagues find that a single nonsynonymous mutation in *Epas1* is associated with the maintenance of heart rate under hypoxia, which is critical maintaining a steady supply of oxygenated blood to the body. Heart rate is but one of a suite of responses to hypoxia regulated by *Epas1*, including red blood cell production, changes to vascular tone (constriction and dilation of blood vessels) and metabolic fuel use. How a single mutation can influence one of many integrated responses, and not the others, is unknown, but may involve compensatory mutations at other interacting loci that change how *Epas1* affects its downstream targets (Figure 1).

The work by Schweizer et al. highlights the ongoing debate about the role of highly pleiotropic loci like transcription factors in adaptive evolution. As evidenced by the ubiquity of selection on *Epas1* (along with other genes with putatively pleiotropic effects on hypoxia signalling; Xiang et al., 2013), adaptive evolution may often act on transcription factors, although only if the antagonistic effects are minimized. Indeed, alternative theory predicts that pleiotropic loci may actually be efficient targets of natural selection, because

changes in signalling and gene regulation can lead to adaptive changes across many phenotypes all at once (Lynch & Wagner, 2008; Wagner, 2012). More studies that characterize the phenotypic effects of mutation at transcription factors, and detail the mechanisms by which their antagonistic effects are minimized, are needed to resolve the debate of when and how pleiotropy constrains or facilitates adaptation. Moreover, how mutational change at multiple loci lead to further constraint or facilitation should, although likely challenging, form the basis of future research.

2.4 | Lesson 4: The translation of genotype to phenotype is context dependent

The environment shapes how genetic variation is expressed. Phenotypic plasticity, when a single genotype produces multiple phenotypes in response to the environment, has well-documented influences on evolution and adaptation (Ghalambor, McKay, Carroll, & Reznick, 2007; Levis & Pfennig, 2016; Pfennig et al., 2010; Pigliucci, Murren, & Schlichting, 2006; Schlichting & Pigliucci, 1998). Organisms also take advantage of predictable environmental variation to time critical life history events. Several speakers at the UNVEIL Symposium highlighted the importance of measuring phenotypes in the environment an organism naturally experiences and how environmentally-dependent phenotypes contribute to adaptive evolution (Figure 1).

Jonathan Velotta (University of Montana) explored the role of plasticity in adaptation to high-altitude in deer mice. In this system, phenotypic plasticity in erythropoiesis (the production of red blood cells) is adaptive under one set of environmental conditions, but maladaptive in another. At low altitude, erythropoiesis improves blood oxygen carrying capacity in response to temporary bouts of tissue-level hypoxia, driven by anaemia for example. At high altitude (>4,000 m above sea level), however, chronically elevated erythropoiesis driven by environmental hypoxia can cause hypertension and cardiac hypertrophy, both of which are associated with high-altitude diseases in lowlanders. Velotta found that high-altitude deer mice have evolved to suppress the erythropoietic response to hypoxia, which is likely adaptive over the long term under the chronically hypoxic conditions experienced at altitude (Velotta, Ivy, Wolf, Scott, & Cheviron, 2018). They suspect that this suppression is the result of compensatory evolution, and not the result of selection directly on the genes involved in erythropoiesis; it is indeed likely that evolution to suppress maladaptive plasticity may in general be achieved by compensatory changes that protect an organism's internal homeostatic environment against perturbations from the external environment (Velotta & Cheviron, 2018).

Studying the role of phenotypic plasticity in thermal adaptation, Kristi Montooth (University of Nebraska, Lincoln) described her work investigating the patterns of cellular membrane adaptation to temperature among populations of *Drosophila melanogaster*. By measuring membrane composition and plasticity in populations experimentally evolved in constant or temporally variable thermal environments, Montooth and her former graduate student, Brandon Cooper (now at the University of Montana) found that laboratory populations evolved in the presence of temporally variable temperatures exhibit greater developmental plasticity in cellular membrane composition and have greater fecundity than the populations evolved at constant temperatures (Condon, Cooper, Yeaman, & Angilletta, 2014; Cooper, Hammad, Fisher, Karty, & Montooth, 2012). She then extended this work to natural populations of *Drosophila melanogaster*, sampled at three different latitudes (Vermont, Indiana, and North Carolina, USA). Consistent with the patterns observed in the laboratory-evolved populations, they found that flies from the most variable thermal environment (Vermont, USA) showed greater plasticity in membrane composition than other populations (Cooper, Hammad, & Montooth, 2014). Combining results from these experiments, they found that the magnitude of developmental plasticity in the natural populations was similar to that of laboratory populations evolved in a temporally variable environment, but was greater than that of populations evolved at constant temperatures. This suggests that membrane plasticity comes at a cost as thermal variability maintained plasticity, while evolution in constant thermal environments relaxed selection on plasticity.

The above examples illustrate how plasticity is involved in adaptation to new or changing environments. Benjamin Blackman (University of California, Berkeley) explored a related question in his keynote address: how do organisms use predictable environmental cues to coordinate development and reproduction? Blackman addressed this question by studying solar tracking in sunflowers (*Helianthus annuus*) and flowering phenology of the yellow monkeyflower (*M. guttatus*) across elevational gradients. In sunflowers, predictable, 24 hr day-night cycles are required for the eponymous solar tracking in developing plants and the cessation of tracking coincides with floral maturation (Atamian et al., 2016). This ensures that flowers face east, warming faster in the morning and attracting more pollinators.

In the yellow monkeyflower, Blackman showed how populations have finely tuned day length requirements for flowering such that higher elevation populations require longer days to flower, consistent with the later start to the growing season at high elevation (Kooyers, Greenlee, Colicchio, Oh, & Blackman, 2015). To map the genetic variation underlying this phenological switch, Blackman and colleagues used QTL mapping in crosses between plants from low- and high-elevation populations across multiple transects in California, finding that the minimum day length requirement for floral induction was a highly polygenic trait across the range. Each individual transect revealed a small number of major QTL (Flagel et al., 2018), but nearly all of these QTL were geographically local. Further, they found little overlap between QTL for critical photoperiod and the QTL for days to flower under an inductive day length indicating that these two aspects of phenology can evolve through independent means. Combined, Blackman's work showed us how plants use the sun as a predictable cue to coordinate and time development and also that, because of heterogeneity in other aspects of the environment, locally adapted populations can evolve different responses to the same cues.

The studies outlined in this section demonstrate how much we have to learn about how organismal experience modulates the connection between genotype and phenotype (see Verhoeven, Vonholdt, & Sork, 2016 and references therein). As these traits are likely to be governed by multiple genes, our capacity to draw connections between genotype, phenotype, and the environment will largely depend on the genetic architecture of plasticity and regulatory networks, which has been hotly debated for years (Via et al., 1995). Large-scale "network-based" or "pathway-based" studies that associate phenotypic variation with variation in gene networks rather than individual genes are promising ways forward to interrogating more complex genotype-to-phenotype maps, particularly in cases where no large-effect loci can be identified (Califano, Butte, Friend, Ideker, & Schadt, 2012). Moreover, the observation that one genotype can produce multiple phenotypes speaks to the broader influence of the environment on gene expression, including the understudied impacts of epigenetic modification and inheritance on adaptive phenotypes.

2.5 | Lesson 5: Gene flow acts as both a sieve and source of large-effect adaptive variation

Understanding the consequences of gene flow for adaptation in populations is a central goal in evolutionary biology. The interplay between gene flow and selection affects how variation is partitioned across geography and among populations. Gene flow homogenizes genetic variation between populations, counteracting divergent selection in local environments and potentially swamping local adaptation (Haldane, 1930). However, theory suggests that alleles with large selective effects are less likely to experience gene swamping and may therefore contribute to adaptation under gene flow (Kawecki & Ebert, 2004; Yeaman & Whitlock, 2011). Furthermore, gene flow can also result in the sharing of large-effect adaptive genetic variation across geographically and evolutionarily distant populations (Hedrick, 2013). Thus, gene flow may play a crucial role in structuring large-effect adaptive variation in populations (Tigano & Friesen, 2016). Speakers at the UNVEIL Symposium explored the extremes of these scenarios: where local adaptation takes place across the distance of a few metres, and where gene flow across species boundaries promotes adaptation to common selective pressures.

Studying yellow monkeyflower populations in Yellowstone National Park, Lila Fishman (University of Montana) described a single locus (*out6*) under strong local selection between plants adapted to lush bog habitats and those adapted to harsh soils near the park's geysers and thermal pools. Variation at *out6* is associated with multiple phenotypes which differ between the thermal plants and nonthermal plants and alternative haplotypes at *out6* have swept through both habitats. In Yellowstone, adaptation truly is local because mosaicism of thermal and nonthermal habitats exists on the scale of metres. The potential for gene flow across such short distances may mean that the only way for adaptation to proceed is through few loci of large effect. These findings provide striking evidence of how populations can undergo finescale local adaptation enabled by selection on large-effect loci.

Matthew Jones (University of Montana) presented work demonstrating a case of introgression in the origin of adaptive variation in snowshoe hares (Jones et al., 2018). Across most of North America, snowshoe hares transition from brown summer coats to white winter coats to match seasonally snow-covered environments. However, populations in the Pacific Northwest, where winters are mild, remain brown in the winter. Using a GWAS of polymorphic populations, Jones identified a single locus that explains winter coat colour: the pigmentation gene *Agouti*. To trace the origins of winter-brown and winter-white coat colour alleles, Jones sequenced the genomes of closely-related species and found that the winter-brown *Agouti* allele in snowshoe hares was unexpectedly similar to the *Agouti* allele in black-tailed jackrabbits. Using population genetic simulations under realistic demographic histories, Jones ruled out incomplete lineage sorting of winter-brown and winter-white alleles, confirming that the evolution of brown winter coats in snowshoe hares arose from hybridization with black-tailed jackrabbits. Furthermore, the winter-brown allele shows strong signatures of recent positive selection in the Pacific Northwest, demonstrating hybridization led to adaptive introgression in mild winter environments.

These studies provide empirical support for the hypothesis that gene flow can favour adaptation via large-effect variants by either swamping small effect alleles (e.g., when gene flow occurs between closely related populations) or by seeding large-effect variation (e.g., when gene flow occurs between distantly related populations or species). Again, our understanding of the genetics of adaptation in the context gene flow remains biased towards detecting large-effect variation, which may skew our inferences of its relative importance. Indeed, some theoretical models suggest that small-effect mutations may contribute to adaptation through gene flow under certain scenarios (Yeaman, 2015). Future research that dissects the variants underlying polygenic adaptation in the face of gene flow remains a pressing need.

3 | THE EVIDENCE FOR THE PREDICTABILITY OF EVOLUTION

An ongoing debate in evolutionary biology is whether, and to what degree, evolution is predictable (Blount, Lenski, & Losos, 2018; Gould, 1989; Lewontin, 1974). Answering this question is fundamental in the era of rapid environmental change, where predicting evolutionary responses may aid management of vulnerable species. Below we offer some insight into the question of evolutionary predictability using examples from the UNVEIL Symposium.

Several studies presented at the symposium demonstrated repeatability in evolutionary outcomes by studying phenotypic convergence, which is strong evidence for adaptation to common selective pressures (Conte, Arnegard, Peichel, & Schluter, 2012; Storz, 2016). What is apparent from this work is that for traits with

both simple and complex genetic architectures, the mutational targets of adaptive phenotypes may be surprisingly consistent across unrelated species.

From Sally Aitken (University of British Columbia), we know that there is a surprising amount of convergence at the genetic level among distantly related species that have adapted to similar temperature regimes: Aitken and her group have shown that genetic variation in a suite of 47 genes is associated with spatial variation in temperature and cold hardiness between two species of conifer trees separated by 150 million years of evolution (Yeaman et al., 2016). This suggests that adaptation of complex physiological traits like cold tolerance might be constrained to specific loci.

Work by Rena Schweizer and Matthew Jones suggest that the same loci of large phenotypic effect are repeatedly used in distantly related species that adapt to similar environmental pressures: selection on *Epas1* in deer mice and *Agouti* in hares join a long list of organisms that have experienced selection at these particular loci in response to high-altitude hypoxia and cryptic coloration, respectively. For *Epas1*, different mutations have been identified in different species (Beall et al., 2010; Li et al., 2014; Newman et al., 2014; VonHoldf, Fan, Vecchyo, & Wayne, 2017; Yi et al., 2010; Zhang et al., 2014), though the effects of these mutations differ among species. The genetic basis of variation in coat colour polymorphism provide clear examples of convergent evolution (Manceau, Domingues, Linnen, Rosenblum, & Hoekstra, 2010) though the mechanisms by which such mutations act to produce phenotypic change are often different across species.

Research presented at the UNVEIL Symposium demonstrates what appears to be a fundamental discovery in studies of convergent evolution: although selection may act on the same gene or suites of genes, it is not always, or even commonly, by way of the same mutation or amino acid change. This is most likely because the functional consequences of a mutation are dependent on the genetic background in which the mutation arises (see *Lesson 2* above; Figure 1). Work from Jay Storz's laboratory, for example, has eloquently demonstrated this point: convergence in haemoglobin-oxygen affinity across high-altitude avian taxa proceeds via unpredictable changes at key amino acid residues, and the functional effect of each mutation depends on the genetic context in which it is expressed (Natarajan et al., 2018). Thus, although the genes that underlie adaptive evolution may be predictable in that they are repeated targets of selection, the precise mechanisms by which any single mutation leads to adaptive trait variation is highly species- and context-dependent.

One take away from the UNVEIL Symposium is that we are beginning to understand the molecular details underlying when and how evolution takes predictable paths. Our hope is that these studies will serve as models for future work. Moving forward, the most challenging aspect of predicting evolution will be to integrate a genomic understanding of variation and its history with an understanding of how new mutations will interact with existing genetic backgrounds and the environment to alter phenotypes in adaptive ways. This, in essence, is the goal and purpose of UNVEIL.

4 | CONSERVATION IMPLICATIONS OF GENOME-TO-PHENOME-TO-FITNESS MAPS

Is it simply an academic exercise to explore the genetic underpinnings of adaptive traits, or can this information have practical relevance for conservation? While debate surrounds the precise role of genomics in conservation (Garner et al., 2016; Kardos & Shafer, 2018; McMahon, Teeling, & Hoglund, 2014; Shafer et al., 2015), understanding adaptive variation and predicting evolutionary responses to environmental change is a fundamental goal shared between evolutionary and conservation biology (Flanagan, Forester, Latch, Aitken, & Hoban, 2018).

Nancy Chen (University of Rochester) presented a keynote address on her work understanding the factors shaping temporal allele frequency changes in the federally threatened Florida Scrub Jay (*Aphelocoma coerulescens*), which have experienced significant population declines over the last century due to habitat destruction and fragmentation (Chen, Cosgrove, Bowman, Fitzpatrick, & Clark, 2016). An ongoing long-term study of population demography at the Archbold Biological Station has monitored and tracked the ancestry of individuals in the study area since 1969. Leveraging this rich pedigree information, Chen has partitioned the variance in allele frequency change through time due to drift and immigration, providing precise measurements of how fragmentation and connectivity between populations influences temporal patterns of genetic variation (Chen et al., 2016, 2019). This expectation for neutral allele frequency change through time – when also combined with individual reproduction, survival, and morphology data – can be used as a powerful approach to identify signatures of rapid adaptation in vulnerable species.

A number of talks at the UNVEIL Symposium bridged basic and applied disciplines to inform conservation. Sally Aitken described how studying selection for adaptive traits in conifers (Yeaman et al., 2016) and engaging directly with foresters helped improve artificial selection for locally adapted seedlings for reforestation efforts (Aitken & Bemmels, 2016). Similarly, Michael Buchalski (California Department of Fish and Wildlife) spoke about work linking local adaptive variation to landscape features in desert bighorn sheep to inform translocation strategies and management unit delineation, providing both basic and applied insights. Marketa Zimova's (University of Montana) presentation on seasonal camouflage in hares highlighted the importance of understanding adaptive variation for predicting biological responses to climate change. Hares suffer severe fitness costs when mismatched against their background and these costs are likely to be more severe under climate change (Zimova, Mills, & Nowak, 2016). Zimova's research into both heritable and plastic variation for coat colour phenology provides fundamental insights into the potential for rapid adaptation to climate change. These talks highlighted the importance of genotype-phenotype-fitness connections for predicting evolutionary responses to climate change and guiding conservation efforts that explicitly consider evolutionary processes and natural genetic and phenotypic variation.

As we learn more about how genetic variation translates to adaptive phenotypic variation, gene-editing technologies (e.g., CRISPR-Cas9) may become appealing conservation tools to directly facilitate rapid adaptation, eliminate disease, or remove invasive species (e.g., using gene drives: Esvelt, Smidler, Catteruccia, & Church, 2014). And while these technologies continue to improve, ethical implications of their use should give us pause (Esvelt & Gemmell, 2017). To explore these implications, the symposium ended with a workshop led by conservation ethicist Dane Scott (University of Montana) and graduate students of the UNVEIL Network who participated in his course on conservation ethics and biotechnology. Scott first introduced the 2009 "President's Commission for the Study of Bioethical Issues" as a framework for discussing and developing ethical guidelines for biotechnology in conservation. With respect to the utility of gene editing for human health, the Commission advocated "prudent vigilance" in the application of these technologies, recognizing both their immense positive potential and their risks. To help define the idea of prudent vigilance as it relates to conservation, graduate students of Dane's conservation bioethics class highlighted potential costs and benefits of gene editing across three conservation case studies: reintroduction of blight-resistant American chestnut, eradication of devil facial tumour disease, and mitigation of coral bleaching. Workshop participants then formed groups to consider the relative merits of gene editing strategies in these cases.

Groups often reached the conclusion, "just because we can doesn't mean we should". For instance, while manipulating blight-resistant genes in American chestnut was generally perceived as both highly effective and with low risk of genetic side-effects, the large-scale reintroduction of this functionally extinct species may come with massive unintended ecological consequences. Similarly, discussions highlighted the challenge of establishing ethical guidelines while technologies remain in flux. A consensus emerged that the current state of gene editing is not sufficiently advanced to alleviate major risks potentially inherent to gene drive, such as incidental spread beyond a target population (Esvelt & Gemmell, 2017). On this point, group discussions further emphasized the importance of democratic deliberation at a global-scale. While the intended effects of gene drives may be local (e.g., removal of invasive rats in New Zealand), the incidental spread of genetically modified organisms harbouring gene drives could have massive global ramifications. Thus, conservation actions involving genetic modifications with the potential of incidental spread should involve a global risk-benefit assessment.

Local discussions of both the goals and implementation of conservation actions are also essential for successful conservation. Levi Collier-Robinson, Aisling Rayne, and Roger Moraga (The University of Canterbury) brought this point home while discussing their conservation work with native New Zealand fauna. A major component of this work involves engagement of local Māori communities, in the conservation and management of taonga (treasured) species. By working with locals to perform minimally invasive sampling of these species, their research is helping to bridge gaps between western science and the culture of native communities. As local communities often have significant associations with species, their voices are likely to provide unique perspectives on species of conservation concern that emphasize their intrinsic or cultural value.

5 | THE UNVEIL SYMPOSIUM: SMALL CONFERENCE, BIG IMPACT

The research presented at the UNVEIL Symposium exemplified the need to explore the genotype-phenotype map in an integrated way to advance our understanding of adaptation and inform conservation outcomes. Scientists seeing bar-headed geese fly over the Himalayas lead to discoveries about structure-function relationships within the haemoglobin molecule. Concerns by conservationists over land use change in the American West lead to a deeper understanding of genetic relatedness among bighorn sheep populations. The success of these studies and of the Symposium as a whole was due in large part to collaborations and conversations between geneticists, molecular biologists, ecologists, and conservationists. The Symposium's small format and diverse scientific program spurred these conversations and allowed participants to reach out beyond their focal discipline. We, the authors, believe that as our efforts to describe the connections between genotype, phenotype, and fitness become more integrative, the field will benefit from small meetings of researchers with diverse interests seeking a more complete understanding of the natural world.

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AUTHOR CONTRIBUTIONS

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