doi:10.1111/evo.14432

Evolution in small steps and giant leaps

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Received October 26, 2021

Accepted January 3, 2022

The first Editor of *Evolution* was Ernst Mayr. His foreword to the first issue of *Evolution* published in 1947 framed evolution as a "problem of interaction" that was just beginning to be studied in this broad context. First, I explore progress and prospects on understanding the subsidiary interactions identified by Mayr, including interactions between parts of organisms, between individuals and populations, between species, and between the organism and its abiotic environment. Mayr's overall "problem of interaction" framework is examined in the context of coevolution within and among levels of biological organization. This leads to a comparison in the relative roles of biotic versus abiotic agents of selection and fluctuating versus directional selection, followed by stabilizing selection in shaping the genomic architecture of adaptation. Oligogenic architectures may be typical for traits shaped more by fluctuating selection and biotic selection. Conversely, polygenic architectures may be typical for traits shaped more by directional followed by stabilizing selection and abiotic selection. The distribution of effect sizes and turnover dynamics of adaptive alleles in these scenarios deserves further study. Second, I review two case studies on the evolution of acquired toxicity in animals, one involving cardiac glycosides obtained from plants and one involving bacterial virulence factors horizontally transferred to animals. The approaches used in these studies and the results gained directly flow from Mayr's vision of an evolutionary biology that revolves around the "problem of interaction."

KEY WORDS: **Adaptation, coevolution, evolutionary biology, genomes, oligogenic, omnigenic.**

Interaction, Adaptation, and Effect Size

In his foreword for the first issue of *Evolution* published on March 1, 1947, Ernst Mayr, the Editor (then at the American Museum of Natural History), wrote (Mayr 1947):

> Evolution is a problem of interaction: interaction of parts in the organism; interaction between individuals and populations; interactions between different species; and interaction of the genetic world with the inorganic. The study of evolution in this broad meaning is still in its infancy.

This framing of evolution as a "problem of interaction" still rings true 75 years later. Mayr echoed the same questions that Charles Darwin (1859) posed and that continue to inspire us: "How have all those exquisite adaptations of one part of the organisation to another part, and to the conditions of life, and of one distinct organic being to another, been perfected? We see these beautiful co-adaptations most plainly in the woodpecker and missletoe; and only a little less plainly in the humblest parasite which clings to the hairs of a quadruped or feathers of a bird…"

With the benefit of hindsight, we might now include as "parts in the organism" interactions between biomolecules within and between cells. One "problem of interaction" nested within this level is epistasis—interaction between proteins, RNA, and DNA as well as interactions within each. The implications of pervasive epistasis in evolution are profound (Phillips et al. 2000). Epistasis was central to Wright's shifting balance theory (Wright 1931; Provine 1971; Wade and Goodnight 1991, 1998; Mallet 2010), a baroque model of adaptation that has been criticized (Whitlock et al. 1995; Coyne et al. 1997; Peck et al. 1998; Coyne et al. 2000). Yet, epistasis is, without question, important in adaptation in a narrower sense: within supergenes and single genes through intramolecular epistasis, and between these loci and the rest of the genome via modifiers (Fisher 1930; Ford 1953; Elena and Lenski 1997a; Kunte et al. 2014; Thompson and Jiggins 2014; Charlesworth 2016; Starr and Thornton 2016).

Dominant among the next two types of Mayr's problems of interaction are those between individuals of the same or different species. Biotic interactions were paramount in Charles Darwin's "struggle for existence" and are at the heart of Van Valen's Law of Constant Extinction and Red Queen Hypothesis (Van Valen 1973). The Red Queen Hypothesis posits that limited resources set up a zero-sum game within ecological communities. Not only are co-occurring lineages all improving in fitness over time, each must also respond to adaptation of the others. Under this model, constant co-adaptation creates similar risks of extinction across lineages. Van Valen explicitly addressed one the most important macroevolutionary problems of all—the persistence of lineages through time—with not just a microevolutionary process in a vague sense, but an ecologically explicit one: reciprocal natural selection.

Pimentel's "genetic feed-back mechanism" (Pimentel 1961), Maynard Smith's elaboration and critique of Van Valen's model (Smith 1976), and Levin and Udovic's model of genetic coevolution (Levin and Udovic 1977) provide a mechanistic foundation for how coevolution might actually unfold in ecological communities. Recognition of the links between microevolutionary process and macroevolution pattern is one of the great achievements of the Evolutionary Synthesis (Charlesworth et al. 1982; Futuyma 2015). Niche construction theory and its descendant (Fututyma 2017), eco-evolutionary feedback theory, are also salient because the abiotic and the biotic are further integrated (Lewontin 1983; Odling-Smee 1988; Odling-Smee et al. 2003; Levins and Lewontin 1985).

Drilling down to lower levels of organization, a model of coevolution could also be applied to independently assorting loci within or between genomes of a cell (Elena and Lenski 1997a; Rand et al. 2004; Reik and Lewis 2005; Wade 2007; Haig 2010; Lynch and Hagner 2015; McLaughlin and Malik 2017; Sloan et al. 2018; Lund-Hansen et al. 2021). This grand view of epistasis, of Mayr's "interaction between parts of the organism," grades into the evolution of conflict and cooperation within and between genes, chromosomes, cells, colonies or tissues, organs, organ systems, individuals, populations, and species (Queller and Strassmann 2009).

A central question arising from Mayr's "problem of interaction" is whether the way evolution unfolds is inherently different depending on which type of interaction is at play (Dittmar et al. 2016). Orr's model of adaptation (Orr 1998, 2005), which builds on Fisher's geometric (Fisher 1930), shows that when the environment suddenly changes, mutations of larger effect fix earlier on in the adaptive walk than later, as the optimum is approached. Pleiotropy is the principal driver. The ingenuity of this model is that it also provides a bridge between the infinitesimal and macromutationist models (Connallon and Hodgins 2021).

The ultimate substrate on which natural selection acts, genetic variation, is also a "problem of interaction." This is evidenced by the high level of genetic variation in fitness in natural populations that may stem in part from balancing selection (Charlesworth 2015), hybridization and resulting adaptive introgression (Hedrick 2013; Marques et al. 2019; Edelman and Mallet 2021; Moran et al. 2021), and a growing appreciation for the role of horizontal gene transfer (Mi et al. 2000; Koonin et al. 2001; Pace et al. 2008; Husnik and McCutcheon 2018; Rossoni et al. 2019), including in our own lineage (Mi et al. 2000). Large phenotypic effects of single alleles cannot easily be dismissed as being unimportant evolutionarily (Orr and Coyne 1992).

If for the sake of argument we consider them as wholly separate environments, the biotic environment experienced by a sessile organism may be more dynamic than the abiotic. Constant ecological and evolutionary change in the local biotic environment creates fitness optima that are perpetually in flux for a focal population (Bell 2010). This population finds itself recurrently climbing the lower flanks of different fitness peaks in a rugged landscape. A population takes larger leaps when first climbing the hill and gets part-way up before tumbling back down to the bottom, only to begin again in a different direction, up a different hill. The Red Queen must continually begin to climb new hills to stay in the same place. Perhaps Orr said it best (Orr 2005): "…it is as though the population is forever taking a first step and the distribution of factors fixed must collapse to that given by Kimura (weighted by the distribution of mutational effects), not an exponential."

Such a scenario is rather different from directional selection and subsequent stabilizing selection in canonical models of adaptation (Dittmar et al. 2016). Under a fluctuating model, whether spatially or temporally varying, or both, instead of directional selection leading to fixation of adaptive alleles, selection is transient (Gillespie 1994). This can drive the maintenance of adaptive variants in a population over time or space (Levene 1953; Gillespie 1978; Gillespie and Turelli 1989; Elena and Lenski 1997b; Gloss et al. 2016; Wittman et al. 2017) even when directional and stabilizing selection are also occurring (Turelli and Barton 2004). Of course, a strongly adaptive allele in one environment, timepoint, or genotype can also be antagonistically pleiotropic or neutral in others (Anderson et al. 2013).

There are important implications if balancing selection in the broadest possible sense is more pervasive than we commonly assume, not simply for the genomic architecture of adaptation (Gloss and Whiteman 2016). Levels of genetic variation for fitness observed in natural populations are not fully explained by mutation-selection balance, recombination, or background selection (Charlesworth 2015). Do our underlying quantitative genetic and adaptive evolutionary models do balancing selection justice? Distinguishing between recent balancing selection and

incomplete selective sweeps is challenging, but deep neural networks show promise in that regard (Isilda et al. 2021).

What do effect size distributions in nature look like under fluctuating versus directional followed by stabilizing selection? It is difficult to know. However, there is some evidence for the conjecture that substitutions of larger effect size should underpin adaptive trait variation under biotic (fluctuating) versus abiotic (directional, followed by stabilizing) selection. Across plant species, including mostly crop or model organisms, quantitative trait loci (QTL) associated with responses to biotic agents of natural selection tend to be of larger effect size than those shaped by abiotic agents (Louthan and Kay 2011). Notwithstanding the confounding effects of QTL studies themselves on questions of effect size (Xu 2003; Beavis 2019), the findings warrant more study and an analysis comparing effect sizes of traits shaped by abiotic versus biotic agents of natural selection should be expanded to other taxa, including microbes and metazoans.

Although I have focused on the dichotomy between biotic and abiotic, as Lewontin noted (Lewontin 1970) in quoting Darwin, "a plant at the edge of a desert is said to struggle for life against the drought." Thus, the abiotic environment is subsumed in the "struggle for existence." However, Lewontin left out the end of Darwin's sentence: "…more properly it should be said to be dependent on the moisture." Still, in any case, Mayr's "inorganic environment" is a complex milieu to parse.

The abiotic environment includes proximal potential agents of natural selection like variation in atmospheric gas ratios, hydrostatic pressure, humidity, edaphic factors, salinity, sunlight, precipitation, prevailing wind, and temperature. Yet each of these is a partial by-product of Earth's climate control and feedback systems that arise from the interaction between the biotic and abiotic (Lovelock et al. 1972). Just as these environments can fluctuate dramatically over short intervals of space and time, similarly, individuals and propagules can move through an astonishing array of different habitats. For example, a bird can travel hundreds of kilometers and thousands of meters in elevation in a single day. It is difficult to disentangle the abiotic from the biotic as proximate drivers of adaptation in such cases.

As the seasons seesaw back and forth at higher latitudes across the year, "adaptive tracking" is the obvious expectation, whether for oligogenic traits like seasonal camouflage in response to predator pressure (Jones et al. 2018) or whole-scale physiological adaptation in response to temperature (Rudman et al. 2021). In the former case, introgression of large-effect loci through hybridization with jackrabbits is the genetic basis of this adaptation in snowshoe hares (Jones et al. 2018). The latter falls under a polygenic model (Rudman et al. 2021), but involves nontrivial effect sizes ($>2\%$) for *many* loci in *Drosophila melanogaster*, although causality of the alleles with respect to adaptation is unresolved.

More generally, the search for causative mutations underlying adaptive phenotypes, "the alleles that matter for evolution," and the underlying oligogenic model (Bell 2009) flowing from Orr (2000) have been criticized (Rockman 2012) for being fraught with "trait selection and publication bias." At the heart of the argument is that the larger, monogenic to oligogenic effect sizes found in textbook examples of adaptive trait evolution are exceptional and therefore unimportant for generally understanding how adaptation proceeds. The implication is that although striking, they are only flashes in the pan at best and products of typology and ascertainment bias at worst.

In Rockman's (2012) critique, another incisive one (Travisano and Shaw 2013), and a counterpoint to the first (Lee et al. 2014), the biotic versus abiotic question was not discussed. Yet, Lee et al. (2014) got close and exerted considerable effort pointing to the role for the QTN program in assessing the role of balancing selection in maintaining functionally important variation.

The dust seemed to settle on the fact that examples of large and intermediate effect size mutations underlying complex trait variation within species (oligogenic alleles) are important but exceptional because polygenic adaptation is the norm. Undergirding new enthusiasm for a return to the infinitesimal was the groundbreaking omnigenic model for complex trait variation (Boyle et al. 2017). This model flowed from the consideration of complex disease risk in humans and its predictions had immediate and important implications for the study of polygenic adaptation.

The omnigenic model posits that nearly universal "network pleiotropy" constrains the genomic architecture of adaptive traits such that "species adapt by small allele frequency shifts of many causal variants across the genome" (Boyle et al. 2017). Yes, core genes with a direct effect turn up in associations, but most are unrelated to the phenotype of interest. Because of pleiotropy, however, these hits are "real" because peripheral loci still coevolve with core genes to which they are peripherally connected, yielding a morass of alleles each of tiny effect on phenotype. The omnigenic model addresses the heart of Mayr's problem of "interaction of parts in the organism."

There was early support for a highly polygenic model in which adaptation is constrained by network pleiotropy center on human height (Turchin et al. 2012; Berg and Coop 2014; Robinson et al. 2015; Field et al. 2016; Racimo et al. 2018). Yet, the dataset was bedeviled by subtle effects of population stratification, which inflated population-level differences (Novembre and Barton 2018; Berg et al. 2019; Edge and Coop 2019; Sohail et al. 2019; Zaidi and Mathieson 2020). However, there may still be hope for using GWAS to dissect the genomic basis of polygenic adaptation: a new full likelihood model may circumvent many thorny confounding variables embedded in these datasets (Stern et al. 2021).

On the other hand, Mathieson (2020) argues that although we expect polygenic adaptation to be common in theory, it has been so hard to uncover because it is uncommon in nature. Three nonmutually exclusive mechanisms are proposed to explain this paradox (Mathieson 2020). In the first, which turns the omnigenic model on its head, oligogenic architecture is more pervasive and emerges from the fact that pleiotropy constrains allele frequency shifts to handfuls of loci. In the second, polygenic adaptation occurs, but is not easily detectable owing to a preponderance of phenomena such as fluctuating selection, gene by environment interaction, overdominance, or epistasis. Finally, variable mutational target sizes (Hermisson and Pennings 2005; Pritchard et al. 2010) and genetic architectures underlying different phenotypic responses to selection can lead to highly divergent outcomes from a genetical perspective—from sweeps to modest frequency shifts in alleles (Höllinger et al. 2019).

An important nuance is that the field has shifted from expecting hard sweeps of de novo mutations of large effect (Voight et al. 2006) to one wherein they are the exception (Hernandez et al. 2011). This shift back to an infinitesimal occurred in roughly the last 15 years.

I am only an observer to this rich field. Yet, from what I see, consilience may be near. Just as Orr's model bridged the infinitesimal and macromutationist models of adaptation (Connallon and Hodgins 2021), the fluctuating model and the directional selection followed by stabilizing selection model of adaptation can be seen as two sides of the same coin.

Hayward and Sella (2021) used simulations of finite populations to study effect size distributions and allele frequency dynamics during the initial adaptive response to the sudden change in an environmental variable. They found two distinct patterns over time. In the first phase of adaptation, large-effect alleles can play an important role in adaptation, but over long periods of time, they are instead entirely supplanted by moderate-effect and then small-effect alleles during an equilibration phase (Hayward and Sella 2021). The equilibration phase takes a very long time to complete ($\sim 2N_e$) generations and so whether supplantation occurs at all depends on how stable the environment is over a very long period of time. Flowing from this, might we expect fundamentally different genetic architectures of polygenic adaptation for populations and species with small versus large N_e?

The result of the analysis by Hayward and Sella (2021) implies that under fluctuating selection, we expect a different polygenic architecture of adaptation than under a directional followed by stabilizing selection scenario. Fluctuating selection imposes a perpetual state of the early stages of adaptation and therefore opens the door to a role for a distribution of effect sizes that has shifted toward larger effect size mutations. This may well explain many of the discrepancies between what appear to be cherry-picked oligogenes that end up in textbook examples of adaptation and what we know from the crop and animal science literature—that most quantitative traits that respond to artificial selection are highly polygenic.

Such a distinction in the genetic architectures of adaptation between continually shifting versus rapidly changing and subsequently stable optima may also explain findings like those of Louthan and Kay (2011) wherein the distribution of QTL skewed toward larger effect sizes for traits shaped by biotic selection (more dynamic) compared to those shaped by abiotic selection (less dynamic). Although shifts toward a continually moving optimum have been modeled (e.g., Matuszewski et al. 2014), applying Hayward and Sella's (2021) simulation approach to more dynamical selection scenarios in *n*-dimensional trait space would be useful. Superficially, the problem is similar to the disparity in the nature of mutations affecting fitness that segregate within populations compared to those that fix between them (Stern and Orgogozo 2008).

Returning to Mayr's "problem of interaction," in my view, the Red Queen helps explain patterns of evolution across the entire expanse of evolutionary time—through hundreds of millions of years in the fossil record to allele frequencies and their effect sizes in extant populations. Biotic interactions can generate rugged fitness landscapes and dynamic evolutionary responses, to which the adaptive response is more likely to have an oligogenic basis. Such a scenario lends itself to experimental dissection, leading to important advances in connecting genotype to phenotype as well as biases (Rockman 2012). Still, for biotic interactions and populations with large effective population sizes, oligogenes underpinning adaptive phenotypes are perhaps justifiably the null expectation. The return to the infinitesimal through the omnigenic is an important advance because of the predictive framework and explicit modeling it affords.

With all of this in mind, I now share insights from my own research on adaptation, which focus on two case studies that bear on several of Mayr's problems of interaction and interface with the specific issues raised above.

A Tale of Two Toxins

Over the past decade, research in our laboratory has focused on the role of toxins of biological origins in mediating interactions between species and particularly the genetic basis of adaptation in animals in response to dietary and pathogenic toxins. This research is highly biased toward studying the nature of alleles with large phenotypic effects on fitness.

In the two cases I will discuss, the toxins have been co-opted by animals. In the first case, the animal sequesters a dietary toxin from a plant, resulting in unpalatability to predators. In the second case, a gene encoding a toxin was horizontally transferred from a phage or bacterium to an animal where it may be used

as an immune effector, but interacted first with animals through bacterial endosymbionts.

Before I dive into the details, it is useful to observe that the evolution of toxicity can be transformative for animal phenotypes. Through it, animal phenotypes evolve from being cryptic to conspicuous, nocturnal to diurnal, small to large, fast to slow, solitary to social, local to widespread, neglectful to doting parents, and from short to long lived (Santos and Cannatella 2011).

On the one hand, organisms are more than the sum of their parts. Evolution somehow elegantly integrates disparate phenotypic modules to produce whole organisms (Wagner et al. 2007). On the other hand, pleiotropy constrains natural selection from simultaneously acting on different phenotypic modules (Stern and Orgogozo 2008). Yet, toxic animals thread this needle as disparate novelties evolve in lockstep, triggered by the invasion of new adaptive zones that protection from enemies allows (Santos and Cannatella 2011). The repeated evolution of acquired toxicity in animals therefore provides opportunity to resolve this important paradox in evolution (Giorgianni et al. 2020).

It seems reasonable to assume that adaptations like the evolution of toxicity will tend to have an oligogenic basis, affording an escape from the pull of omnigenic pleiotropy and mitigating the costs that complexity exacts (Orr 2000). A textbook example of oligogenic adaptation revolves around the use of cardiac glycoside (CG) heart poisons as chemical defenses and its study touches on many of Mayr's problems of interaction. Miriam Rothschild and collaborators in 1968 found that CGs are sequestered by larval monarch butterflies (*Danaus plexippus*) as they feed on CG-producing milkweed (Apocynaceae) plants (Reichstein et al. 1968). That same year, Lincoln Brower and collaborators discovered that CG levels in the monarchs could predict their degree of unpalatability to birds (Brower et al. 1968). The conclusion is that CGs protect monarchs from bird predation, which can explain the evolution of warning coloration in the milkweed butterfly lineage.

The intergenerational migration of monarchs from the northern prairies of the eastern United States and Canada to the oyamel fir forests of Mexico and back again is a wonder of the natural world. This migration is enabled by protection from predation afforded by the sequestered CGs. However, nature finds a way, and mixed flocks of black-backed orioles (*Icterus abeillei*) and black-headed grosbeaks (*Pheucticus melanocephalus*) may consume up to 9% of these butterflies in some of the overwintering colonies in Mexico, which translates to over 2 million individual monarchs per year (Fink and Brower 1981; Brower and Calvert 1985).

The foundation for unraveling how monarch butterflies could resist CGs was laid by William Withering, who in 1785 reported that extracts of foxglove (*Digitalis purpurea*) could successfully treat heart failure, in what would be the first human clinical trial in the European medical literature (Withering 1785). In 1960, Jens Skou discovered that the Na^+/K^+-ATPase (sodium pump) could be blocked by the CG g-strophanthin or ouabain, derived from waabaayo, Somali for arrow poison (Jez 2021), from *Acokanthera* and *Strophanthus* trees in East Africa. Skou (1960) suspected that this was "presumably by interfering with the binding of the cations for the enzyme…"

In 1992, Ferdinand Holzinger and colleagues reported that the monarch's sodium pump activity was not altered by the presence of ouabain, unlike that of the control species, the tobacco hornworm (*Manduca sexta*) that did not eat CG-containing plants (Holzinger et al. 1992). When they sequenced the first extracellular loop of the sodium pump (ATPα), where ouabain was thought to bind, a histidine (H) had replaced an asparagine (N) at position 122, in the monarch relative to the other species. They suspected that this substitution might alter the binding affinity of ouabain. Then in 1996, Holzinger and Michael Wink addressed this hypothesis by swapping the asparagine for the histidine at position 122 using the native *D. melanogaster ATPα* copy and then tested for CG-resistance in vitro after transfecting the mutant construct in human embryonic kidney cells (Holzinger and Wink 1996). The cells expressing ATPα with the histidine at 122 were highly resistant to ouabain, unlike those carrying the asparagine.

In 2012, two research teams identified convergently evolved amino acid substitutions in the sodium pump across insects that fed on CG-producing plants (Aardema et al. 2012; Dobler et al. 2012; Zhen et al. 2012). Paralogs were also discovered encoding sodium pump copies that were expressed differentially across tissues, suggesting the potential for subfunctionalization and performance-resistance trade-offs (Zhen et al. 2012).

Particularly striking were parallel changes at positions 111 and 122 across a diversity of CG-feeding insects. Substitutions at these and other sites evolved in a stepwise fashion within the milkweed butterflies, producing sodium pump enzymes that generally also showed stepwise increases in resistance when isolated from representative species. Some fed but did not sequester CGs, others sequestered at lower levels, culminating with the monarch, which sequestered the most (Petschenka et al. 2013).

Although it was clear that amino acid substitutions at ATPα positions 111 and 122 were critical to CG-resistance in vitro and when expressed heterologously in cell lines, it was not clear if they were sufficient to explain adaptation to the high levels of CGs present in sequestering species (Petschenka and Agrawal 2015), which exceed the level of digitalin used to treat human patients, nor was it clear why what appeared to be the most resistant substitution to a histidine at 122 evolved last.

To address these questions, we mapped amino acid ancestral character states in the sodium pump across the insect phylogeny and identified which had evolved to derived character states when

CG feeding or sequestration arose (Karageorgi et al. 2019). We found other amino acid sites that coevolved with those that fixed as CG feeding and sequestration evolved, including position 119. This opened the door to the possibility that substitutions at 119 (Crambert et al. 2004; Pegueroles et al. 2016; Yang et al. 2019) were somehow directly or indirectly evolved in the adaptive walk that in the milkweed butterflies began with changes at 111 and 119 followed by those at 122.

Using two rounds of CRISPR-Cas9-mediated homology-directed repair, we swapped the derived mutations that fixed in the monarch lineage because it diverged from a common ancestor with the ithiomiine butterflies into the native *D. melanogaster ATPα* as the background. We found (Karageorgi et al. 2019) that substitutions at three amino acid positions (111, 119, and 122) in ATPα fixed in a predictable fashion in insect species that fed on CG-bearing plants, which resulted in full resistance to CGs in vivo in *D. melanogaster*.

The adaptive walk taken by the monarch butterfly because its lineage diverged from a common ancestor shared with the ithiomiines was recapitulated in vivo in an animal with no historical association with CG-bearing plants. Flies carrying the monarch's three substitutions at 111, 119, and 122 in native ATPα copies were nearly completely resistant to dietary ouabain levels that were as high as 30 mM. Structural variation in CGs other than ouabain causes marked variability in inhibition of the sodium pump across CG-adapted insect species, but their effects on *D. melanogaster* are not well known (Groen et al. 2017; Petschenka et al. 2018).

We also went back in evolutionary time (Dean and Thornton 2007) and engineered single and double *D. melanogaster ATPα* mutants that evolved along the way to the monarch lineage, as well as those that never evolved, yet conferred CG resistance. The paradox of why H122 conferred the most resistance but evolved last can be resolved if this mutation is also costly pleiotropically. A neurological phenotype (bang-induced seizure sensitivity [Schubiger et al. 1994]) exacted by H122 may be partially ameliorated through epistasis with S119 (Karageorgi et al. 2019; Taverner et al. 2019). Such intramolecular epistasis may be common in the evolution of target site insensitivity to toxins (Tarvin et al. 2017).

Epistasis of another kind is required to explain why the effect size of L111 and S119 on CG resistance when they occurred together was far more than that conferred by either mutation alone. Positive sign epistasis between L111 and S119 may also help to explain the order of the substitutions in the monarch and the other CG-resistant insects with parallel changes.

Both additivity and positive epistasis contributed to CG resistance as the milkweed butterflies climbed up the CG-resistance fitness peak. Our results were complementary to an independent study that also used *D. melanogaster* as an in vivo model to test hypotheses of adaptation to CGs in insects (Taverner et al. 2019).

We recently reported another set of parallel CG-resistance substitutions at these same sites in natural enemies of CG-feeding insects (Groen and Whiteman 2021). It was known that the black-headed grosbeak was physiologically resistant to CGs in the monarchs on which they feasted, but the mechanism was unclear (Fink and Brower 1981). The next question was whether there were also convergently evolved resistance substitutions in this bird. Frogs and snakes that feed on toads that produce CGs themselves have evolved parallel amino acid changing substitutions (Ujvari et al. 2015; Mohammadi et al. 2021).

In the case of the monarchs, however, the toxins must traverse an additional trophic level: from plant to herbivore and then to predator. Among >150 birds species with genome sequences available on GenBank, two of the four ATPα copies in the black-headed grosbeak carried previously studied CG-resistance amino acid changing substitutions at 111 and/or 119. This includes two substitutions (L111 and S119) in one of the black-headed grosbeak ATPα copies that are also found in the ATPα of *Euploea* spp. butterflies that attack CG-bearing Apocynaceae. These two substitutions confer a large step in CG resistance through positive sign epistasis when engineered into *D. melanogaster* (Karageorgi et al. 2019).

The process of one adaptive substitution building on those that came before, through both additivity and intramolecular epistasis, was a gradual one in the milkweed butterflies. Turning to Mayr's "problem of interaction," a set of species interactions and interactions between amino acids in the molecular target all revolve around a toxin produced by a plant.

As the CGs flow through the ecosystem (Ferrer and Zimmer 2012), they apparently exert strong enough selective pressure to recurrently fix CG-resistance mutations across at least two higher trophic levels. This is in part explained by the extraordinary constraint imposed by the sodium pump's canonical function, which results in few allowable paths to target site insensitivity and many forbidden ones (Storz 2016; Ogbunugafor and Eppstein 2019). Intramolecular interactions between amino acid residues in the first extracellular loop of the sodium pump as well as interactions between those residues and the hydrogen bonds with the toxin are required to explain resistance to CGs (Crambert et al. 2004; Ogawa et al. 2009; Dobler et al. 2012; Zhen et al. 2012; Karageorgi et al. 2019).

An evolutionary cascade in response to an ecological one is echoed by newts and their snake predators, which have both adapted to tetrodotoxin found in the skin of newts through substitutions in toxin's target, voltage-gated sodium channels. The sequestered tetrodotoxin may be obtained through symbiotic bacteria living in the tissues of the newts (Geffeney et al. 2005; Vaelli et al. 2020). Interaction is the evolutionary leitmotif across and

within many levels of biological organization in these dietary-derived toxin systems.

Another toxin-mediated adaptation we have studied involves the *cytolethal distending toxin subunit B* (*cdtB*) gene. We discovered that this gene, which is widespread in Proteobacteria and Actinobacteria, was independently horizontally transferred multiple times into the genomes of insects. Specifically, *cdtB* was horizontally transferred ffrom bacteria or lysogenic bacteriophages into an ancestor of *Scaptomyza* and *Drosophila primaeva*, an ancestor of the *Drosophila ananassae* species subgroup, and an ancestor of *Drosophila biarmipes*, all in the Drosophilidae (Verster et al. 2019). This gene was also horizontally transferred to an ancestor of the black cherry and peach aphids in the genus *Myzus* and is a pseudogene in the Russian Wheat Aphid, *Diuraphis noxia*, a member of the same tribe as *Myzus*. Remarkably, the *cdtB* copies in the non-*Scaptomyza* drosophilids and aphids encode the same intron-exon splice junctions. This gene has also been found in the genomes of cecidomyiid gall midges and thrips (Verster et al. 2021).

CDTB is the active subunit of the tripartite Cytolethal Distending Toxin (CDT) holotoxin. CDT is well-studied because it has been linked to gastrointestinal illness, including cancers, in humans (Talley et al. 2019). The *cdtB* gene was also found in the genome sequences of the secondary endosymbiotic bacterium *Hamiltonella defensa* that infects pea aphids (*Acyrthosiphon pisum*) and several other sap-feeding insects (Moran et al. 2005; Rouïl et al. 2020; Boyd et al. 2021).

Previously *cdtB* was known only from diverse mammalian-associated Proteobacteria and Actinobacteria, including *Escherichia coli*. Moran et al. found that *cdtB* was one of several cargo genes within a toxin cassette of the associated bacteriophage (named APSE-2). Experiments pea aphids infected with *H. defensa* strains positive for the toxin cassette containing *cdtB* were then found to be resistant to parasitoid wasp attack (Oliver et al. 2009; Brandt et al. 2017). Given this context, when we discovered horizontally transferred copies of *cdtB* in insect genomes, it was convenient that it was already being used to address a number of fundamental biological questions that intersected with evolutionary biology.

CDTB functions as a DNase I and/or phosphatase when imported into eukaryotic cells, resulting in cell cycle arrest and apoptosis (Nesić et al. 2004). We hypothesized that the horizontally transferred *cdtB* genes may protect flies and aphids from parasitoid wasp attack as it likely does when expressed in *H. defensa* (Verster et al. 2019). Is this a potential case of saltational evolution in the narrow sense? A bacterial toxin gene is introduced several times independently from prokaryotes, resulting in the instantaneous evolution of a new enzyme in the animals.

Saltational evolution is a multifarious term that has taken on many forms over time, but most conceptions of it have been rightly abandoned (Futuyma 2015). Another way of looking at *cdtB* is that this gene evolved gradually in prokaryotes over deep evolutionary time and was then horizontally transmitted to a diverse suite of insects as a macromutation, resulting in a new physiological trait. In this light, although both small steps and giant leaps describe the nature of this potential adaptation, the entire picture is wholly consistent with the Evolutionary Synthesis. A research frontier in this system is in understanding how a new gene encoding a eukaryotic toxin became integrated into an animal gene network repeatedly (Husnik and McCutcheon 2018).

Returning to Mayr's "problem of interaction"—in this case, the system is one of interactions across many levels of biological organization: between the insect host and parasitoid wasp enemy; between insect host and bacterial symbiont; between bacterial symbiont and bacteriophage; between *cdtB* and other genes in the toxin cassette; between *cdtB* and other genes in the insect genome after horizontal gene transfer (HGT); between DNA and DNAase; and between *cdtB* (and CDTB) and other insect-encoded gene and their products in the network in which it has evolved. Top-down pressure from parasitoids or other enemies may have driven its evolution in insect symbionts, which are now obviated through HGT of genes carried in toxic "cargo." The five independent origins of *cdtB* in insects are probably the tip of the iceberg. As in the case of the CGs, a toxin that first evolved in lower trophic levels was harnessed by higher trophic levels that is potentially repurposed in defense of the organism, and the same evolutionary path is taken by different lineages.

Anastomoses between vertically descending branches of the tree of life form through hybridization, horizontal gene transfer events, and symbioses (Clausen 1951). These mechanisms were once thought to be of little importance in adaptation, especially in animals, but this conclusion, in my view, is no longer justified. The tree of life may be more accurately conceived of as a braided river of life.

I used Mayr's "problem of interaction" as a window into understanding the extent to which evolution is gradual or saltational in these two case studies of defensive toxicity in insects—one in which toxins are obtained from plants and the other in which a gene encoding a toxic enzyme was obtained from prokaryotes. In the first example, we see that when gradual evolution proceeds via both smaller and larger steps through additivity and intramolecular epistasis. Genotype-dependent or "background" effects such as positive intramolecular epistasis serve as a phenotypic rachet, pulling a population up the lower flanks of a fitness peak—just as *Euploea* spp. butterflies and black-headed grosbeaks seem to have done with the same amino acid substitutions L111 and S119 in the sodium pump. In the second example, HGT is a mechanism through which a macromutation may confer a new trait in an animal lineage, but at the same time, the gene first evolved gradually in bacteria and their phages. The

importance of lateral movement of genes sensu lato in adaptation is now dogma in microbiology (through HGT; Arnold et al. 2021) and botany (through hybridization; Stebbins 1950; Anderson and Stebbins 1954), but its importance in animals has been less appreciated until recently.

Conclusion

The dialectical nature of Mayr's "problem of interaction" remains the *sine qua non* of evolutionary biology. Over deep evolutionary time, an explicit coevolutionary model arising from a zero-sum game is a useful heuristic because it can be explained by a microevolutionary process. Interactions between species produce a rugged fitness landscape that may produce a genomic architecture of adaptation distinct from that expected by directional selection followed by stabilizing selection. A higher proportion of mutations of larger effect may typify responses to selection driven by fluctuations in optima as a result. Top-down and bottom-up ecological forces as well as intramolecular epistasis shape the evolution of acquired toxicity. Predictable evolutionary cascades are triggered by ecological ones.

Returning to our 75th anniversary theme, the Evolutionary Synthesis solidified the dogma that *natura non facit saltus* (Darwin 1859). In other words, evolutionary change is gradual. Genomics and genome editing have allowed not only the ability to measure effect sizes of mutations fixed along adaptive walks in vivo, but the nature of the alleles themselves, both their provenance and phenotypic effects. These new tools have reaffirmed gradual nature of evolution by natural selection overall (Linnen et al. 2013), a tenet of the Evolutionary Synthesis (Smocovitis 2009). However, they have also unearthed new insights into old questions on the Mayr's synthetic "problem of interaction," particularly regarding the roles of pleiotropy, epistasis, laterally acquired genes, and predictability itself in adaptation.

AUTHOR CONTRIBUTIONS

NKW was involved in all aspects of analysis and writing.

ACKNOWLEDGMENTS

C. Miller, M. Nachman, J. Peláez, C. Rushworth, and V. B. Smocovitis provided constructive feedback on earlier drafts of the manuscript. NKW was supported by a grant from the National Institute of General Medical Sciences of the National Institutes of Health (award no. R35GM119816).

DATA ARCHIVING

There are no data to be archived.

LITERATURE CITED

Aardema, M. L., Y. Zhen, and P. Andolfatto. 2012. The evolution of cardenolide-resistant forms of Na+,K+ -ATPase in Danainae butterflies. Mol. Ecol. 21:340–349.

Anderson, E., and G. L. Stebbins Jr. 1954. Hybridization as an evolutionary stimulus. Evolution 8:378–388.

Anderson, J. T., C.-R. Lee, C. A. Rushworth, R. I. Colautti, and T. Mitchell-Olds. 2013. Genetic trade-offs and conditional neutrality contribute to local adaptation. Mol. Ecol. 22:699–708.

Arnold, B. J., I.-T. Huang, and W. P. Hanage. 2021. Horizontal gene transfer and adaptive evolution in bacteria. Nat. Rev. Microbiol. https://doi.org/10.1038/s41579-021-00650-4.

Beavis, W. D. 2019. QTL analyses: power, precision, and accuracy. Pp. 145–162 *in* A. H. Paterson, ed. Molecular dissection of complex traits. CRC Press, Boca Raton, FL.

Bell, G. 2010. Fluctuating selection: the perpetual renewal of adaptation in variable environments. Philos. Trans. R. Soc. Lond. B Biol. Sci. 365: 87–97.

Berg, J. J., and G. Coop. 2014. A population genetic signal of polygenic adaptation. PLoS Genet. 10:e1004412.

Berg, J. J., A. Harpak, N. Sinnott-Armstrong, A. M. Joergensen, H. Mostafavi, Y. Field, E. A. Boyle, X. Zhang, F. Racimo, J. K. Pritchard, et al. 2019. Reduced signal for polygenic adaptation of height in UK Biobank. eLife 8:e39725.

Boyd, B. M., G. Chevignon, V. Patel, K. M. Oliver, and M. R. Strand. 2021. Evolutionary genomics of APSE: a tailed phage that lysogenically converts the bacterium *Hamiltonella defensa* into a heritable protective symbiont of aphids. Virol. J. 18:219.

Boyle, E. A., Y. I. Li, and J. K. Pritchard. 2017. An expanded view of complex traits: from polygenic to omnigenic. Cell 169:1177–1186.

Brandt, J. W., G. Chevignon, K. M. Oliver, and M. R. Strand. 2017. Culture of an aphid heritable symbiont demonstrates its direct role in defence against parasitoids. Proc. R. Soc. B Biol. Sci. 284:20171925.

Brower, L. P. and W. H. Calvert. 1985. Foraging dynamics of bird predators on overwintering monarch butterflies in Mexico. Evolution 39: 852–868.

Brower, L. P., N. R William, L. L. Coppinger, and S. C. Coppinger. 1968. Ecological chemistry and palatability spectrum. Science 161: 1349–1350.

Charlesworth, B. 2015. Causes of natural variation in fitness: evidence from studies of *Drosophila* populations. Proc. Natl. Acad. Sci. USA 112:1662–1669.

Charlesworth, B., R. Lande, and M. Slatkin. 1982. A neo-Darwinian commentary on macroevolution. Evolution 36:474–498.

Charlesworth, D. 2016. The status of supergenes in the 21st century: recombination suppression in Batesian mimicry and sex chromosomes and other complex adaptations. Evol. Appl. 9:74–90.

Clausen, J. 1951. Stages in the evolution of plant species. Cornell Univ. Press, Ithaca, NY.

Connallon, T., and K. A. Hodgins. 2021. Allen Orr and the genetics of adaptation. Evolution 75:2624–2640.

Coyne, J. A., N. H. Barton, M. Turelli. 1997. Perspective: A critique of Sewall Wright's shifting balance theory of evolution. Evolution 51 (3): 643–671. https://doi.org/10.2307/2411143

Coyne, J. A., N. H. Barton, and M. Turellli. 2000. Perspective: a critique of Sewall Wright's shifting balance theory of evolution. Evolution 51:343–671.

Crambert, G., D. Schaer, S. Roy, and K. Geering. 2004. New molecular determinants controlling the accessibility of ouabain to its binding site in human Na,K-ATPase alpha isoforms. Mol. Pharmacol. 65:335–341.

Darwin, C. 1859. On the origin of species. John Murray, Lond.

Dean, A. M., and J. W. Thornton. 2007. Mechanistic approaches to the study of evolution: the functional synthesis. Nat. Rev. Genet. 8: 675–688.

Dittmar, E. L., C. G. Oakley, J. K. Conner, B. A. Gould, and D. W. Schemske. 2016. Factors influencing the effect size distribution of adaptive substitutions. Proc. R. Soc. B Biol. Sci. 283:20153065.

Dobler, S., S. Dalla, V. Wagschal, and A. A. Agrawal. 2012. Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na,K-ATPase. Proc. Natl. Acad. Sci. USA 109:13040–13045.

Edelman, N. B., and J. Mallet. 2021. Prevalence and adaptive impact of introgression. Annu. Rev. Genet. 55:265–283.

Edge, M. D., and G. Coop. 2019. Reconstructing the history of polygenic scores using coalescent trees. Genetics 211:235–262.

Elena, S. F., and R. E. Lenski. 1997a. Test of synergistic interactions among deleterious mutations in bacteria. Nature 390:395–398.

———. 1997b. Long-term experimental evolution in *Escherichia coli*. VII. Mechanisms maintaining genetic variability within populations. Evolution 51:1058–1067.

Ferrer, R. P., and R. K. Zimmer. 2012. Community ecology and the evolution of molecules of keystone significance. Biol. Bull. 223:167–177.

Field, Y., E. A. Boyle, N. Telis, Z. Gao, K. J. Gaulton, D. Golan, L. Yengo, G. Rocheleau, P. Froguel, M. I. McCarthy, et al. 2016. Detection of human adaptation during the past 2000 years. Science 354:760–764.

Fink, L. S., and L. P. Brower. 1981. Birds can overcome the cardenolide defence of monarch butterflies in Mexico. Nature 291:67–70.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon, Oxford, U.K.

Ford, E. B. 1953. The genetics of polymorphism in the Lepidoptera. Adv. Genet. 5:43–87.

Futuyma, D. J. 2017. Evolutionary biology today and the call for an extended synthesis. Interface Focus 7(5):20160145. https://doi.org/10.1098/rsfs.2016.0145

Futuyma, D. J. 2015. Can modern evolutionary theory explain macroevolution? Pp. 29–85 *in* E. Serrelli and N. Gontier, eds. Macroevolution: explanation, interpretation and evidence. Springer International Publishing, Cham, Switzerland.

Geffeney, S. L., E. Fujimoto, E. D. Brodie, and P. C. Ruben. 2005. Evolutionary diversification of TTX-resistant sodium channels in a predator–prey interaction. Nature 434:759–763.

Gillespie, J. H. 1978. A general model to account for enzyme variation on natural populations. V. The SAS-CFF model. Theor. Popul. Biol. 14:1–45.

———. 1994. The causes of molecular evolution. Oxford Univ. Press, Oxford, U.K.

Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121:129–138.

Giorgianni, M. W., N. L. Dowell, S. Griffin, V. A. Kassner, J. E. Selegue, and S. B. Carroll. 2020. The origin and diversification of a novel protein family in venomous snakes. Proc. Natl. Acad. Sci. USA 117:10911–10920.

Gloss, A. G., and N. K. Whiteman. 2016. Balancing selection: walking a tightrope. Curr. Biol. 26:R73–R76.

Gloss, A. G., S. C. Groen, and N. K. Whiteman. 2016. A genomic perspective on the generation and maintenance of genetic diversity in herbivorous insects. Ann. Rev. Ecol. Evol. Syst. 47:165–187.

Groen, S. C., and N. K. Whiteman. 2021. Convergent evolution of cardiac-glycoside resistance in predators and parasites of milkweed herbivores. Curr. Biol. 31:R1465–R1466.

Groen, S. C., E. R. LaPlante, N. M. Alexandre, A. A. Agrawal, S. Dobler, and N. K. Whiteman. 2017. Multidrug transporters and organic anion transporting polypeptides protect insects against the toxic effects of cardenolides. Insect Biochem. Mol. Biol. 81:51–61.

Haig, D. 2010. Colloquium papers: transfers and transitions: parent-offspring conflict, genomic imprinting, and the evolution of human life history. Proc. Natl. Acad. Sci. USA 107:1731–1735.

Hayward, L. K., and G. Sella. 2021. Polygenic adaptation after a sudden change in environment. bioRxiv. https://doi.org/10.1101/792952.

Hedrick, P. W. 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol. Ecol. 22:4606–4618.

Hermisson, J., and P. S. Pennings. 2005. Soft sweeps: molecular population genetics of adaptation from standing genetic variation. Genetics 169:2335–2352.

Hernandez, R. D., J. L. Kelley, E. Elyashiv, S. C. Melton, A. Auton, G. McVean, 1000 Genomes Project, G. Sella, and M. Przeworski. 2011. Classic selective sweeps were rare in recent human evolution. Science 331:920–924.

Höllinger, I., P. S. Pennings, and J. Hermisson. 2019. Polygenic adaptation: from sweeps to subtle frequency shifts. PLoS Genet. 15:e1008035.

Holzinger, F., and M. Wink. 1996. Mediation of cardiac glycoside insensitivity in the monarch butterfly (*Danaus plexippus*): role of an amino acid substitution in the ouabain binding site of Na+,K+-ATPase. J. Chem. Ecol. 22:1921–1937.

Holzinger, F., C. Frick, and M. Wink. 1992. Molecular basis for the insensitivity of the Monarch (*Danaus plexippus*) to cardiac glycosides. FEBS Lett. 314:477–480.

Husnik, F., and J. P. McCutcheon. 2018. Functional horizontal gene transfer from bacteria to eukaryotes. Nat. Rev. Microbiol. 16:67–79.

Isilda, U., A. Stella, and M. Fumagalli. 2021. Distinguishing between recent balancing selection and incomplete sweep using deep neural networks. Mol. Ecol. Resour. 21:2706–2718.

Jez, J. 2021. Encyclopedia of biological chemistry. 3rd ed. Elsevier, Amsterdam, The Netherlands.

Jones, M. R., L. S. Mills, P. C. Alves, C. M. Callahan, J. M. Alves, D. J. R. Lafferty, F. M. Jiggins, J. D. Jensen, J. Melo-Ferreira, and J. M. Good. 2018. Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. Science 360:1355–1358.

Karageorgi, M., S. C. Groen, F. Sumbul, J. N. Pelaez, K. I. Verster, J. M. Aguilar, A. P. Hastings, S. L. Bernstein, T. Matsunaga, M. Astourian, et al. 2019. Genome editing retraces the evolution of toxin resistance in the monarch butterfly. Nature 574:409–412.

Koonin, E. V., K. S. Makarova, and L. Aravind. 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Annu. Rev. Microbiol. 55:709–742.

Kunte, K., W. Zhang, A. Tenger-Trolander, D. H. Palmer, A. Martin, R. D. Reed, S. P. Mullen, and M. R. Kronforst. 2014. *doublesex* is a mimicry supergene. Nature 507:229–232.

Lee, Y. W., B. A. Gould, and J. R. Stinchcombe. 2014. Identifying the genes underlying quantitative traits: a rationale for the QTN programme. AoB Plants 6:plu004.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331-333.

Levin, S. A., and J. D. Udovic. 1977. A mathematical model of coevolving populations. Am. Nat. 111:657–675.

Levins, R., and R, Lewontin. 1985. The Dialectical Biologist. Harvard University Press, Cambridge.

Lewontin, R. C. 1983. Gene, Organism and Environment, In D. S. Bendall (ed.), Evolution: from Molecules to Men, Cambridge University Press, Cambridge.

Lewontin, R. C. 1970. The units of selection. Annu. Rev. Ecol. Syst. 1:1–18.

Linnen, C. R., Y. P. Poh, B. K. Peterson, R. D. Barrett, J. G. Jensen, and H. E. Hoekstra. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science 339:1312–1316.

Louthan, A. M., and K. M. Kay. 2011. Comparing the adaptive landscape across trait types: larger QTL effect size in traits under biotic selection. BMC Evol. Biol. 11:60.

Lovelock, J. E., R. J. Maggs, and R. A. Rasmussen. 1972. Atmospheric dimethyl sulphide and the natural sulphur cycle. Nature 237:452–453.

Lund-Hansen, K. K., C. Olito, E. H. Morrow, and J. K. Abbott. 2021. Sexually antagonistic coevolution between the sex chromosomes of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 118:e2003359118.

Lynch, M., and K. Hagner. 2015. Evolutionary meandering of intermolecular interactions along the drift barrier. Proc. Natl. Acad. Sci. USA 112:E30–E38.

Mallet, J. 2010. Shift happens: shifting balance and the evolution of diversity in warning colour and mimicry. Ecol. Entomol. 35:90–104.

Marques, D. A., J. I. Meier, and O. Seehausen. 2019. A combinatorial view on speciation and adaptive radiation. Trends Ecol. Evol. 34:531–544.

Mathieson, I. 2020. Human adaptation over the past 40,000 years. Curr. Opin. Genet. Dev. 62:97–104.

Matuszewski, S., J. Hermisson, and M. Kopp. 2014. Fisher's geometric model with a moving optimum. Evolution 68:2571–2588.

Mayr, E. 1947. Foreword. Evolution 1:i–ii.

McLaughlin, R. N., Jr., and H. S. Malik. 2017. Genetic conflicts: the usual suspects and beyond. J. Exp. Biol. 220:6–17.

Mi, S., X. Lee, X. Li, G. M. Veldman, H. Finnerty, L. Racie, E. LaVallie, X. Y. Tang, P. Edouard, S. Howes, et al. 2000. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature 403:785–789.

Mohammadi, S., L. Yang, A. Harpak, S. Herrera-Álvarez, M. del Pilar Rodríguez-Ordoñez, J. Peng, K. Zhang, J. F. Storz, S. Dobler, and A. J. Crawford. 2021. and Others. Concerted evolution reveals co-adapted amino acid substitutions in Na+ K+-ATPase of frogs that prey on toxic toads. Curr. Biol. 31:P2530–P2538.

Moran, B. M., C. Payne, Q. Langdon, D. L. Powell, Y. Brandvain, and M. Schumer. 2021. The genomic consequences of hybridization. eLife 10:e69016.

Moran, N. A., P. H. Degnan, S. R. Santos, H. E. Dunbar, and H. Ochman. 2005. The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. Proc. Natl. Acad. Sci. USA 102:16919–16926.

Nesić, D., Y. Hsu, and C. E. Stebbins. 2004. Assembly and function of a bacterial genotoxin. Nature 429:429–433.

Novembre, J., and N. H. Barton. 2018. Tread lightly interpreting polygenic tests of selection. Genetics 208:1351–1355.

Odling-Smee, F. J. 1988. Niche constructing phenotypes. In: Plotkin, H.C. (ed) The role of behavior in evolution. MIT Press, Cambridge.

Odling-Smee, F. J., K. N. Laland, and M. W. Feldman. 2003. Niche construction: the neglected process in evolution. In:Monographs in population biology 37. Princeton University Press, Princeton.

Ogawa, H., T. Shinoda, F. Cornelius, and C. Toyoshima. 2009. Crystal structure of the sodium-potassium pump (Na+,K+-ATPase) with bound potassium and ouabain. Proc. Natl. Acad. Sci. USA 106:13742–13747.

Ogbunugafor, C. B., and M. J. Eppstein. 2019. Genetic background modifies the topography of a fitness landscape, influencing the dynamics of adaptive evolution. IEEE Access 7:113675–113683.

Oliver, K. M., P. H. Degnan, M. S. Hunter, and N. A. Moran. 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325:992–994.

Orr, H. A. 2000. Adaptation and the cost of complexity. Evolution 54:13–20.

Orr, H. A. 1998. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52:935–949.

Orr, H. A., and J. A Coyne. 1992. The genetics of adaptation: a reassessment. Am. Nat. 140:725–742.

Orr, H. A. 2005. Theories of adaptation: what they do and don't say. Genetica 123:3–13.

Pace, J. K., 2nd, C. Gilbert, M. S. Clark, and C. Feschotte. 2008. Repeated horizontal transfer of a DNA transposon in mammals and other tetrapods. Proc. Natl. Acad. Sci. USA 105:17023–17028.

Peck, S. L., S. P. Ellner, and F. Gould. 1998. A spatially explicitstochastic model demonstrates the feasibility of Wright's shifting balance theory. Evolution 52:1834–1839.

Pegueroles, C., A. Ferrés-Coy, M. Martí-Solano, C. F. Aquadro, M. Pascual, and F. Mestres. 2016. Inversions and adaptation to the plant toxin ouabain shape DNA sequence variation within and between chromosomal inversions of *Drosophila subobscura*. Sci. Rep. 6: 23754.

Petschenka, G., and A. A. Agrawal. 2015. Milkweed butterfly resistance to plant toxins is linked to sequestration, not coping with a toxic diet. Proc. R. Soc. B Biol. Sci. 282:20151865.

Petschenka, G., S. Fandrich, N. Sander, V. Wagschal, M. Boppré, and S. Dobler. 2013. Stepwise evolution of resistance to toxic cardenolides via genetic substitutions in the Na + /K + ATPase of milkweed butterflies (Lepidoptera: Danaini): cardenolide resistance in milkweed butterflies. Evolution 67:2753–2761.

Petschenka, G., C. S. Fei, J. J. Araya, S. Schröder, B. N. Timmermann, and A. A. Agrawal. 2018. Relative selectivity of plant cardenolides for Na+/K+-ATPases from the monarch butterfly and non-resistant insects. Front. Plant Sci. 9:1424.

Phillips, P. C., S. P. Otto, and M. C. Whitlock. 2000. Beyond the average: the evolutionary importance of gene interactions and variability of epistatic effects. Pp. 21–38 *in* J. B. Wolf, E. D. Brodie III, and M. J. Wade, eds. Epistasis and the evolutionary process. Oxford Univ. Press, Oxford, U.K.

Pimentel, D. 1961. Animal population regulation by the genetic feed-back mechanism. Am. Nat. 95:65–79.

Pritchard, J. K., J. K. Pickrell, and G. Coop. 2010. The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. Curr. Biol. 20:R208–R215.

Provine, W. B. 1971. The origins of theoretical population genetics. Univ. of Chicago Press, Chicago.

Queller, D. C., and J. E. Strassmann. 2009. Beyond society: the evolution of organismality. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364:3143–3155.

Racimo, F., J. J. Berg, and J. K. Pickrell. 2018. Detecting polygenic adaptation in admixture graphs. Genetics 208:1565–1584.

Rand, D. M., R. A. Haney, and A. J. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. 19:645–653.

Reichstein, T., J. von Euw, J. A. Parsons, and M. Rothschild. 1968. Heart poisons in the monarch butterfly. Science 161:861–866.

Reik, W., and A. Lewis. 2005. Co-evolution of X-chromosome inactivation and imprinting in mammals. Nat. Rev. Genet. 6:403–410.

Robinson, M. R., G. Hemani, C. Medina-Gomez, M. Mezzavilla, T. Esko, K. Shakhbazov, J. E. Powell, A. Vinkhuyzen, S. I. Berndt, S. Gustafsson, et al. 2015. Population genetic differentiation of height and body mass index across Europe. Nat. Genet. 47:1357–1362.

Rockman, M. V. 2012. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution 66:1–17.

Rossoni, A. W., D. C. Price, M. Seger, D. Lyska, P. Lammers, D. Bhattacharya, and A. P. M. Weber. 2019. The genomes of polyextremophilic cyanidiales contain 1% horizontally transferred genes with diverse adaptive functions. eLife 8:e45017.

Rouïl, J., E. Jousselin, A. Coeur d'acier, C. Cruaud, and A. Manzano-Marín. 2020. The protector within: comparative genomics of APSE phages across aphids reveals rampant recombination and diverse toxin arsenals. Genome Biol. Evol. 12:878–889.

Rudman, S. M., S. I. Greenblum, S. Rajpurohit, N. J. Betancourt, J. Hanna, S. Tilk, T. Yokoyama, D. A. Petrov, and P. Schmidt. 2021. Direct observation of adaptive tracking on ecological timescales in Drosophila. bioRxiv. https://doi.org/10.1101/2021.04.27.441526.

Santos, J. C., and D. C. Cannatella. 2011. Phenotypic integration emerges from aposematism and scale in poison frogs. Proc. Natl. Acad. Sci. USA 108:6175–6180.

Schubiger, M., Y. Feng, D. M. Fambrough, and J. Palka. 1994. A mutation of the *Drosophila* sodium pump alpha subunit gene results in bang-sensitive paralysis. Neuron 12:373–381.

Skou, J. C. 1960. Further investigations on a Mg++ + Na+-activated adenosintriphosphatase, possibly related to the active, linked transport of Na+ and K+ across the nerve membrane. Biochim. Biophys. Acta 42:6–23.

Sloan, D. B., J. M. Warren, A. M. Williams, Z. Wu, S. E. Abdel-Ghany, A. J. Chicco, and J. C. Havird. 2018. Cytonuclear integration and co-evolution. Nat. Rev. Genet. 19:635–648.

Smith, J. M. 1976. A comment on the Red Queen. Am. Nat. 110:325–330.

Smocovitis, V. B. 2006. Keeping up with Dobzhansky: G. Ledyard Stebbins, Jr., plant evolution, and the evolutionary synthesis. Hist. Philos. Life Sci. 28:9-47.

———. 2009. The "Plant Drosophila": E. B. Babcock, the genus "Crepis, and the evolution of a genetics research program at Berkeley, 1915–1947. Hist. Stud. Nat. Sci. 39:300-355.

Sohail, M., R. M. Maier, A. Ganna, A. Bloemendal, A. R. Martin, M. C. Turchin, C. W. Chiang, J. Hirschhorn, M. J. Daly, N. Patterson, et al. 2019. Polygenic adaptation on height is overestimated due to uncorrected stratification in genome-wide association studies. eLife 8:e39702.

Starr, T. N., and J. W. Thornton. 2016. Epistasis in protein evolution. Protein Sci. 25:1204–1218.

Stebbins, G. L., Jr. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.

Stern, A. J., L. Speidel, N. A. Zaitlen, and R. Nielsen. 2021. Disentangling selection on genetically correlated polygenic traits via whole-genome genealogies. Am. J. Hum. Genet. 108:219–239.

Stern, D. L., and V. Orgogozo. 2008. The loci of evolution: how predictable is genetic evolution? Evolution 62:2155–2177.

Storz, J. F. 2016. Causes of molecular convergence and parallelism in protein evolution. Nat. Rev. Genet. 17:239–250.

Talley, N. J., G. Holtmann, M. M. Walker, G. Burns, M. Potter, A. Shah, M. Jones, N. A. Koloski, and S. Keely. 2019. Circulating anti-cytolethal distending toxin B and anti-vinculin antibodies as biomarkers in community and healthcare populations with functional dyspepsia and irritable bowel syndrome. Clin. Transl. Gastroenterol. 10:e00064.

Tarvin, R. D., C. M. Borghese, W. Sachs, J. C. Santos, Y. Lu, L. A. O'Connell, D. C. Cannatella, R. A. Harris, and H. H. Zakon. 2017. Interacting amino acid replacements allow poison frogs to evolve epibatidine resistance. Science 357:1261–1266.

Taverner, A. M., L. Yang, Z. J. Barile, B. Lin, J. Peng, A. P. Pinharanda, A. S. Rao, B. P. Roland, A. D. Talsma, D. Wei, et al. 2019. Adaptive substitutions underlying cardiac glycoside insensitivity in insects exhibit epistasis in vivo. eLife 8:e48224.

Thompson, M. J., and C. D. Jiggins. 2014. Supergenes and their role in evolution. Heredity 113:1–8.

Travisano, M., and R. G. Shaw. 2013. Lost in the map. Evolution 67: 305–314.

Turchin, M. C., C. W. K. Chiang, C. D. Palmer, S. Sankararaman, D. Reich, Genetic Investigation of ANthropometric Traits (GIANT) Consortium, and J. N. Hirschhorn. 2012. Evidence of widespread selection on standing variation in Europe at height-associated SNPs. Nat. Genet. 44:1015–1019.

Turelli, M., and N. H. Barton. 2004. Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and G x E interactions. Genetics 166:1053–1079.

Ujvari, B., N. R. Casewell, K. Sunagar, K. Arbuckle, W. Wüster, N. Lo, D. O'Meally, C. Beckmann, G. F. King, E. Deplazes, et al. 2015. Widespread convergence in toxin resistance by predictable molecular evolution. Proc. Natl. Acad. Sci. USA 112: 11911–11916.

Vaelli, P. M., K. R. Theis, J. E. Williams, L. A. O'Connell, J. A. Foster, and H. L. Eisthen. 2020. The skin microbiome facilitates adaptive tetrodotoxin production in poisonous newts. eLife 9:e53898.

Van Valen, L. 1973. A new evolutionary law. Evol. Theory 1:1–30.

Verster, K. I., J. H. Wisecaver, M. Karageorgi, R. P. Duncan, A. D. Gloss, E. Armstrong, D. K. Price, A. R. Menon, Z. M. Ali, and N. K. Whiteman. 2019. Horizontal transfer of bacterial cytolethal distending toxin B genes to insects. Mol. Biol. Evol. 36:2105–2110.

Verster, K. I., R. L. Tarnopol, S. M. Akalu, and N. K. Whiteman. 2021. Horizontal transfer of microbial toxin genes to gall midge genomes. Genome Biol. Evol. 13:1–13.

Voight, B. F., S. Kudaravalli, X. Wen, and J. K. Pritchard. 2006. A map of recent positive selection in the human genome. PLoS Biol. 4: e72.

Wade, M. J. 2007. The co-evolutionary genetics of ecological communities. Nat. Rev. Genet. 8:185–195.

Wade, M. J., and C. J. Goodnight. 1991. Wright's shifting balance theory: an experimental study. Science 253:1015–1018.

———. 1998. Perspective: the theories of Fisher and Wright in the context of metapopulations: when nature does many small experiments. Evolution 52:1537–1553.

Wagner, G. P., M. Pavlicev, and J. M. Cheverud. 2007. The road to modularity. Nat. Rev. Genet. 8:921–931.

Whitlock, M. C., P. C. Phillips, F. B.-G. Moore, and S. J. Tonsor. 1995. Multiple fitness peaks and epistasis. Annu. Rev. Ecol. Syst. 26:601–629.

Withering, W. 1785. An account of the foxglove, and some of its medical uses: with practical remarks on dropsy and other diseases. M Swinney, Birmingham, U.K.

Wittman, M. J., A. O. Bergland, M. W. Feldman, P. S. Schmidt, and D. A. Petrov. 2017. Seasonally fluctuating selection can maintain polymorphism at many loci via segregation lift. Proc. Natl. Acad. Sci. USA 114:E9932–E9941.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Xu, S. 2003. Theoretical basis of the Beavis effect. Genetics 165:2259–2268.

Yang, L., N. Ravikanthachari, R. Mariño-Pérez, R. Deshmukh, M. Wu, A. Rosenstein, K. Kunte, H. Song, and P. Andolfatto. 2019. Predictability in the evolution of Orthopteran cardenolide insensitivity. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374:20180246.

Zaidi, A. A., and I. Mathieson. 2020. Demographic history mediates the effect of stratification on polygenic scores. eLife 9:e61548.

Zhen, Y., M. L. Aardema, E. M. Medina, M. Schumer, and P. Andolfatto. 2012. Parallel molecular evolution in an herbivore community. Science 337:1634–1637.