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Authors

Bolnick, Daniel I
Barrett, Rowan DH
Oke, Krista B
et al.

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(Non)Parallel Evolution

Daniel I. Bolnick[1,2*], Rowan Barrett[3], Krista Oke[3,4] , Diana J. Rennison[5] , Yoel E. Stuart[1]

[1] Department of Integrative Biology, University of Texas at Austin, Austin TX 78712, USA; danbolnick@utexas.edu; yestuart@utexas.edu

[2] Department of Ecology and Evolution, University of Connecticut, Storrs CT 06268, USA (as of 07/2018)

[3] Redpath Museum, McGill University, Montreal, Quebec, Canada; rowan.barrett@mcgill.ca

[4] Department of Ecology and Evolutionary Biology, University of California Santa Cruz, Santa Cruz, CA, 95060, USA; kristaoke@gmail.com

[5] Institute of Ecology and Evolution, University of Bern, 3012 Bern, Switzerland; diana.rennison@iee.unibe.ch

* Corresponding author: Email: danbolnick@utexas.edu Telephone: +011 (512) 471-2824

Running title: “(Non)Parallel Evolution”

Abstract

Parallel evolution across replicate populations has provided evolutionary biologists with iconic examples of adaptation. When multiple populations colonize seemingly similar habitats, they may evolve similar genes, traits, or functions. Yet, replicated evolution in nature or in the lab often yields inconsistent outcomes: some replicate populations evolve along highly similar trajectories, whereas other replicate populations evolve to different extents or in atypical directions. To understand these heterogeneous outcomes, biologists are increasingly treating parallel evolution not as a binary phenomenon but rather as a quantitative continuum ranging from nonparallel to parallel. By measuring replicate populations' positions along this "(non)parallel" continuum, we can test hypotheses about evolutionary and ecological factors that influence the likelihood of repeatable evolution. We review evidence regarding the distribution of (non)parallel evolution in the laboratory and in nature and enumerate the many genetic and evolutionary processes that contribute to variation in the extent of parallel evolution.

Key Words: Adaptation, Convergence, Divergence, Many-to-One Mapping, Nonparallel, Parallel Evolution

I. INTRODUCTION

Parallel evolution holds a special place in the annals of evolutionary biology because it provides strong evidence for adaptation. The replicated independent evolution of similar traits leads us to infer that evolution was driven by a deterministic process, most likely natural selection (Harvey & Pagel 1991). Biologists therefore use the repeated, parallel evolution of genes, phenotypes, or ecotypes to infer that (i) similar environments impose similar natural selection, (ii) there exist few solutions to this selection, and (iii) the traits or genes that evolve in parallel are adaptations. These inferences offer the hope that, in some situations, evolution may even be predictable enough that we can anticipate evolution of pests or disease-causing agents, or evolutionary responses to anthropogenic environmental change (Agrawal 2017, Day 2012, de Visser & Krug 2014, Langerhans 2017). However, this optimistic goal of predicting future evolution is only plausible if parallel evolution is common and strong.

There are many textbook cases of parallel evolution that have rightfully received a lot of attention (e.g., Colosimo et al 2005, Elmer et al 2014, Khaitovich et al 2005, Thompson et al 1997). But, are these representative of replicated evolution more generally, or have we given undue attention to a few exceptionally parallel genes, traits, or species? If we objectively surveyed replicate populations in similar habitats, how common and how extensive would parallel evolution be? What fraction of replicate populations would evolve in parallel, for what number of traits? Conversely, how often would replicate populations diverge genetically or phenotypically despite experiencing similar environments?

As we describe in this review, there is widespread evidence that replicate populations in similar environments sometimes evolve similar traits (or genes) and sometimes evolve dissimilar traits (or genes). Thus, we argue here that parallel evolution is best viewed as an extreme end of a quantitative continuum of '(non)parallel evolution' (see Fig. 1 for a visual glossary). Section II provides examples of this continuum of (non)parallel evolution, drawn from settings of practical interest (e.g., disease, agriculture) to motivate study of (non)parallelism. After addressing some semantics (Section III), we then describe approaches to quantify (non)parallel evolution (Section IV), what those measures have revealed (Section V), and what we learn about evolutionary biology more generally as a result (Section VI). Throughout this essay, we seek answers to questions such as: What evolutionary forces generate variation in (non)parallelism among replicate populations? What kinds of traits are more or less parallel? Perhaps most fundamentally: when we see deviations from parallel evolution, what are we to conclude about adaptation? Biologists use parallel evolution as evidence of adaptation, but

when evolution in similar environments falls toward the nonparallel end of the continuum, should we infer there is maladaptation, neutral evolution, or adaptation?

II. INCOMPLETELY PARALLEL EVOLUTION

Our first goal for this review is to motivate it. That is, we must establish that evolution is often less parallel than we might have reasonably expected. Intuitively, we expect that initially similar populations that are exposed to similar selection pressures will evolve similar phenotypic adaptations. As we show in this section, however, in many contexts this expectation is only partly true, and the examples of nonparallel evolution described here illustrate the need for quantitative rather than binary approaches to studying parallel evolution. In presenting these cases of (non)parallel evolution, we focus on evolution in highly applied contexts to convey the point that this evolutionary continuum has very practical consequences and should be considered in an interdisciplinary way.

II.1 (Non)parallelism in cancer

Cancer tumors are evolving populations of cells (Burrell et al 2013, Nowell 1976, Shpak & Lu 2016, Swanton 2014). Tumors originate when somatic mutations confer an 'escape' from normal cell cycle regulation. Growing tumors contain multiple genetically divergent cell lines that differ in their ability to proliferate, evade the immune system, resist chemotherapy, and metastasize. This genetic variation can therefore be subject to strong selection within a tumor. Typically, each cancer patient is an independent, replicated case of one or more oncogenic mutations that initiate a tumor and the subsequent clonal selection on additional mutations. If tumor evolution is highly parallel, then the same mutations in the same genes should evolve repeatedly in most or all patients. It is increasingly clear, however, that ostensibly similar tumors (i.e., same tissue and histology) often comprise fundamentally different mutations across patients.

In an experimental evolution study, Tegze et al. (2012) applied identical selection (18 months of chemotherapy) to 29 identical artificial tumors that were all derived from one breast cancer cell line. Only 18 of the 29 replicates evolved resistance, and within those resistant replicates, the underlying genetic changes were nonparallel, affecting different cell functions (Tegze et al 2012). This result highlights some key themes: first, even identical starting populations subjected to identical selection can exhibit nonparallel evolutionary responses.

Second, parallel evolution of resistance (an emergent function) occurred without parallel evolution of the underlying genes.

Such evolutionary inconsistency also occurs in real cancer patients. Takahashi et al (2007) compared allele frequency differences between primary versus metastatic lung tumor genomes to find targets of selection during metastasis. Most of these rapidly evolving genes experienced selection in only one or a few patients, and the rest were never shared by more than half the patients (Takahashi et al 2007). This (non)parallel evolution is why cancer treatment is increasingly reliant not on tissue type or histological traits but rather on personalized genomics to tailor therapies to the particular causal gene(s) in an individual (Abbosh et al 2017).

II.2 (Non)parallel evolution in pathogens

Like cancer, human pathogens show (non)parallel evolution in response to therapies and host immunity. In HIV patients with low viral load during drug therapy, an interruption to therapy often results in a rapid rebound of viral load. One study of 12 chronic HIV patients revealed that the HIV-1 gp120 gene evolved rapidly in each patient when they experienced this viral rebound (Martinez-Picado et al 2002). If gp120 evolved in parallel following therapy-interruption, we could potentially develop drugs targeting the gp120 variants that facilitate rapid viral rebound. However, for unknown reasons, different mutations contributed to this rebound in each patient, so we cannot develop therapies that anticipate gp120 evolution following treatment interruption.

Human macrophages protect against pathogenic strains of *Escherichia coli*, but this bacterium sometimes evolves immune-escape variants, leading to life-threatening illness. *In vitro* experimental evolution of *E.coli* in macrophage culture led to recurrent evolution of bacteria with increased resistance to macrophage attack (Ramiro et al 2016). But, the magnitude of this resistance differed among replicates, highlighting yet another major pattern of (non)parallel evolution. That is, although most replicate populations evolved resistance, the magnitude of resistance differed among cultures. This quantitative variation was attributed to the evolution of different genes within each replicate (i.e., nonparallel genetics), although most causal genes were part of the electron transport chain (i.e., parallel at the level of biochemical pathways). Notably, through pleiotropy, these electron transport changes made all resistant strains more sensitive to certain antibiotics (Ramiro et al 2016). This parallel pleiotropic change offers a therapeutic strategy for anticipating and combating evolution of *E.coli* resistance to macrophage attack.

II.3 (Non)parallelism in agriculture

Agricultural pests frequently evolve new mechanisms to subvert the herbicides and pesticides we use to control them. For example, *QoI* fungicides act to inhibit *cytochrome bc1* function in the mitochondria of fungi that damage crops. Several pathogenic fungi have evolved *QoI* resistance, using at least four independent mutations at the same cytochrome b codon (Torriani et al 2008). From this perspective, *QoI* resistance has evolved in parallel in two respects (the same phenotype caused by mutation to the same coding locus), but nonparallel in another (each of the four mutations are at separate SNPs), highlighting the general point that the extent of parallel change may differ across biological levels of organization. In this case, highly parallel evolution at the gene level makes it easier to monitor the spread of resistance through genetic screens, and to perhaps develop fungicides that target the new mutation as well. However, this parallel evolution is limited to certain pathogen species; in other fungal species nonparallel mutations confer resistance to *QoI* (Fernandez-Ortuno et al 2008).

Parallel evolution of domesticated species could reveal useful traits and genes for breeding strategies. The common bean was domesticated twice from wild *Phaeseolus vulgaris*, once in Mexico and once in the Andes (Bitocchi et al 2013), providing an unusual opportunity to consider (non)parallelism in the origins of a major agricultural resource (albeit with N=2). Across 27,197 genes surveyed, 1,835 and 748 exhibited signatures of selection in these respective geographic replicates, but only 59 appear to be selected in both regions (0.2% of all genes, which does not exceed null expectations) (Schmutz et al 2014). An equivalent result was seen for two independent instances of maize domestication at high altitude (Takuno et al 2015). Thus, artificial selection for domestication has involved largely nonparallel genomic changes in the few crops for which data are available. It would be fascinating to extend this type of analysis to more instances of domestication (e.g., replicate origins of fish aquaculture) to locate essential domestication genes as those evolving in parallel, or to identify nonparallel changes that might be combined for further improvements.

The cases described above illustrate several recurring themes in (non)parallel evolution. Most notably, when similar populations are exposed to similar selection pressures, only a subset of the replicates might experience evolution in the same way. That is, the magnitude and direction of evolution can differ among replicates, among traits, and across biological levels of organization (gene, pathway, trait, function). The same themes frequently apply to wild populations (e.g., CITATIONS). This multi-level continuum of (non)parallel evolution offers opportunities to learn more about evolutionary processes, as we describe below. To do so,

however, we first need clear terminology, and the quantitative tools for measuring where traits and populations fall along the (non)parallel continuum.

III. AN ASIDE ON TERMINOLOGY

The study of (non)parallel evolution has been the source of recurrent semantic disagreements. In the 150-year history of evolutionary biology, 'parallelism' first described simultaneous fossil record transitions across many continents (Darwin 1859). Later, evolutionary biologists used 'parallelism' to describe the similarity between embryological development and paleontological transitions (Cope 1876, Cope & Kingsley 1891, Packard 1898, Wilson 1941). The standard modern use of 'parallelism' emerged in the early 1900's (Nichols 1916, Osborn 1900, Vavilov 1922) following observations of recurrent similar mutations in *Oenothera* flowers (Gates 1912). This led Dobzhansky (1933) to suggest that "the essential similarity of the germ-plasm" predisposed related species to have similar mutations. However, Gates (1936) cautioned that this conclusion was premature: "In very few instances, either in plants or animals, has it been shown genetically that these parallelisms are due to the same gene in related species".

During this time, convergence was often conflated with parallelism (Haas & Simpson 1945), until Carl Hubbs clarified the distinction between homology and homoplasy (Hubbs 1944). G.G. Simpson (1961) provided a modern definition of parallel evolution as "the independent occurrence of similar changes in groups with a common ancestry and *because* they had a common ancestry." Common ancestry was crucial in Simpson's view, because it implied that initially similar populations evolved similar adaptations. This is in contrast to convergent evolution, which entails similar evolution but from initially dissimilar (less related) taxa (Gould 2002). The boundary between 'common ancestry' versus 'less related' is unclear, which has long blurred the distinction between parallel and convergent evolution (Arendt & Reznick 2008, Scotland 2011, Wake 1999). There is some debate whether common ancestry is even an important criterion. That is, phylogenetically closely related taxa are more likely to use similar genes to produce similar phenotypes (Conte et al 2012), whereas distantly related taxa more often use different genes when they converge phenotypically. But, there are examples of distantly related species that nevertheless use the same genes to adapt to the same challenge (Rosenblum et al 2010), and closely related populations that use different genes for the same phenotype (Sturm & Duffy 2012). This decoupling of shared genetics from recent ancestry has led some biologists to argue that there is no clear distinction between parallel and convergent

evolution (Arendt & Reznick 2008, Manceau et al 2011).

Developmental biologists, meanwhile, have used 'convergent' to describe the evolution of similar phenotypes but with different underlying genes or developmental pathways (Abouheif 2008; Baguñà & Garcia-Fernàndez 2003). From this point of view, ancestry is irrelevant, and the key distinction between convergent and parallel has to do with genetic mechanism. Evolution is parallel when the same gene caused the evolution of similar phenotypes in different groups (Rosenblum et al 2014). But, there is again a grey area between parallel and convergent: what constitutes sufficiently similar molecular explanations (Losos 2011, Wake et al 2011). For instance, evolution can be due to repeated change at the same gene but not the same nucleotide (Storz 2016). Or, for polygenic traits, evolution may reflected repeated changes at some causal loci but divergent evolution at others (Elmer & Meyer 2011).

Given the semantic ambiguities described above, some researchers have argued we should always just apply 'convergent' when talking about phenotypes, and 'parallel' to describe genes (Rosenblum et al 2014, Scotland 2011). Other researchers advocate dropping the term 'parallel' entirely (Arendt & Reznick 2008). An emerging alternative view is that the terms parallel and convergent (and their antonyms, nonparallel and divergent), can be defined in terms of the geometry of evolution in trait space (Fig. 1). *Parallel evolution* can then be defined as evolution of two (or more) populations in very similar directions in trait space (Fig. 1e). *Nonparallel evolution* is when populations evolve in different directions in trait space, which can encompass anything from weakly similar directions (Fig. 1d), orthogonal directions (Fig. 1c), to opposite directions (*antiparallel*; Fig. 1a). Finally, *(non)parallel* denotes the entire continuum illustrated in Fig. 1a-e). In contrast, convergent evolution occurs when derived populations are phenotypically more similar than their ancestral states were (Fig. 1g); divergence is the reverse (Fig. 1f).

IV. QUANTIFYING (NON)PARALLEL EVOLUTION

The semantic challenges in defining parallel or convergent evolution are, in part, a consequence of trying to make a binary decision (e.g., "parallel or not?") to describe a quantitative, multivariate, and multi-scale phenomenon. Therefore, a promising solution is to augment the binary approach with quantitative measures of *how* parallel or nonparallel evolution has been (Langerhans 2017, Oke et al 2017, Speed & Arbuckle 2017, Stuart et al 2017). Below, we summarize three widely-used approaches to quantifying where replicatens fall along this (non)parallel continuum. By quantifying (non)parallelism across many replicate populations, researchers can ask questions such as, "How do abiotic conditions, community ecology,

historical events, and genetic processes generate variation along this continuum?" We focus on phenotypic traits hereafter, with the understanding that the methods we describe can also be applied to other traits including protein structures (Rokas & Carroll 2008, Storz 2016), allele frequencies (Jones et al 2012), gene expression (Cooper et al 2003, Manousaki et al 2013, Velotta et al 2017), QTL effects (Conte et al 2015), etc..

IV.A Counting.

The simplest strategy when quantifying (non)parallelism is to 'vote count', estimating the probability that a given trait evolves in parallel (Orr 2005). For a given trait (and only one at a time), measured in multiple independently established populations, one can quantify the fraction of evolutionary transitions that go in a particular direction. This approach was used in the cancer and pathogen evolution examples described above. When 100% of the replicate populations evolve in the same direction, the case for parallel evolution seems clear (given enough populations). It may be more typical for only a subset of populations evolve in the same direction.

When interpreting vote counts, it is important to clearly define a null hypothesis. For a single quantitative trait evolving strictly neutrally, we would expect half the replicate populations to evolve in the same direction by chance. Using a sign test, one needs a minimum of 6 replicate populations to all evolve in the same direction for a given trait to reject the null hypothesis of random evolutionary change at a significance threshold of 0.05. For instance, in 16 replicate comparisons of parapatric lake and stream stickleback, in half the replicate pairs, stream fish had higher suction feeding ability than lake fish (Thompson et al 2017), no different from the null expectation. Thus, it was unclear whether suction feeding capacity was evolving neutrally or whether it was adaptive but selection itself was inconsistent among watersheds. In contrast, lake fish had more gill rakers than stream fish in 14 of 16 lake-stream pairs (Fig. 2A) (Stuart et al 2017).

IV.B Variance partitioning.

Vote-counting ignores variation in effect size. Populations might all evolve in the same direction but to different magnitudes. One approach to account for effect sizes was popularized by Langerhans and Dewitt (2004), assuming a researcher has quantitative trait data for one or more traits for multiple individuals in each of two (or more) categorically defined habitats. These habitats must be replicated across multiple locations (e.g., different islands, watersheds). One then estimates a statistical model that partitions trait variance among habitats, locations, and

habitat*location interactions. The main effect of habitat measures the extent to which between-habitat evolutionary divergence is shared across replicate locations (Fig. 2) and thus measures parallel evolution. The location effect summarizes properties unique to different replicates (e.g., different islands). Last, the habitat*location interaction measures how the direction or magnitude of between-habitat divergence is inconsistent among replicate populations, implying nonparallel evolution. A closely related method focuses on 'exchangeability' – a quantitative measure of the extent to which statistical classification tools correctly or incorrectly assign individuals to the correct habitat or location (Hendry et al 2013); high exchangeability implies strongly parallel evolution across independent replicate populations.

Variance partitioning has been applied to a wide variety of measures of population divergence including karyotopes (Dunn et al 2005), genomes (Ravinet et al 2016), physiology (Pfenninger et al 2015), and morphology (Langerhans & DeWitt 2004). For instance, an experimental comparison of inland versus coastal California poppies (*Eschscholzia californica*) in California and their invasive range in Chile found equally large effects of habitat, and habitat*location interactions, indicating that some different traits contributed to inland-coastal divergence in each region (Leger & Rice 2007).

This analytical approach is appealing because it builds on familiar statistical tools and provides multivariate, quantitative estimates of each effect: percent partial variance (Langerhans & DeWitt 2004) or r_2 (Langerhans 2017). The approach's weaknesses include ambiguity in interpreting the habitat*location interaction. A significant interaction could stem from variance in the direction of evolution, the magnitude of evolution, or both.

IV.C Vector analysis.

'Phenotypic Change Vector Analysis' (PCVA) offers a geometric definition of (non)parallelism (Adams & Collyer 2009, Collyer & Adams 2007, Collyer et al 2015) that we illustrate in Figure 3. Unlike variance partitioning, PCVA separately measures both magnitude and direction of evolution. For instance, Stuart et al (2017) used PCVA to show that the direction of phenotypic divergence between lake and stream stickleback depended on environmental variation, whereas the magnitude of divergence was best explained by gene flow (or the lack thereof).

PCVA requires replicate population pairs (e.g., ancestral and derived populations) that span some putative evolutionary change or habitat contrast. For each population, one calculates the phenotypic centroid in multivariate trait space (or the centroids for breeding values, gene expression, genomic data, etc.). The vector connecting one population's centroid to the other population's centroid gives a formal measure of the direction and magnitude of divergence

through trait-space (Fig. 3A). The longer the vector, the more divergent the paired populations are, while the orientation of a vector in trait-space describes the relative contributions of different traits to divergence between that pair of populations. To quantify (non)parallel evolution, one needs two such vectors representing replicated, independent trajectories (Fig. 3A) from which one calculates two metrics: the angle between the vectors, θ, and the difference in their magnitudes, ΔL (Fig. 3A). A definition of parallel evolution, then, is that replicate vectors point in the same direction so that the angle between them is near zero (Fig. 3). Evolutionary change is literally parallel in the geometric sense of the word. For instance, two sister species of *Brachyrhaphis* fishes diverged in multivariate behavior; the direction of this divergence was similar across independent watersheds (low θ) (Ingley et al 2014). The greater the angle between two vectors, the less parallel their evolution. The point here is to avoid artificially discretizing the (non)parallel continuum. But, if we must use categorical descriptions, parallel evolution has occurred when θ is statistically indistinguishable from zero (assuming decent power), and nonparallel when θ significantly exceeds zero. Several subgroups along the continuum might also be useful (Fig. 1): acute nonparallel when the vectors proceed in roughly the same direction with $0 < \theta < 90$; orthogonal nonparallel when $\theta \sim 90$; obtuse nonparallel when $90 < \theta < 180$; antiparallel—a standard mathematical term—when vectors point in opposing directions ($\theta \sim 180$).

A more stringent definition of parallel evolution could also require that the vectors have similar magnitudes (the difference in lengths is near zero). For example, in the *Brachyrhaphis* example discussed above, the magnitude of divergence was inconsistent between watersheds (large ΔL), suggesting some nonparallel evolution. An even stricter criterion could require the two vectors begin and/or end close together in morphospace (e.g., the Euclidian distances between starting points of any two vectors (S_D), and/or the distance between their ending points (E_D), have near-zero lengths; Fig. 3B). These alternatives highlight a benefit of PCVA: we can simultaneously quantify parallel evolution, convergence vs. divergence, and the magnitude of change (Fig. 3C). For example, with replicate ancestor-descendent pairs, evolution is divergent when descendent populations are farther apart than the ancestral populations ($S_D < E_D$) while convergence has occurred when $S_D > E_D$. Note also that convergence or divergence can result from parallel or nonparallel evolution (Fig. 3C). In PCVA terminology, parallelism and convergence are neither mutually exclusive nor redundant terms. Thus, PCVA provides substantially more information than vote counting or variance partitioning approaches.

PCVA is best applied to ancestor-descendant pairs, because the resulting vector represents an evolutionary trajectory through time. This is possible when the ancestor is still extant (largely

unchanged), or when fossil data, ancient DNA, or phylogenetic reconstructions can be used to infer ancestral states. Unfortunately, such data are rare. Therefore, many researchers apply PCVA in other contexts such as comparing replicate, extant population pairs in different habitats. The vector then represents evolutionary divergence between sister populations, rather than a trajectory through time. We can compare replicate contemporary population pairs to ask the extent to which between-habitat divergence proceeds in similar directions. PCVA can also be extended to describe more continuous evolutionary trajectories through time or along a cline (Phenotypic Trajectory Analysis, PTA (Adams & Collyer 2009, Lohman et al 2017)). Because summary statistics from PCVA can be collected for any kind of multivariate data, it is possible to compare the extent of (non)parallel evolution across biological levels (Stuart et al 2017).

PCVA has drawbacks. First, interpreting angle and length differences between multivariate vectors and translating those differences back to real traits is not always intuitive to biologists whose mathematical training often emphasizes statistical tests rather than geometry. For instance, a given angle between two vectors can be achieved many different ways through divergence in different combinations of traits across different replicate pairs. Interpretation is especially challenging for high-dimensional data because the mathematical measures of (non)parallel evolution might be insufficiently explained by 2- or 3-dimensional graphics. Moreover, PCVA vector angles are not useful alone, but must be considered with vector lengths: two vectors can share very similar (or highly different) trajectories through trait space but be biologically uninteresting if vector lengths are near zero.

A second unresolved challenge entails development and testing of biologically useful null hypotheses. The initial implementations of PCVA provided a permutation-based test for whether two vectors had a non-zero angle (Collyer & Adams 2007). One problem is that the randomization procedure has very low power. Another problem is that this permutation test treats perfect parallel change as the null hypothesis, whereas for many researchers parallel change is the alternative hypothesis they seek to demonstrate. Should the null instead be that the vectors are orthogonal? Or, should we test whether vectors are randomly oriented in multivariate trait space? New techniques that use Bayesian methods to estimate the posterior probability distribution of θ, or that compare support for alternative models of θ are needed.

Finally, perhaps the biggest problem with PCVA is that angle and length metrics may be sensitive to one's choice of trait space. Sampling more traits may change vector orientations and the angles between them (Carscadden et al 2017). The implication is that researchers' decisions about what and how many traits to measure might substantially alter PCVA interpretation.

V. HOW (NON)PARALLEL IS EVOLUTION?

Disagreements over the prevalence of parallel evolution are as old as the discipline itself. Darwin was keenly aware of nonparallel evolution: “There is hardly a climate or condition in the Old World which cannot be paralleled in the New… Notwithstanding this general parallelism in the conditions of the Old and New Worlds, how widely different are their living productions!” (Darwin 1859; Chapter 12). Similarly, Calman (1935) argued that parallel evolution was the exception rather than the rule, with divergent evolution far more common. Yet other researchers felt that parallel evolution was widespread (Muller 1939, Rensch 1939).

This long-standing debate is likely to see substantial progress as the analytical tools described above are widely adopted to quantify (non)parallel evolution, rather than counting examples. For examples of this quantitative approach, see (Conte et al 2015, Conte et al 2012, Eroukhmanoff et al 2009, Evans et al 2013, Fitzpatrick et al 2014, Kaeuffer et al 2012b, Langerhans & Makowicz 2009, Laporte et al 2015, Manousaki et al 2013, McGee et al 2016, Oke et al 2017, Perreault-Payette et al 2017, Perrier et al 2013, Pfenninger et al 2015, Pujolar et al 2017, Ravinet et al 2016, Rosenblum & Harmon 2011, Siwertsson et al 2013, Stuart et al 2017). Below, we describe examples of how these and other studies have provided valuable insights into how strong, and how variable, parallel evolution can be in natural populations. In the subsequent section (**VI**), we describe the biological processes underlying (and revealed by) this (non)parallel continuum.

V.A Evolution in replicate populations is often nonparallel

Studies of parallel evolution often note inconsistencies or variation among replicate populations pairs without directly explaining them (e.g., (Brinsmead & Fox 2002, Gíslason et al 1999, Hoekstra & Nachman 2003). Recently these inconsistencies have become an area of research in their own right, to describe the extent of (non)parallel evolution and explain heterogeneity along this continuum. A recent study of Bahamian mosquitofish in high versus low predation environments used variance partitioning methods to show that more than half of the overall among-population phenotypic variation (of 90 traits) was driven by something other than shared selection arising from predation regime (Langerhans 2017). In a meta-analysis of parallel evolution in many species of fishes, Oke et al. (2017) found large variation within and among species in the extent of parallel evolution among replicated conspecific populations. Here, variance partitioning found that fish ecotype (presumably evolved in parallel in shared environments) accounted for less than 10% of the partial variance of morphology in some

systems, to over 90% in others. The nonparallel cases tended to be more common. Oke reached the same result using PCVA or PTA results, which were applicable to 14 fish systems with paired populations replicated across habitat boundaries (e.g. benthic-limnetic stickleback, lake-stream stickleback, dwarf-normal whitefish). Of these 14, only 4 had a consistent trend towards parallel divergence across a boundary ($\theta < 90°$ for all pairwise vector comparisons).

Perhaps the strongest evidence for (non)parallelism comes from laboratory experimental evolution studies (see Sidebar). Researchers have subjected replicate laboratory populations (e.g., of bacteria, *Drosophila*, etc.) to identical artificial selection and then evaluated the repeatability of subsequent evolution (Box 1; Cooper et al 2003, Ferea et al 1999, Fong et al 2005, Roberge 2006). However, most of these studies used vote-counting as their measure of parallel evolution. For example, Ferea et al (1999) raised three replicate yeast cultures, selected to live in glucose-limited media, and identified several hundred genes that evolved the same expression changes in all three populations. A similar experiment with *E.coli* found 59 genes (out of the entire genome) that evolved strongly and in the same direction in 2 replicate populations (Cooper et al 2003). Both studies support parallel evolution, but in their reliance on vote-counting from a few replicates makes it more likely that parallel changes are coincidental.

V.B Evolution across traits is often (non)parallel

Traits vary in the extent of (non)parallel evolution

We expect natural selection to act more strongly on some traits than others. Or, a trait subject to selection may be highly correlated with some traits but not others. Still other traits may be subject to divergent natural selection between superficially similar habitat replicates. This variation in (correlated) selection strength should cause some traits to diverge, and others to converge, evolve in parallel, or evolve neutrally. Within a given study system, it is often the case that some traits will show parallel change, while others show nonparallel change or even no evolution at all (Oke et al 2017). For example, In lake-stream pairs of stickleback, a study of 86 phenotypic traits found that the effect of crossing the lake-stream habitat boundary explained 0% of variation in some traits but over 20% of variation in others (Stuart et al 2017). Similarly, ninety traits measured in high- and low-predation Bahamian mosquitofish varied from highly parallel divergence between high and low regimes to nonparallel changes that didn't match the predator differences (Langerhans 2017). Neither study found any evidence that certain categories of traits (e.g., trophic, locomotion, defense) were more strongly parallel than others.

V.C (Non)parallel evolution across biological scales: genotype versus phenotype

To what extent does (non)parallelism at one biological scale necessarily correlate with (non)parallelism at other biological scales? We may be able to predict this in some cases. For example, because parallel phenotypic evolution is mostly attributed to selection, we would not expect parallel evolution for neutral genetic markers. This expectation was corroborated by the study of lake-stream stickleback mentioned above (Fig. 2). Focusing on putatively neutral markers (by excluding SNPs in the top 5% of lake-stream F_{ST} values), the orientation of genomic PCVA vectors was unrelated to the orientation of phenotypic trait PCVA vectors (Stuart et al 2017). That is, the combination of neutral SNPs that diverged did not predict the combination of traits that diverged, likely because these neutral SNPs are shouldn't be important for lake-stream divergence. However, the magnitude of trait divergence (ΔL) was strongly positively correlated with measures of genomic divergence (e.g., F_{ST}, or coalescent estimates of Nm). This positive relationship is consistent with the hypothesis that gene flow between adjoining habitats constrains lake-stream divergence. When gene flow differs between replicate watersheds, it creates variance in the magnitude of trait divergence (ΔL) and thus (non)parallelism.

The same study found a different result for putatively non-neutral genetic markers (top 5% of lake-stream F_{ST} outliers). Replicate watersheds that shared more outlier SNPs were more phenotypically parallel (though the trend was marginally significant). The authors inferred that phenotypically parallel change reflects parallel change at particular genes targeted by lake-stream divergent selection. In a study of two benthic-limnetic species pair lakes, Conte et al. (2015) found that 76% of 42 morphological traits diverged in parallel between benthic and limnetic forms. These parallel traits were controlled by 43 identifiable chromosomal regions (QTL), but only 49% of these QTL evolved in parallel in both lakes. Like the lake-stream system, evolution was less parallel at the genetic level than the phenotypic level (Conte et al 2015). This pattern is also found in repeated coastal ecotypes of *Senecio* that exhibit only partial re-use of QTL among replicate populations (Roda et al 2017).

Another strategy for comparing across levels is, for example, to deliberately focus only on strongly parallel evolution at the phenotypic level and ask to what extent it is underlain by parallel genetic changes (e.g., Colosimo et al 2005). This has been done in studies of lodgepole pine vs. interior spruce (Yeaman et al 2016); wild vs. weedy sunflower (Lai et al 2008); dwarf vs. normal whitefish ecotypes (Derome et al 2006); and Midas cichlid ecotypes (Manousaki et al 2013). Using F_{ST} outliers to detect putative genomic targets of selection, these studies showed that phenotypically very-parallel populations often share only a small proportion of their F_{ST} outliers (e.g., Westram et al 2014; Le Moan et al 2016; Kautt et al 2012). For highly parallel

traits in two pairs of benthic-limnetic stickleback, only 32% of the underlying QTL loci are shared (Conte et al 2012). Thus, even dramatically parallel phenotypes can be generated by a continuum of (non)parallelism at the genetic level.

V.D (Non)parallel evolution among species

This review has focused on replicated evolution of multiple populations within a species. However, textbook cases of parallel evolution often come from inter-specific comparisons, where replicated geographic areas (e.g. islands or lakes) promote the repeated evolution of independent sets of species, each set containing similar 'ecotypes' that are adapted to specific habitats, suggesting that ecological conditions on the four islands generate adaptive landscapes with similar selective optima, resulting in convergent evolution: e.g., African Rift Lake Cichlids (Kocher et al 1993), Hawaiian Silverswords (Baldwin & Sanderson 1998), and Tetragnathan spiders (Gillespie 2004). Many of these replicated adaptive radiations also contain species that don't fall neatly into ecotype categories (Leal et al 2002). This suggests that comparative phylogenetic methods could be applied to measure (non)parallelism at a higher taxonomic scale than we considered above (Pérez-Pereira et al 2017).

Such phylogenetic methods have been used to study (non)parallelism in Anolis lizards of the Greater Antilles. Anoles have repeatedly evolved island communities containing four to six morphologically distinctive habitat specialists termed 'ecomorphs' (Langerhans et al 2006, Losos 2009). However, of the 120 *Anolis* species in the Greater Antilles, 25 do not fall into a classic ecomorph category (Losos 2009), nor do the several hundred species found across the Lesser Antilles and mainland Central and South America. This vote-counting measure of (non)parallelism raises the question of whether the ecomorphs are really phenotypic clusters arising from parallel evolution and whether unique species are due to unique selection pressures. To address these questions, Ingram and Mahler developed a phylogentic comparative method that tests whether trait distributions are best explained by genetic drift or stabilizing selection around one or more phenotypic optima (Ingram & Mahler 2013, Mahler et al 2013). Mahler et al (2013) modeled phenotypic evolution on the *Anolis* phylogeny, contrasting alternative hypotheses of Brownian motion alone, Brownian motion around a single optimum (an Ornstein-Uhlenbeck process), or multiple optima. The empirical data best matched a model with multiple adaptive optima corresponding to different ecomorphs that evolved independently on different islands (and in different sub-clades) (Mahler et al 2013). Yet, the analysis confirmed that some unique species do not fit any broader ecomorph type. These unique species were mostly confined to the two largest Greater Antillean islands, suggesting the occasional cases of

nonparallel *Anolis* evolution require particular biogeographic or ecological settings (e.g., context-dependent evolution). Phylogenetic comparative methods like these allow us to quantify (non)parallel evolution above the population level, and do not require paired populations that span some sort of habitat boundary, unlike the quantitative methods described above. However, these methods do not consider parallel evolution in the strict sense of similar trajectories of trait change, which is an area where more progress might be made.

VI. WHY IS THERE VARIATION ALONG THE (NON)PARALLEL CONTINUUM?

From relatively early in the Modern Synthesis, researchers interpreted parallel evolution as evidence for similar natural selection (Muir 1924, Simpson 1953) because few if any other evolutionary forces can produce such deterministic outcomes. In contrast, many evolutionary forces can give rise to nonparallel evolution. So, observing nonparallel evolution does not clearly demonstrate any one evolutionary process. Most biologists' first instinct may be to explain nonparallel evolution by invoking a non-adaptive process (Losos 2011, Rosenblum et al 2014). However, stochastic forces in evolution mean that even replicated artificial selection on identical starting populations in highly controlled settings can yields some nonparallel results (Cooper et al 2003, Ferea et al 1999, Fong et al 2005, Roberge 2006). Thus, stochasticity can be important even when replicate populations experience similar selection (Orr 2005), especially in concert with less controlled natural settings, where replicate populations will also vary with respect to demographic factors like population size, connectivity, constraints from genetic architecture, plasticity, or many-to-one mapping (Alfaro et al 2004, Kolbe et al 2012, Leinonen et al 2012, Nosil & Crespi 2004, Oke et al 2017, Stayton 2008, Stuart et al 2017, Thompson et al 2017). On the other hand, (non)parallelism could also be adaptive, if selection differs among qualitatively similar environments (Kaeuffer et al 2012, Landry & Bernatchez 2010, Landry et al 2007, Langerhans & DeWitt 2004, Stuart et al 2017). In this section, we expand on these topics to address the question "why is evolution (non)parallel where we might reasonably have expected parallel change?"

VI.A Population size

In small populations, enhanced genetic drift will reduce the extent of parallel change across replicate populations (Szendro et al 2013). Small populations maintain lower genetic diversity, reducing the probability that the same alleles are available for selection in replicate populations (Chevin et al 2010, Feiner et al 2017, Gompel & Brud'homme 2009, MacPherson & Nuismer

2017). Small populations also have lower rates of mutational input to enable responses to selection (Barrett & Schluter 2008, Coyle et al 2007). Stochastic allele frequency changes reduce the efficacy of natural selection, so drift decreases the likelihood that initially similar populations fix the same alleles in response to similar selection (Kimura 1964, Orr 2005). Note that selection also reduces effective population size (Charlesworth 2013), so strong selection can induce drift that inhibits populations' subsequent adaptive capacity.

VI.B History

The direction of evolution is contingent on populations' initial genetic conditions: available genetic diversity upon which selection can act, linkage between loci, and epistatic interactions. These conditions are likely to differ if two populations are initially genetically divergent, and populations will therefore respond in different ways even if selection is identical. Accordingly, studies in the field and lab have shown that more recently-diverged populations are more likely to use the same alleles or loci during adaptation to a particular environment (Bollback & Huelsenbeck 2009, Conte et al 2012).

Many phenotypes are controlled by epistatically interacting networks of genes. The phenotypic effect of any one allele is therefore contingent on the genotypic state at other loci (Cohen 1967, Costanzo et al 2016). Even mutations at different positions within a single gene will interact epistatically (Sailer & Harms 2017). Thus, the fitness effects and evolutionary trajectory of a single mutation will differ among populations, depending on their genotypes at other loci with which the mutant allele interacts. The importance of epistatic contingency has been confirmed by artificial selection experiments that yield nonparallel results (Jerison & Desai 2015, Vogwill et al 2014) and is sometimes called a 'mutation order' effect because the same mutations may lead to very different evolutionary results depending on the order in which they arise and (perhaps) fix (Gerstein et al 2012).

The historical duration of evolutionary divergence is also relevant to (non)parallelism (Lucek et al 2014). Populations that have been diverging for more time have more scope for genetic drift to introduce stochastic differences into replicate populations' evolutionary trajectories. This is, after all, why Brownian motion models of evolution lead to greater divergence through time (Ord & Summers 2015). Yet, if evolution is mutation-limited, then older populations will have had more time to accumulate similar adaptive mutations needed to converge on similar phenotypic solutions to a given environment (Orr 2005, Whitlock & Gomulkiewicz 2005).

VI.C Selection landscape

It is intuitive that replicate populations in more similar environments should experience more similar selection and evolve more parallel traits. However, few studies have tested this inference directly. Theoretical studies of parallel evolution typically assume that selection is identical and constant across all replicate populations (Orr 2005). Lab studies of experimental evolution attempt to impose identical selection regimes across replicate populations experiencing the same treatment (Wichman et al 1999). Even field studies often focus on comparisons between apparently discrete habitat categories (e.g., lake versus stream), implicitly assuming that variation within habitat categories is minimal. However, natural selection is unlikely to be exactly replicated, due to unrecognized site-to-site environmental differences, community structure differences, or fluctuating selection through time (Siepielski et al 2009). Thus, environmental heterogeneity among ostensibly replicate habitats might contribute to nonparallel evolution. For example, replicate lake whitefish populations in eastern Canada have repeatedly diverged into coexisting dwarf and normal ecotypes that evolved (non)parallel morphology. Dwarf-normal pairs are more phenotypically (and genetically) divergent in lakes with greater seasonal variation in oxygen (Landry et al 2007), and larger diet differentiation (Landry & Bernatchez 2010), while nonparallel evolution of immunologically important *MHCIIb* genes is linked to nonparallel parasite communities (Pavey et al 2013). Thus, lake-to-lake environmental differences influence lake-to-lake differences in how dwarf and normal ecotypes diverge. Similar enviroment-dependent (non)parallelism has been demonstrated in whitefish in Europe (Siwertsson et al 2013), lake-stream stickleback (Stuart et al 2017) and in Trinidadian guppies (Fitzpatrick et al 2014).

Finally, natural selection fluctuates over time in nature (Siepielski et al 2009). Abiotic conditions change from year to year, and as a result, replicate populations may experience different selection in any one year. Even if populations experience similar selection, they will tend to diverge over time in a drift-like process driven by fluctuating selection (Gillespie 1994). For example, antagonistic coevolution (e.g., between predator and prey, host and parasite or between males and females) can generate fluctuating selection, as initially winning defensive strategies become targets for attack by the antagonist and lose their advantage (Ellner et al 2011, Tellier & Brown 2007). If replicate populations' eco-evolutionary cycles are out of phase, they may be phenotypically nonparallel at any one instant in time, yet experience similar cyclical dynamics over long time-scales (Auld & Brand 2017).

VI.D Gene flow

(Non)parallelism should also depend on levels of population connectivity. To our knowledge, there has been little study of how migration rates alter the extent of parallel evolution, but the theoretical expectations are intuitive. Gene flow typically constrains divergence between populations (Lenormand 2002, Slatkin 1985). Therefore, gene flow between replicate populations in the same habitat type should make them more genetically similar and hence facilitate more parallel evolution.

Gene flow across habitat types, however, tends to constrain local adaptation. This constraint will hinder parallel evolution among replicate populations adapting to a particular habitat. That is, if gene flow is stronger across the habitat boundary for some pairs, but weaker in other pairs, then evolution will be more strongly constrained in some replicates than in others, which should contribute to deviations from strictly parallel evolution (Hendry & Taylor 2004, Moore et al 2007, Stuart et al 2017), especially the magnitude of change (PCVA vector lengths). For example, gene flow between lake and stream stickleback is strong in some watersheds (constraining trait divergence), and weak in others (permitting trait divergence), explaining some of the variation in the magnitude of lake-stream divergence (Stuart et al 2017).

VI.E Many-to-one mapping

Natural selection acts on morphological traits indirectly via traits' functional output (Arnold 1983, Lauder 1981, Wainwright 1996, Walker 2007). If there is a simple 1:1 relationship between form and function, then replicated selection on function will favor the evolution of similar underlying phenotypes. However, many physiological or biomechanical functions have many-to-one mapping, where different trait combinations can generate the same functional output. Such redundancy allows trait divergence (and nonparallel evolution) even when stabilizing selection favors a single function (Alfaro et al 2005, Wainwright et al 2005). Hence, many-to-one mapping enables nonparallel evolution of structural traits even when the emergent functional traits are evolving in parallel. Consistent with this theory, some studies have found that functional trait evolution is more predictable (i.e., has a higher percent variance explained by ecotype) than the underlying structural traits (Thompson et al 2017). This observation highlights the importance of distinguishing between the extent of (non)parallel evolution at different levels of biological organization.

VI.F Genomic architecture

Replicate populations' (non)parallel response to selection also depends on their respective genetic architectures (e.g., recombination rates, mutation rates, chromatin packing, and

epigenetic modifications), which can vary among populations and across the genome (Hodgkinson & Eyre-Walker 2011, Nachman 2002).

Mutational hotspots within the genome (Burch & Chao 2000, Holland et al 1982) harbor greater genetic variation and thus present more fodder for natural selection. Because mutational hot-spots are more evolvable, they increase the probability that mutations arise independently in the same hot-spot genes, facilitating parallel evolution at the genetic level across independent taxa. For example, *Pitx1* resides in a fragile region of the stickleback genome and has independently mutated in multiple independent populations to confer a reduced pelvis, which selection then fixed (Chan et al 2010, Coyle et al 2007). Remarkably, this mutational bias confirms Dobzhansky's early explanation for parallel evolution (Dobzhansky 1933).

Empirical work suggests that shared adaptive alleles tend to be found more often in regions of low recombination, particularly during divergence-with-gene-flow (Roesti et al 2013, Samuk et al 2017). The most dramatic version of this effect entails chromosomal inversions segregating within populations. Inversions usually suppress recombination, creating linked groups of co-adapted alleles at various loci. Selection acts on these loci as a group, facilitating parallel adaptation to new environments when inversions are shared among founder populations (Terekhanova et al 2014).

Polygenic traits enable a many-to-one mapping of genotype to phenotype. So, much like the many-to-one form-to-function mapping discussed above, parallel genetic evolution is more likely when only a single gene underlies an evolving trait (Orr 2005). Nonetheless, parallel genomic evolution has been found even when there are multiple mutations in many genes that can produce similar phenotypic changes (e.g., *Frigida*, for flowering time (Levy & Dean 1998, Shindo et al 2005)).

Mutations that improve fitness through one trait might have deleterious effects via a different trait. This negative pleiotropy reduces the likelihood that the mutation will persist in a population and eventually fix (Cooper et al 2007, Otto 2004). If negative pleiotropy is common, then replicate populations are less likely to have the same genetic variants available for adaptation and evolution will be more nonparallel. Alternatively, pleiotropy may constrain the number of plausible evolutionary trajectories, increasing the extent of parallel change. There is little empirical evidence to distinguish these opposing hypotheses, though one study found that more pleiotropic genes exhibited less parallel evolution of gene expression (Papakostas et al 2014).

Pleiotropy may also reduce the likelihood of parallel evolution through correlated selection. Basic quantitative genetics tells us that the direction and speed of evolution of a focal

trait depends on selection that might act on other genetically correlated traits. A focal trait may be subject to parallel selection, but if correlated traits experience inconsistent selection among replicate populations, then even the focal trait will not evolve in parallel (Brodie 1992, Falconer 1952, Gratten et al 2008, Lande & Arnold 1983, Thompson et al 2017).

In our introduction, we posed the question, “When we see deviations from parallel evolution, what are we to conclude about adaptation?” The material reviewed above makes it clear that there is no single answer. Nonparallel evolution may or may not be adaptive. But, when replicate populations vary along the (non)parallel continuum, these variable evolutionary outcomes can provide an opportunity to test the alternative models of evolution described above.

VII. WHERE NEXT?

In a replicated study of bacteriophage evolution under selection in the lab, only 25% to 50% of genetic substitutions in any one replicate population also evolved in at least one other replicate (Wichman et al 1999). This is more parallel than expected by chance, but certainly less than 100%. Such inconsistent responses to selection are common in nature, as our review has made clear. Thus, Wichman and colleagues’ closing question, “Why is parallel evolution not complete?”, remains germane. We now have a wide array of plausible answers to Wichman’s question, but many important questions remain unanswered. In this final section we summarize some next steps.

First, we must improve quantitative approaches for describing the continuum of (non)parallel evolution and statistically distinguishing different patterns of parallel and nonparallel evolution (Figure 2). The multivariate vector-based approach (PCVA) is a useful tool, but problems remain with statistical power, defining suitable null hypotheses, sensitivity to the number of measured phenotypes, and reliance on pairwise comparisons. Nevertheless, PCVA has proved to be an effective took for making evolutionary inferences (e.g., Stuart et al 2017), so we advocate applying this method to more research systems in the lab and wild. An intriguing future direction is to apply PCVA to population triplets using vectors to connect an ancestral population to two descendant populations that have diverged in different habitats. This latter option offers a more complex geometry (a triangle of vectors) that describes the temporal trajectories of between-population divergence.

Second, we need formal tools for comparing measures of (non)parallelism across levels of biological organization. One clear theme in the existing literature is that evolution may be

718 parallel for a higher-level trait (e.g., phenotype or function), but nonparallel for lower level traits
719 (e.g., physiological processes, biochemistry, genes). Understanding how (non)parallel evolution
720 correlates across levels may increase our ability to predict evolutionary change.
721 Third, the vast majority of studies of (non)parallelism focus on wild-caught individuals
722 whose traits are affected by phenotypic plasticity that may exaggerate or obscure patterns of
723 parallel evolution (Oke et al 2015). The obvious solution is to evaluate (non)parallelism based
724 on trait measurements taken in common-garden settings or from quantitative genetic estimates
725 of breeding value. Of course, an important open question concerns the contribution of plasticity
726 and genotype by environment interactions to parallel trait change (Mazzarella et al 2015).
727 Fourth, most studies of (non)parallelism examine extant populations, rather than
728 ancestor-descendent pairs. The field would benefit from temporal transects that trace replicate
729 trajectories of evolutionary change through time. This requires fossil and sub-fossil samples to
730 measure phenotypes (or ancient DNA genotypes) to calculate evolutionary vectors through time
731 (Bell et al 2004). For most taxa (and most traits), the fossil record is too sparse, generates small
732 sample sizes, or is entirely absent. However, in exceptional cases where we can measure many
733 individuals continuously through time, we will surely find that evolution traces non-linear paths
734 through trait space over time, which would complicate geometric measures of “parallel”
735 evolution (Adams & Collyer 2009). Such non-linear multivariate trajectories have been
736 observed across spatial transects (Lohman et al 2017), but temporal trajectories that might arc
737 through trait space have not been integrated into (non)parallel evolution studies. Plant
738 domestication offers an exceptionally promising venue for this work because archaeological
739 studies provide temporal transects of food plant materials (Fuller et al 2014). Trajectories
740 through time could also be studied using ‘resurrection studies’, where ancestral populations can
741 be recreated from seed or egg banks. But,
742 Fifth, we need to explain variation in the extent of (non)parallelism among evolutionary
743 replicates. This requires investigation of the ecological, genetic, and historical mechanisms that
744 lead to that pattern in the first place. For instance, we tend to assume that similar environments
745 impose similar selection pressures, but we need to test this explicitly by measuring selection on
746 populations that are more and less parallel. Better still, experimental manipulation of selective
747 forces to track parallel responses to selection are an important future direction. Furthermore, a
748 mechanistic understanding of evolutionary genetics and how traits are constructed may be
749 necessary to effectively account for nonparallel evolution. Functional genetics studies that
750 dissect the specific pathways by which traits are built during development will be needed to
751 understand how genes and traits respond to (non)parallel selection. In particular, it is

increasingly clear that epistasis is common and strongly influences evolution. To what extent is epistasis responsible for nonparallel genetic (or phenotypic) evolution when selection would otherwise favor parallel change?

Sixth, biomedical and agricultural practices increasingly draw on genome-wide association studies (GWAS) that pinpoint genetic variants that are correlated with traits. A common approach is to obtain genomic SNP data for a large number of individuals from many populations, then identify SNPs correlated with an environment or trait (Coop et al 2010; Davey et al 2011). Genetic nonparallel evolution undermines the strength of these correlations, reducing the power of GWAS. At the extreme, GWAS would fail if each population evolved a given trait via unique genes or alleles, as in HIV-1's gp120 gene (Martinez-Picado et al 2002).

Last, we need to expand research on the practical consequences of variation along the (non)parallel continuum. In the introduction to this review, we summarized a variety of studies related to medicine or agriculture. To make our basic research useful, we must consider how to apply the perspectives discussed here to solve real-world challenges. The evolution of tumors, pathogens, weeds, and pests pose major health and economic burdens. When a pest's evolution is strongly parallel, we might effectively anticipate future changes and thereby develop therapies to preemptively combat any ill effects of evolution. In contrast, nonparallel evolution will prove harder to anticipate. The (non)parallel continuum also has implications for other applied concerns. To mitigate extinction risk, conservation biologists and managers sometimes transfer organisms from healthy populations into declining populations to boost their abundance and genetic diversity (Rinkevich 2005). When replicate populations have evolved in parallel, they are pre-adapted to each others' habitats, and so may be especially well suited to rescuing declining populations. However, nonparallel local adaptation results in non-interchangeable populations, in which case transplants may undermine population viability (Kenkel et al 2015, Stockwell et al 2003).

VIII. CONCLUSIONS

Evolution is often described as being parallel, convergent, or divergent. These semantic designations draw us into binary thinking about evolutionary processes and their resulting patterns. The reality is wonderfully more subtle and complex: the evolution of multiple phenotypes or genes in replicate populations is best described by a quantitative continuum from parallel to antiparallel and convergent to divergent. Some populations will be highly parallel to each other, while other populations will follow unique trajectories, and some phenotypes and

genes are more prone to parallel evolution than others. A growing number of studies have embraced this complexity, recognizing that parallel evolution is a measurable continuum along which populations and traits and genes will vary. This quantitative view of a (non)parallel continuum opens up new opportunities to study the processes that generate heterogeneity in the extent of parallel evolution.

In the past, biologists have used parallel evolution to argue that evolution can be (sometimes) predictable. Yet, growing evidence suggests that deviations from parallel evolution can also be deterministic, so nonparallel change need not imply unpredictable evolution. Many research opportunities lie ahead for biologists seeking to develop tools to explain why evolution generates a continuum of (non)parallel results. With these tools, we hope to improve our ability to predict the future course of evolution.

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804 **LITERATURE CITED**

805

806 Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, al. e. 2017. Phylogenetic ctDNA analysis
807 depicts early-stage lung cancer evolution. *Nature* 545: 446-51
808 Abouheif E. 2008. Parallelism as the pattern and process of mesoevolution. *Evolution and*
809 *Development* 10: 3-5
810 Adams DC, Collyer ML. 2009. A general framework for the analysis of phenotypic trajectories in
811 evolutionary studies. *Evolution* 65: 1143-54
812 Agrawal AA. 2017. Toward a predictive framework for convergent evolution: integrating natural
813 history, genetic mechanisms, and consequences for the diversity of life. *American*
814 *Naturalist* 190: S1-S12
815 Alfaro M, Bolnick DI, Wainwright PC. 2004. Evolutionary dynamics of complex biomechanical
816 traits: an example from the 4-bar lever system of labrids. *Evolution* 58: 495-503
817 Alfaro M, Bolnick DI, Wainwright PC. 2005. Evolutionary consequences of many-to-one
818 mapping of jaw morphology to mechanics in labrid fishes. *American Naturalist* 165: e140
819 – e54
820 Arendt J, Reznick DN. 2008. Convergence and parallelism reconsidered: what have we learned
821 about the genetics of adaptation? *Trends In Ecology & Evolution* 23: 26-32
822 Arnold SJ. 1983. Morphology, performance, and fitness. *American Zoologist* 23: 347-61
823 Auld SKJR, Brand J. 2017. Environmental variation causes different (co) evolutionary routes to
824 the same adaptive destination across parasite populations. *Evolution Letters*
825 Baguñà J, Garcia-Fernàndez J. 2003. Evo-Devo: the long and winding road. *International*
826 *Journal of Developmental Biology* 47: 705-13
827 Bailey SF, Blanquart F, Bataillon T, Kassen R. 2017. What drives parallel evolution? .
828 *BioEssays* 39: e201600176–9
829 Bailey SF, Rodrigue N, Kassen R. 2015. The effect of selection environment on the probability
830 of parallel evolution. *Molecular Biology and Evolution* 32: 1436-48
831 Baldwin BG, Sanderson MJ. 1998. Age and rate of diversification of the Hawaiian silversword
832 alliance (Compositae). *Proceedings of the National Academy of Sciences* 95: 9402-06
833 Barrett RDH, Schluter D. 2008. Adaptation from standing genetic variation. *Trends in Ecology*
834 *and Evolution* 23: 38-44
835 Bell MA, Aguirre WE, Buck NJ. 2004. Twelve years of contemporary armor evolution in a
836 threespine stickleback population. *Evolution* 58: 814-24
837 Bitocchi E, Bellucci E, Giardini A, Rau D, Rodriguez M, et al. 2013. Molecular analysis of the
838 parallel domestication of the common bean (*Phaseolus vulgaris*) in Mesoamerica and
839 the Andes. *New Phytologist* 197: 300-13
840 Blair GR, Quinn TP. 1991. Homing and spawning site selection by sockeye salmon
841 (*Oncorhynchus nerka*) in Iliamna Lake, Alaska. *Canadian Journal of Zoology* 69: 176-81
842 Blair GR, Rogers DE, Qiunn TP. 2011. Variation in life history characteristics and morphology of
843 sockeye salmon in the Kvichak River system, Bristol Bay, Alaska. *Transactions of the*
844 *American Fisheries Society* 122: 550-59
845 Bollback JP, Huelsenbeck JP. 2009. Parallel genetic evolution within and between
846 bacteriophage species of varying degrees of divergence. *Genetics* 181: 225-34
847 Brinsmead J, Fox MG. 2002. Morphological variation between lake- and stream-dwelling rock
848 bass and pumpkinseed populations. *Journal of Fish Biology* 61: 1619-38
849 Brodie EDB. 1992. Correlational selection for color pattern and antipredator behavior in the
850 garter snake *Thamnophis ordinoides*. *Evolution* 46: 1284-98
851 Burch CL, Chao L. 2000. Evolvability of an RNA virus is determined by its mutational
852 neighborhood. *Nature* 406: 625-28

853 Burrell RA, McGranahan N, Bartek J, Swanton C. 2013. The causes and consequences of
854 genetic heterogeneity in cancer evolution. *Nature* 501: 338–45
855 Calman WT. 1935. Presidential Address: The meaning of biological classification. *Proceedings*
856 *of the Linnean Society of London* 1934-35: 145-58
857 Carscadden KA, Cadotte MW, Gilbert B. 2017. Trait dimensionality and population choice alter
858 estimates of phenotypic dissimilarity. *Ecology and Evolution* 7: 2273-85
859 Chan YF, Marks ME, Jones FC, Villarreal G, Shapiro MD, et al. 2010. Adaptive evolution of
860 pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* 327:
861 302-05
862 Charlesworth B. 2013. Why we are not dead one hundred times over. *Evolution* 67: 3354-61
863 Chevin L-M, Lande R, Mace GM. 2010. Adaptation, plasticity, and extinction in a changing
864 environment: towards a predictive theory. *PLoS Biology* 8: e1000357
865 Cohen D. 1967. Optimizing reproduction in a randomly varying environment whe a correlation
866 may exist between the conditions at the time a choice has to be made and the
867 subsequent outcome. *Journal of Theoretical Biology* 16: 1-14
868 Collyer ML, Adams DC. 2007. Analysis of two-state multivariate phenotypic change in ecological
869 studies. *Ecology* 88: 683-92
870 Collyer ML, Sekora DJ, Adams DC. 2015. A method for analysis of phenotypic change for
871 phenotypes described by high-dimensional data. *Heredity* 115: 357-65
872 Colosimo PA, Hosemann KE, Balabhadra S, Villarreal G, Dickson A, et al. 2005. Widespread
873 parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*
874 307: 1928-33
875 Conte GL, Arnegard ME, Best J, Chan YF, Jones FC, et al. 2015. Extent of QTL Reuse During
876 Repeated Phenotypic Divergence of Sympatric Threespine Stickleback. *Genetics* 201:
877 1189-200
878 Conte GL, Arnegard ME, Peichel CL, Schluter D. 2012. The probability of genetic parallelism
879 and convergence in natural populations. *Proceedings of the Royal Society of London*
880 *Series B.* 279: 5039-47
881 Coop G, Witonsky D, Di Rienzo A, Pritchard JK. 2010. Using environmental correlations to
882 identify loci underlying local adaptation. *Genetics* 185: 1411-23
883 Cooper TF, Ostrowski EA, Travisano M. 2007. A negative relationship between mutation
884 pleiotropy and fitness effect in yeast. *Evolution* 61: 1495-99
885 Cooper TF, Rozen DE, Lenski RE. 2003. Parallel changes in gene expression after 20,000
886 generations of evolution in Escherichia coli. *Proceedings of the National Academy of*
887 *Sciences* 100: 1072-77
888 Cope ED. 1876. The progress of discovery of the laws of evolution. *American Naturalist* 10:
889 218-21
890 Cope ED, Kingsley JS. 1891. Editorial. *American Naturalist* 25: 558-60
891 Costanzo M, Van der Sluis B, Koch EN, Baryshnikova A, al. e. 2016. A global genetic
892 interaction network maps a wiring diagram of cellular function. *Science* 353: aaf1420
893 Coyle SM, Huntingford FA, Peichel CL. 2007. Parallel evolution of Pitx1 underlies pelvic
894 reduction in Scottish threespine stickleback (*Gasterosteus aculeatus*). *Journal of*
895 *Heredity* 98: 581-86
896 Darwin C. 1859. *On the Orign of Species*. Cambridge, MA.: Harvard University Press.
897 Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. 2011. Genome-wide
898 genetic marker discovery and genotyping using next-generation sequencing. *Nature*
899 *Reviews Genetics* 12: 499-510
900 Day T. 2012. Computability, Godel's incompleteness theorem, and an inherent limit on the
901 predictability of evolution. *Journal of the Royal Society Interface* 9: 624-39
902 de Visser JA, Krug J. 2014. Empirical fitness landscapes and the predictability of evolution.
903 *Nature Reviews Genetics* 15: 480-90

Derome N, Duchesne P, Bernatchez L. 2006. Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. *Molecular Ecology* 15: 1239-49

Dobzhansky T. 1933. Geographical variation in lady-beetles. *American Naturalist* 67: 97-126

Douglas SM, Chubiz LM, Harcombe WR, Ytreberg FM, Marx CJ. 2016. Parallel mutations result in a wide range of cooperation and community consequences in a two-species bacterial consortium. *PLoS One* 11: e0161837

Dunn B, Levine RP, Sherlock G, 2005. Microarray karyotyping of commercial wine yeast strains reveals shared, as well as unique, genomic signatures. BMC Genomics. 6:53.

Ellner SP, Geber MA, Hairston NG. 2011. Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecology Letters* 14: 603-14

Elmer KR, Fan S, Kusche H, Spreitzer ML, Kautt AF, et al. 2014. Parallel evolution of Nicaraguan crater lake cichlid fishes via non-parallel routes. *Nature Communications* 5: 5168

Elmer KR, Meyer A. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence *Trends in Ecology and Evolution* 26: 298-306

Eroukhmanoff F, Hargeby A, Arnberg NN, Hellgren O, Bensch S, Svensson EI. 2009. Parallelism and historical contingency during rapid ecotype divergence in an isopod. *Journal of Evolutionary Biology* 22: 1098-110

Evans ML, Chapman LJ, Mitrofanov I, Bernatchez L. 2013. Variable extent of parallelism in respiratory, circulatory, and neurological traits across lake whitefish species pairs. *Ecology and Evolution* 3: 546-57

Falconer DS. 1952. The problem of environment and selection. *American Naturalist* 86: 293-98

Feiner N, Rago A, While GM, Uller T. 2017. Signatures of selection in embryonic transcriptomes of lizards adapting in parallel to cool climate. *Evolution* Online Early: DOI: 10.1111/evo.13397

Ferea TL, Botstein D, Brown PO, Rosenzweig RF. 1999. Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proceedings of the National Academy of Sciences* 96: 9721-26

Fernandez-Ortuno D, Tores JA, de Vicente A, Perez-Garcia A. 2008. Field resistance to QoI fungicides in Podosphaera fusca is not supported by typical mutations in the mitochondrial cytochrome b gene. *Pest Management Science* 64: 694-702

Fitzpatrick SW, Torres-Dowdall J, Reznick DN, Ghalambor CK, Funk WC. 2014. Parallelism isn't perfect: could disease and flooding drive a life-history anomaly in Trinidadian guppies? *American Naturalist* 183: 290-300

Fong SS, Joyce AR, Palsson B. 2005. Parallel adaptive evolution cultures of Escherichia coli lead to convergent growth phenotypes with different gene expression states. *Genome Research* 2005: 1365-72

Fuller DQ, Denham T, Arroyo-Kalin M, Lucas L, Stevens CJ, et al. 2014. Convergent evolution and parallelism in plant domestication revealed by an expanding archaeological record. *Proceedings of the National Academy of Sciences* 111: 6147-52

Gates RR. 1912. Parallel mutations in *Oenothera biennis*. *Nature* 89: 659-60

Gates RR. 1936. Mutations and natural selection. *American Naturalist* 70: 505-16

Gerstein AC, Lo DS, Otto SP. 2012. Parallel genetic changes and nonparallel gene-environment interactions characterize the evolution of drug resistance in yeast. Genetics 192:241-252.

Gillespie JH. 1994. *The Causes of Molecular Evolution*. Oxford, U.K.: Oxford University Press.

Gillespie R. 2004. Community assembly through adaptive radiation in Hawaiian spiders. *Science* 303: 356-59

954 Gíslason D, Ferguson MM, Skulason S, Snorrason SS. 1999. Rapid and coupled phenotypic
955 and genetic divergence in Icelandic arctic char (*Salvelinus alpinus*). *Canadian Journal of*
956 *Fisheries and Aquatic Sciences* 56: 2229-34.
957 Gompel N, Brud'homme B. 2009. The causes of repeated genetic evolution. *Developmental*
958 *Biology* 332: 36-47
959 Gould SJ. 2002. *The Structure of Evolutionary Theory*. Cambridge, MA: Harvard University
960 Press.
961 Gratten J, Wilson A, McRae A, Beraldi D, Visscher P, et al. 2008. A localized negative genetic
962 correlation constrains microevolution of coat color in wild sheep. *Science* 319: 318-20
963 Graves JL, Jr., Hertweck KL, Phillips MA, Han MV, Cabral LG, et al. 2017. Genomics of parallel
964 experimental evolution in Drosophila. *Molecular Biology and Evolution* 34: 831-42
965 Haas O, Simpson GG. 1945. Analysis of some phylogenetic terms, with attempts at redefinition.
966 *Proceedings of the American Philosophical Society* 90: 319-49
967 Harvey PH, Pagel MD. 1991. *The Compative Method in Evolutionary Biology*. New York, NY:
968 Oxford University Press.
969 Hendry AP, Kaeuffer RE, Crispo E, Peichel CL, Bolnick DI. 2013. Evolutionary inferences from
970 exchangeability: individual classification approaches based on the ecology, morphology,
971 and genetics of lake-stream stickleback population pairs. *Evolution* 67: 3429-41
972 Hendry AP, Taylor EB. 2004. How much of the variation in adaptive divergence can be
973 explained by gene flow? An evaluation using lake-stream stickleback pairs. *Evolution* 58:
974 2319-31
975 Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP. 2000. Rapid evolution of reproductive
976 isolation in the wild: Evidence from introduced salmon. *Science* 290: 516-18.
977 Herron MD, Doebeli M. 2013. Parallel evolutionary dynamics of adaptive diversification in
978 *Escherichia coli*. *PLoS Biology* 11: e10001490
979 Hodgkinson A, Eyre-Walker A. 2011. Variation in the mutation rate across mammalian
980 genomes. *Nature Reviews Genetics* 12: 756-66
981 Hoekstra HE, Nachman MW. 2003. Different genes underlie adaptive melanism in different
982 populations of rock pocket mice. *Molecular Ecology* 12: 1185-94
983 Holland J, Spindler K, Horodyski F, Grabau E, Nichol S, Van de Pol S. 1982. Rapid evolution of
984 RNA genomes. *Science* 215: 1577-85
985 Hubbs CL. 1944. Concepts of homology and analogy. *American Naturalist* 78: 289-307
986 Ingley SJ, Billmann EJ, Hancock C, Johnson JB. 2014. Repeated geographic divergence in
987 behavior: a case study employing phenotypic trajectory analyses. *Behavioral Ecology*
988 *and Sociobiology* 68: 1577-87
989 Ingley SJ, Billmann EJ, Belk MC, Johnson JB. 2014. Morphological divergence drivn by
990 predation environment within and between species of *Brachyrhaphis* fishes. PLoS One.
991 9: e90274.
992 Ingram T, Mahler DL. 2013. SURFACE: detecting convergent evolution from comparative data
993 by fitting Ornstein-Uhlenbeck models with stepwise Akaike Information Criterion.
994 *Methods in Ecology and Evolution* 4: 416-25
995 Jerison ER, Desai MM. 2015. Genomic investigations of evolutionary dynamics and epistasis in
996 microbial evolution experiments. *Current Opinion in Genetics and Development* 35: 33-9
997 Jones FC, Grabherr MG, Chan YF, Russell P, Mauceli E, et al. 2012. The genomic basis of
998 adaptive evolution in threespine sticklebacks. *Nature* 484: 55-61
999 Josephides C, Swain PS. 2017. Predicting metabolic adaptation from networks of mutational
1000 paths. *Nature Communications* 8: 685
1001 Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP. 2012. Parallel and nonparallel aspects of
1002 ecological, phenotypic, and genetic divergence across replicate population pairs of lake
1003 and stream stickleback. *Evolution* 66: 402-18

1004 Kautt AF, Elmer KR, Meyer A. 2012. Genomic signatures of divergent selection and speciation
1005 patterns in a 'natural experiment', the young parallel radiations of Nicaraguan crater lake
1006 cichlid fishes. *Molecular Ecology* 21: 4770-86
1007 Kenkel CD, Almanza AT, Matz MV. 2015. Fine-scale environmental specialization of reef-
1008 building corals might be limiting reef recovery in the Florida Keys. *Ecology* 96: 3197-212
1009 Khaitovich P, Hellmann I, Enard W, Nowick K, Leinweber M, et al. 2005. Parallel patterns of
1010 evolution in the genomes and transcriptomes of humans and chimpanzees. *Science*
1011 309: 1850-54
1012 Kimura M. 1964. Diffusion models in population genetics. *Journal of Applied Probability* 1: 177-
1013 232
1014 Kocher TD, Conroy JA, McKaye KR, Stauffer JR. 1993. Similar morphologies of cichlid fish in
1015 Lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and*
1016 *Evolution* 2: 158-65.
1017 Kolbe JJ, Leal M, Schoener TW, Spiller DA, Losos JB. 2012. Founder effects persist despite
1018 adaptive differentiation: a field experiment with lizards. *Science* 335: 1086-89
1019 Lai Z, Kane NC, Zou Y, Rieseberg LH. 2008. Natural variation in gene expression between wild
1020 and weedly populations of *Helianthus annuus*. *Genetics* 179: 1881-90
1021 Lande R, Arnold SJ. 1983. The measurement of selection on correlated characters. *Evolution*
1022 37: 1210-26
1023 Landry L, Bernatchez L. 2010. Role of epibenthic resource opportunities in the parallel evolution
1024 of lake whitefish species pairs (Coregonus sp.). *Journal of Evolutionary Biology* 23:
1025 2602-13
1026 Landry L, Vincent WF, Bernatchez L. 2007. Parallel evolution of lake whitefish dwarf ecotypes in
1027 association with limnological features of their adaptive landscape. *Journal of*
1028 *Evolutionary Biology* 20: 971-84
1029 Langerhans RB. 2017. Predictability and parallelism of multi-trait adaptation. *Journal of Heredity*
1030 Online Early: esx043
1031 Langerhans RB, DeWitt T. 2004. Shared and unique features of evolutionary diversification.
1032 *American Naturalist* 164: 335-49
1033 Langerhans RB, Knouft JH, Losos JB. 2006. Shared and unique features of diversification in
1034 greater Antillean Anolis ecomorphs *Evolution* 60: 362-69
1035 Langerhans RB, Makowicz AM. 2009. Shared and unique features of morphological
1036 differentiation between predator regimes in *Gambusia caymanensis*. *Journal of*
1037 *Evolutionary Biology* 22: 2231-42
1038 Laporte M, Rogers SM, Dion-Cote AM, Normandeau E, Gagnaire PA, et al. 2015. RAD-QTL
1039 mapping reveals both genome-level parallelism and different genetic architecture
1040 underlying the evolution of body shape in lake whitefish (*Coregonus clupeaformis*)
1041 species pairs. *G3* 5: 1481-91
1042 Lauder GV. 1981. Form and function: structural analysis in evolutionary morphology.
1043 *Paleobiology* 7: 430-42
1044 Le Moan A, Gagnaire P-A, Bonhomme F. 2016. Parallel genetic divergence among coastal–
1045 marine ecotype pairs of European anchovy explained by differential introgression after
1046 secondary contact. *Molecular Ecology* 25: 3187-202
1047 Leal M, Knox AK, Losos JB. 2002. Lack of convergence in aquatic *Anolis* lizards. *Evolution* 56:
1048 785-91
1049 Leger EA, Rice KJ. 2007. Assessing the speed and predictability of local adaptation ininvasive
1050 California poppies (*Eschscholzia californica*). *Journal of Evolutionary Biology* 20: 1090-
1051 103
1052 Leinonen T, McCairns RJ, Herczeg G, Merila J. 2012. Multiple evolutionary pathways to
1053 decreased lateral plate coverage in freshwater threespine sticklebacks. *Evolution* 66:
1054 3866-75

1055 Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and*
1056 *Evolution* 17: 183-89
1057 Lenski RE. 2017. Convergence and divergence in a long-term experiment with bacteria.
1058 *American Naturalist* 190: S57-S68
1059 Levy YY, Dean C. 1998. Control of flowering time. *Current Opinion in Plant Biology* 1: 49-54
1060 Lin J, Quinn TP, Hilborn R, Hauser L. 2008. Fine-scale differentiation between sockeye salmon
1061 ecotypes and the effect of phenotype on straying. *Heredity* 101: 341-50
1062 Lohman B, Berner D, Bolnick DI. 2017. Clines arc through multivariate morphospace. *American*
1063 *Naturalist* 189: 354-67
1064 Losos JB. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*.
1065 University of California Press.
1066 Losos JB. 2011. Convergence, adaptation, and constraint. *Evolution* 65: 1827-40
1067 Lucek K, Sivasundar A, Kristjánsson BK, Skúlason S, Seehausen O. 2014. Quick divergence
1068 but slow convergence during ecotype formation in lake and stream stickleback pairs of
1069 variable age. *Journal of Evolutionary Biology* 27: 1878-92
1070 MacPherson A, Nuismer SL. 2017. The probability of parallel genetic evolution from standing
1071 genetic variation. *Journal of Evolutionary Biology* 30: 326-37
1072 Mahler DL, Ingram T, Revell LJ, Losos JB. 2013. Exceptional convergence on the
1073 macroevolutionary landscape in island lizard radiations. *Science* 341: 292-95
1074 Manceau M, Domingues VS, Mallarino R, Hoekstra HE. 2011. The developmental role of Agouti
1075 in color pattern evolution. *Science* 331: 1062-65
1076 Manousaki T, Hull PM, Kusche H, Machado-Schiaffino G, Franchini P, et al. 2013. Parsing
1077 parallel evolution: ecological divergence and differential gene expression in the adaptive
1078 radiations of thick-lipped Midas cichlid fishes from Nicaragua. *Molecular Ecology* 22:
1079 650-69
1080 Martinez-Picado J, Frost SDW, Izquierdo N, Morales-Lopetegi K, Marfil S, et al. 2002. Viral
1081 evolution during structured treatment interruptions in chronically Human
1082 Immunodeficiency Virus-infected individuals. *Journal of Virology* 76: 12344-48
1083 Mazzarella AB, Voje KL, Hansson TH, Taugbøl A, Fischer B. 2015. Strong and parallel salinity-
1084 induced phenotypic plasticity in one generation of threespine stickleback. *Journal of*
1085 *Evolutionary Biology* 28: 667-77
1086 McGee MD, Neches RY, Seehausen O. 2016. Evaluating genomic divergence and parallelism
1087 in replicate ecomorphs from young and old cichlid adaptive radiations. *Molecular*
1088 *Ecology* 25: 260-8
1089 Meyer JR, Agrawal AA, Quick RT, Dobias DT, Schneider D, Lenski RE. 2010. Parallel changes
1090 in host resistance to viral infection during 45,000 generations of relaxed selection.
1091 *Evolution* 64: 3024-34
1092 Moore J-S, Gow JL, Taylor EB, Hendry AP. 2007. Quantifying the constraining influence of gene
1093 flow on adaptive divergence in the lake-stream threespine stickleback system *Evolution*
1094 61: 2015-26
1095 Muir F. 1924. Homoplasmy or convergent development in evolution. *Proceedings of the*
1096 *Hawaiian Entomological Society* 5: 473-83
1097 Muller HJ. 1939. Reversibility in evolution considered from the standpoint of genetics. *Biological*
1098 *Reviews of the Cambridge Philosophical Society* 14: 261-80
1099 Nachman MW. 2002. Variation in recombination rate across the genome: evidence and
1100 implications. *Current Opinion in Genetics and Development* 12: 657-63
1101 Nichols JT. 1916. On primarily unadaptive variants. *American Naturalist* 50: 565-74
1102 Nosil P, Crespi BJ. 2004. Does gene flow constrain adaptive divergence or vice versa? a test
1103 using ecomorphology and sexual isolation in *Timema cristinae* walking sticks. *Evolution*
1104 58: 102-12
1105 Nowell PC. 1976. The clonal evolution of tumor cell populations. *Science* 194: 23-28

Oke K, Bukhari M, Kaueffer R, Rolshausen G, Rasanen K, et al. 2015. Plasticity enhances phenotypic parallelism: evidence from lake-stream stickleback. *Journal of Evolutionary Biology* 29: 126-43

Oke KB, Rolshausen G, LeBlond C, Hendry AP. 2017. How parallel Is parallel evolution? a comparative analysis in fishes. *American Naturalist* 190: 1-16

Ord TJ, Summers TC. 2015. Repeated evolution and the impact of evolutionary history on adaptation. *BMC Evolutionary Biology* 15: 137

Orr HA. 2005. The probability of parallel evolution. *Evolution* 59: 216-20

Osborn HF. 1900. The geological and faunal relations of Europe and America during the Tertiary period and the theory of the successive invasions of an African fauna. *Science* 11: 561-74

Otto SP. 2004. Two steps forward, one step back: the pleiotropic effects of favoured alleles. *Proceedings of the Royal Society of London Series B.* 271: 705-14

Packard AS. 1898. The philosophical views of Agassiz. *American Naturalist* 32: 159-64

Papakostas S, Vøllestad LA, Bruneaux M, Aykanat T, Vanoverbeke J, et al. 2014. Gene pleiotropy constrains gene expression changes in fish adapted to different thermal conditions. *Nature Communications* 5: 4071

Pavey SA, Sevellec M, Adam W, Normandeau E, Lamaze FC, et al. 2013. Nonparallelism in MHCIIbeta diversity accompanies nonparallelism in pathogen infection of lake whitefish (*Coregonus clupeaformis*) species pairs as revealed by next-generation sequencing. *Molecular Ecology* 22: 3833-49

Pérez-Pereira N, Quesada H, Caballero A. 2017. Can parallel ecological speciation be detected with phylogenetic analyses? *Molecular Phylogenetics and Evolution* 116: 149-56

Perreault-Payette A, Muir AM, Goetz F, Perrier C, Normandeau E, et al. 2017. Investigating the extent of parallelism in morphological and genomic divergence among lake trout ecotypes in Lake Superior. *Molecular Ecology* 26: 1477-97

Perrier C, Bourret V, Kent MP, Bernatchez L. 2013. Parallel and nonparallel genome-wide divergence among replicate population pairs of freshwater and anadromous Atlantic salmon. *Molecular Ecology* 22: 5577-93

Peterson DA, Hilborn R, Hauser L. 2014. Local adaptation limits lifetime reproductive success of dispersers in a wild salmon metapopulation. *Nature Communications* 5: 3696

Pfenninger M, Patel S, Arias-Rodriguez L, Feldmeyer B, Riesch R, Plath M. 2015. Unique evolutionary trajectories in repeated adaptation to hydrogen sulphide-toxic habitats of a neotropical fish (*Poecilia mexicana*). *Molecular Ecology* 24: 5446-59

Pujolar JM, Ferchaud AL, Bekkevold D, Hansen MM. 2017. Non-parallel divergence across freshwater and marine three-spined stickleback *Gasterosteus aculeatus* populations. *Journal of Fish Biology* 91: 175-94

Ramiro RS, Costa H, Gordo I. 2016. Macrophage adaptation leads to parallel evolution of genetically diverse Escherichia coli small-colony variants with increased fitness in vivo and antibiotic collateral sensitivity. *Evolutionary Applications* 9: 994-1004

Ravinet M, Westram A, Johannesson K, Butlin R, Andre C, Panova M. 2016. Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. *Molecular Ecology* 25: 287-305

Rensch B. 1939. Typen der Art-bildung. *Reviews of the Cambridge Philosophical Society* 14: 180-222

Rinkevich B. 2005. Conservation of coral reefs through active restoration measures: recent approaches and last decade progress. *Environmental Science and Technology* 39: 4333-42

Roberge C. 2006. Rapid parallel evolutionary changes of gene transcription profiles in farmed Atlantic salmon. *Molecular Ecology* 15: 9-20

1156 Roda F, Walter GM, Nipper R, Ortiz-Barrientos D. 2017. Genomic clustering of adaptive loci
1157 during parallel evolution of an Australian wildflower. *Molecular Ecology* 14: 3687-99
1158 Roesti M, Moser D, Berner D. 2013. Recombination in the threespine stickleback genome--
1159 patterns and consequences. *Molecular Ecology* 22: 3014-27
1160 Rokas A, Carroll SB. 2008. Frequent and widespread parallel evolution of protein sequences.
1161 *Molecular Biology and Evolution* 25: 1943-53
1162 Rosenblum EB, Harmon LJ. 2011. "Same same but different": replicated ecological speciation
1163 at White Sands. *Evolution* 65: 946-60
1164 Rosenblum EB, Parent CE, Brandt EE. 2014. The molecular basis of phenotypic convergence.
1165 *Annual Reviews of Ecology Evolution and Systematics* 45: 203-26
1166 Rosenblum EB, Römpler H, Schöneberg T, Hoekstra HE. 2010. Molecular and functional basis
1167 of phenotypic convergence in white lizards at White Sands. *Proceedings of the National*
1168 *Academy of Sciences* 107: 2113-17
1169 Sailer ZR, Harms MJ. 2017. Molecular ensembles make evolution unpredictable. *Proceedings*
1170 *of the National Academy of Sciences* Online Early
1171 Samuk K, Owens GL, Delmore KE, Miller SE, Rennison DJ, Schluter D. 2017. Gene flow and
1172 selection interact to promote adaptive divergence in regions of low recombination.
1173 *Molecular Ecology* 26: 4378-90
1174 Schmutz J, McClean PE, Mamidi S, Wu GA, Cannon SB, et al. 2014. A reference genome for
1175 common bean and genome-wide analysis of dual domestications. *Nature Genetics* 46:
1176 707-13
1177 Scotland RW. 2011. What is parallelism? *Evolution and Development* 13: 214-27
1178 Shindo C, Aranzana MJ, Lister C, Baxter C, Nicholls C, et al. 2005. Role of FRIGIDA and
1179 FLOWERING LOCUS C in Determining Variation in Flowering Time of Arabidopsis.
1180 *Plant Physiology* 138: 1163-73
1181 Shpak M, Lu J. 2016. An evolutionary genetic perspective on cancer biology. *Annual Reviews of*
1182 *Ecology Evolution and Systematics* 47: 25-49
1183 Siepielski AM, DiBattista JD, Carlson SM. 2009. It's about time: the temporal dynamics of
1184 phenotypic selection in the wild. *Ecology Letters* 12: 1261-76
1185 Simpson GG. 1953. *The Major Features of Evolution*. Columbia University Press.
1186 Simpson GG. 1961. *Principles of Animal Taxonomy*. New York: Columbia University Press.
1187 Siwertsson A, Knudsen R, Adams CE, Praebel K, Amundsen PA. 2013. Parallel and non-
1188 parallel morphological divergence among foraging specialists in European whitefish
1189 (*Coregonus lavaretus*). *Ecology and Evolution* 3: 1590-602
1190 Slatkin M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics*
1191 16: 393-430
1192 Speed MP, Arbuckle K. 2017. Quantification provides a conceptual basis for convergent
1193 evolution. *Biological Reviews* 92: 815-29
1194 Stayton CT. 2008. Is convergence surprising? An examination of the frequency of convergence
1195 in simulated datasets. *Journal of Theoretical Biology* 252: 1-14
1196 Stockwell CA, Hendry AP, Kinnison MT. 2003. Contemporary evolution meets conservation
1197 biology. *Trends in Ecology and Evolution* 18: 94-101
1198 Storz JF. 2016. Causes of molecular convergence and parallelism in protein evolution. *Nature*
1199 *Reviews Genetics* 17: 239-50
1200 Stuart YE, Veen T, Weber JN, Hanson D, Lohman BK, et al. 2017. Contrasting effects of
1201 environment and genetics generate a continuum of parallel evolution. *Nature Ecology*
1202 *and Evolution* 1: 0158
1203 Sturm RA, Duffy DL. 2012. Human pigmentation genes under environmental selection. *Genome*
1204 *Biology* 13: 248
1205 Swanton C. 2014. Cancer evolution: the final frontier of precision medicine? *Annals of Oncology*
1206 25: 549-441

Szendro IG, Franke J, de Visser JAGM, Krug J. 2013. Predictability of evolution depends nonmonotonically on population size. *Proceedings of the National Academy of Sciences* 110: 571-76

Takahashi K, Kohno T, Matsumoto S, Nakanishi Y, Arai Y, et al. 2007 Clonal and parallel evolution of primary lung cancers and their metastases revealed by molecular dissection of cancer cells. *Human Cancer Biology* 13: 111-20

Takuno S, Ralph P, Swarts K, Elshire RJ, Glaubitz JC, et al. 2015. Independent molecular basis of convergent highland adaptation in maize. *Genetics* 207: 1297-312

Tegze B, Szállási Z, Haltrich I, Pénzváltó Z, Tóth Z, et al. 2012. Parallel Evolution under Chemotherapy Pressure in 29 Breast Cancer Cell Lines Results in Dissimilar Mechanisms of Resistance. *PLoS One* 7: e30804

Tellier A, Brown JK. 2007. Polymorphism in multilocus host parasite coevolutionary interactions. *Genetics* 177: 1777-90

Tenaillon O, Barrick J, Ribeck N, Deatherage DE, Blanchard JL, et al. 2016. Tempo and mode of genome evolution in a 50,000-generation experiment. *Nature* 536: 165-70

Terekhanova NV, Logacheva MD, Penin AA, Neretina TV, Barmintseva AE, et al. 2014. Fast evolution from precast bricks: genomics of young freshwater populations of threespine stickleback *Gasterosteus aculeatus*. *PLoS Genetics* 10: e1004696

Thompson C, Ahmed N, Veen T, Peichel CL, Hendry AP, et al. 2017. Many-to-one form-to-function mapping weakens parallel morphological evolution. *Evolution*

Thopmson CE, Taylor EB, McPhail JD. 1997. Parallel evolution of lake-stream pairs of threespine sticklebacks (*Gasterosteus*) inferred from mitochondrial DNA variation. *Evolution* 51: 1955-65

Torriani SFF, Brunner PC, McDonald BA, Sierotzki H. 2008. QoI resistance emerged independently at least 4 times in European populations of Mycosphaerella graminicola. *Pest Management Science* 65: 155-62

Vavilov NI. 1922. Journal of Genetics. *The law of homologous series in variation* 12: 47-89

Velotta JP, Wegrzyn JL, Ginzburg S, Kang L, Czesny S, et al. 2017. Transcriptomic imprints of adaptation to fresh water: parallel evolution of osmoregulatory gene expression in the Alewife. *Molecular Ecology* 26: 831-48

Vogwill T, Kohadinovic M, Furió V, MacLean RC. 2014. Testing the role of genetic background in parallel evolution using the comparative experimental evolution of antibiotic resistance. *Molecular Biology and Evolution* 31: 3314-23

Wainwright PC. 1996. Ecological explanation through functional morphology: The feeding biology of sunfishes. *Ecology* 77: 1336-43.

Wainwright PC, Alfaro M, Bolnick DI, Hulsey CD. 2005. Many-to-one mapping of form to function: a general principle in organismal design. *Integrative and Comparative Biology* 45: 256–62

Wake DB. 1999. Homoplasy, homology and the problem of 'sameness' in biology In *Homology*, ed. GR Bock, G Cardew, pp. 24-46. New York: John Wiley & Sons

Wake DB, Wake MH, Specht CD. 2011. Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science* 331: 1032-35

Walker JA. 2007. A general model of functional constraints on phenotypic evolution. *American Naturalist* 170: 681-89

Westram AM, Galindo J, Rosenblad MA, Grahame JW, Panova M, Butlin RK. 2014. Do the same genes underlie parallel phenotypic divergence in different *Littorina saxatilis* populations? *Molecular Ecology* 23: 4603-16

Westram AM, Panova M, Galindo J, Butlin RK. 2016. Targeted resequencing reveals geographical patterns of differentiation for loci implicated in parallel evolution. *Molecular Ecology* 25: 3169-86

1257 Whitlock MC, Gomulkiewicz R. 2005. Probability of fixation in a heterogeneous environment.
1258 *Genetics* 171: 1407-17
1259 Wichman HA, Badgett MR, Scott LA, Boulianne CM, Bull JJ. 1999. Divergent trajectories of
1260 parallel evolution during viral adaptation. *Science* 285
1261 Wilson HV. 1941. The recapitulation theory or biogenetic law in embryology. *American*
1262 *Naturalist* 75: 20-30
1263 Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, et al. 2016. Convergent local
1264 adaptation to climate in distantly related conifers. *Science* 353: 1431-33
1265

TERMS AND DEFINITIONS

Parallel evolution (standard) – evolution of similar phenotypes or genotypes in multiple independent populations, in response to similar selection pressures, from *similar* initial conditions.

Convergent evolution (standard) – evolution of similar phenotypes or genotypes in multiple independent populations, in response to similar selection pressures, from *different* initial conditions.

Parallel evolution (geometric) - a low angle ($\theta \sim 0°$) between evolutionary trajectories of independent replicates through trait (or genotype) space (Fig. 1A).

Nonparallel evolution – evolutionary vectors of two replicates are not parallel ($\theta >> 0°$), potentially resulting in convergent or divergent evolution (Fig 1A).

Antiparallel evolution – most extreme nonparallelism, when replicate vectors point in exactly opposite directions (Fig. 1A; $\theta \sim 180°$)

(Non)parallel evolution – shorthand for the distribution of outcomes across populations and traits forming a continuum from parallel, to orthogonal, or even antiparallel evolution.

Convergent evolution (geometric) – when the endpoints of two evolutionary vectors are closer together than the vectors origins (Fig. 1B).

Divergent evolution – the evolution of increased distance between populations in phenotype or genotype space (Fig. 1B).

Many-to-one mapping – when many distinct genotypes can yield the same phenotype, or many distinct phenotypes can yield the same function.

PCVA – Phenotypic Change Vector Analysis is a multivariate approach to measuring trait change or (non)parallel evolution by quantitatively comparing change vectors.

PTA – Phenotypic Trajectory Analysis entails a series of head-to-tail PCVA vectors forming an evolutionary trajectory through trait space.

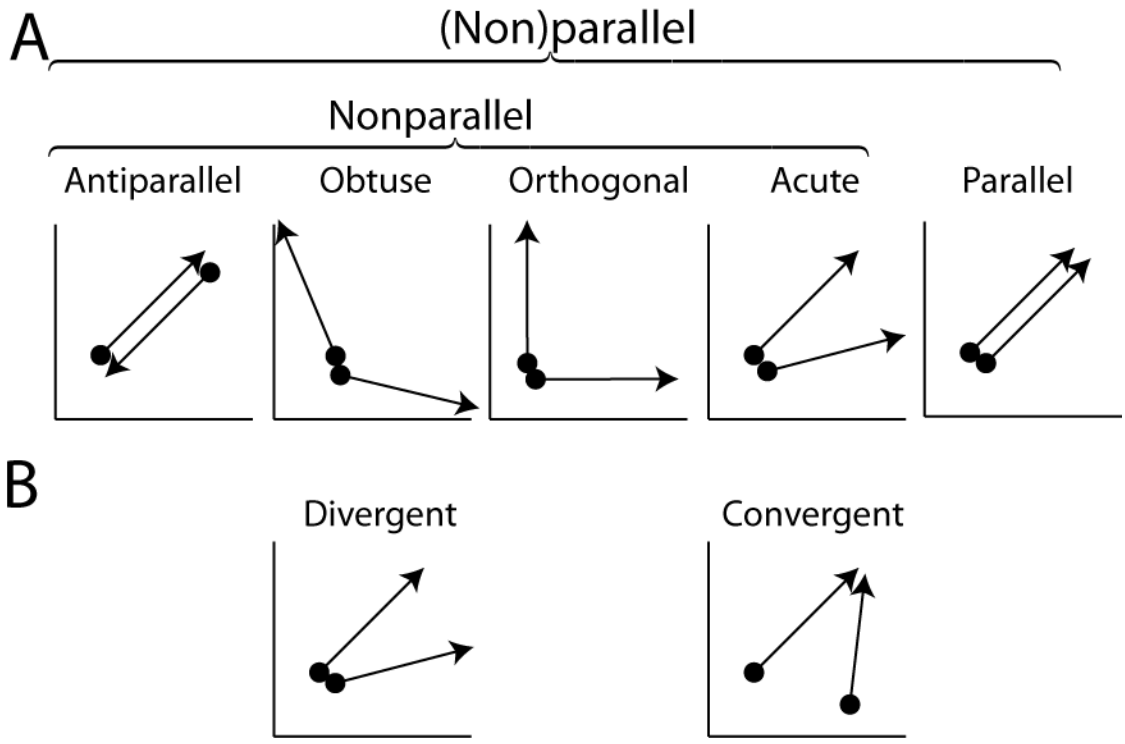
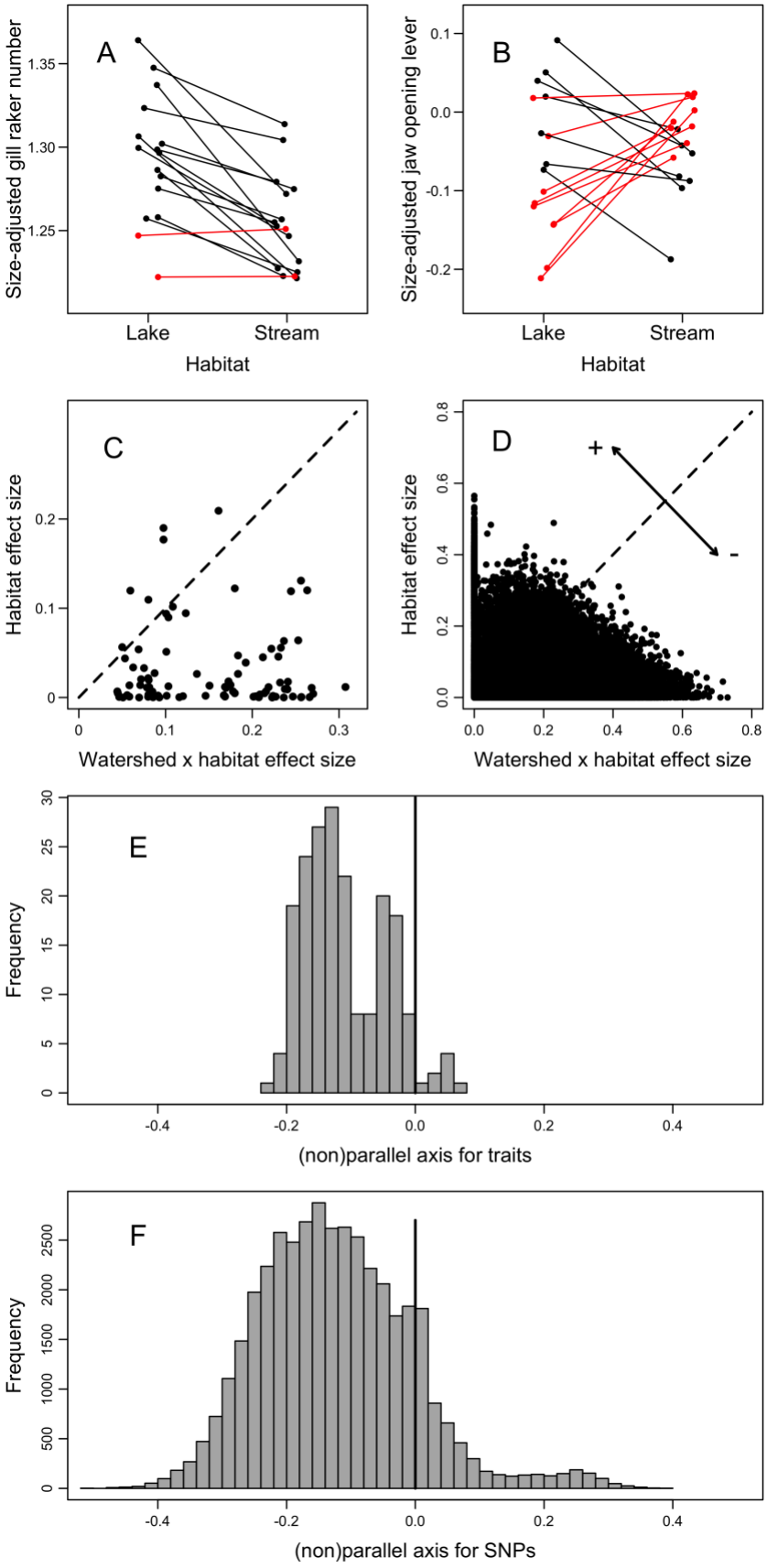
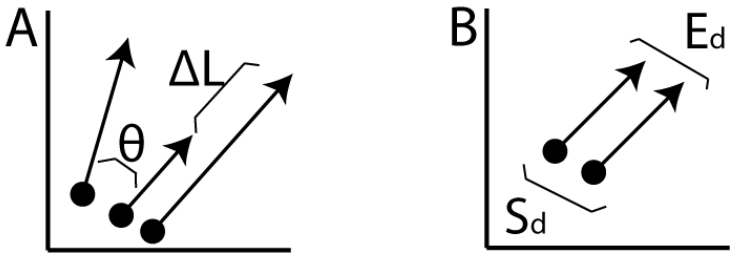


Figure 1. A visual glossary illustrating our use of terms. Each panel represents two replicate evolutionary trajectories (e.g., from ancestor to descendent) plotted as arrows in multivariate trait space. Drawing on geometric definitions, evolution can range from parallel (arrows pointing in the same direction) to antiparallel (arrows that point in opposite directions) and various angles in between. We use 'nonparallel' to refer to the logical complement of 'parallel', and '(non)parallel' to refer to the entire continuum. Continuing with this geometric theme, convergent and divergent are separate concepts from (non)parallelism, having more to do with whether or not descendents are more similar to each other than ancestors. The relationship between the (non)parallel continuum, and the convergence-divergence continuum is illustrated in more detail in Fig. 3.


A
Size-adjusted gill raker number
1.35
1.30
1.25
Lake
Stream
Habitat
B
Size-adjusted jaw opening lever
0.1
0.0
-0.1
-0.2
Lake
Stream
Habitat
C
Habitat effect size
0.2
0.1
0
0
0.1
0.2
0.3
Watershed x habitat effect size
D
+
-
Habitat effect size
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
Watershed x habitat effect size
E
Frequency
30
25
20
15
10
5
0
-0.4
-0.2
0.0
0.2
0.4
(non)parallel axis for traits
F
Frequency
2500
2000
1500
1000
500
0
-0.4
-0.2
0.0
0.2
0.4
(non)parallel axis for SNPs

1304

Figure 2. An example of variation along the (non)parallel continuum in 16 lake-stream pairs of threespine stickleback (modified from Stuart et al. 2017). (A) Gill raker number (size-standardized) shows strong parallel changes with more gill rakers in lake fish in 14 out of 16 pairs (red lines indicate contrary directions), resulting in a strong main effect of habitat (shared change). (B) Lower jaw opening kinematic transmission (kt) exhibits little parallel evolution with equal numbers of cases of lake or stream fish having higher mean kt, resulting in a strong habitat*watershed interaction (unique change). To summarize this variation, Stuart et al plotted habitat versus habitat*watershed effect sizes (partial η_2) for (C) all 86 morphological traits and (D) 74,000 SNPs from ddRADseq. Points lie mostly below the dashed line of equal effect, indicating that unique evolution is typically stronger than shared evolution. To view this variation along a single nonparallel / parallel axis, we calculated each trait or SNP's distance from the line of equal effect (positive values above/left of the line denote more parallel evolution, negative values below/right the line indicate more nonparallel evolution). We plot histograms of traits (E) and SNPs (F) on this (non)parallel axis, to illustrate the point that evolution at both levels is primarily nonparallel, but a small number of traits and SNPs form a distinct peak of parallelism, likely representing targets of parallel selection.


A
ΔL
θ
B
Ed
Sd

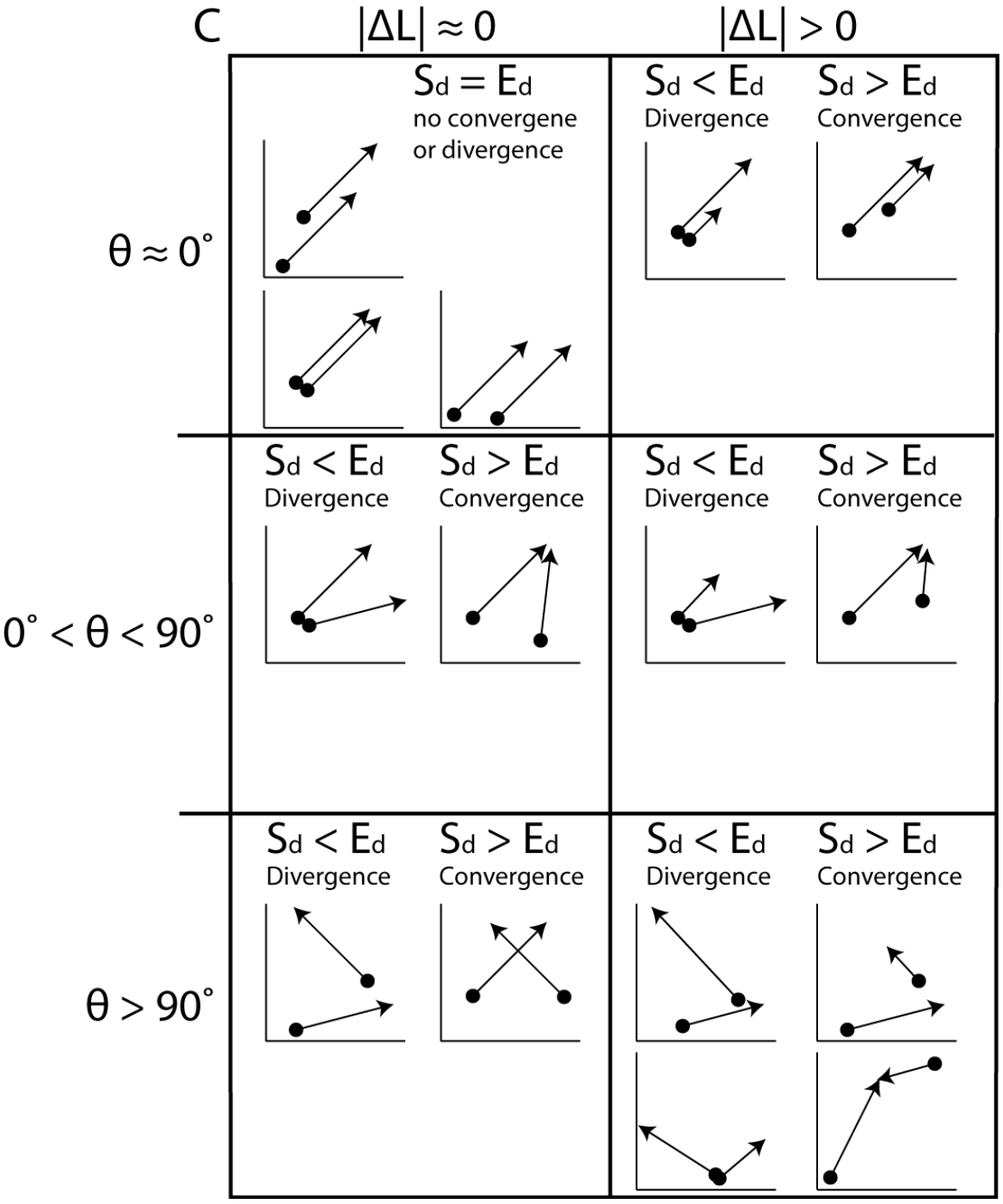

C
|ΔL| ≈ 0
|ΔL| > 0
θ ≈ 0°
0° < θ < 90°
θ > 90°
Sd = Ed
no convergene
or divergence
Sd < Ed
Divergence
Sd > Ed
Convergence

Figure 3. Use of Phenotypic Change Vector Analysis (PCVA) to quantify (non)parallel evolution as well as divergence or convergence. We illustrate the approach using the evolution of two quantitative traits (x and y axes on the small graphs). (A) The trajectory of evolution can be represented in morphospace as a vector connecting the centroids of two paired from different habitats. Each evolutionary replicate pair constitutes its own vector (here, we plot vectors for three such pairs). Any two replicate evolutionary trajectories can be compared to calculate an angle θ and a length difference ΔL. (B) In addition to calculating measures of parallelism, we can measure the extent of convergence or divergence. We define S_d as the distance between two replicates' starting points; and E_d as the distance between ending points. The two vectors diverge if the end points are farther apart than the starting points ($S_d < E_d$), and converge if $S_d > E_d$. Panel (C) presents various combinations of scenarios for (non)parallelism and convergence or divergence. Two replicate evolutionary trajectories are highly parallel when the angle between them (θ) is near zero (top row); they are acute nonparallel when they point in roughly the same direction but with some moderate angle (e.g., $\theta < 90°$; middle row), and obtuse nonparallel or even antiparallel when the replicates evolve in opposite directions ($\theta >> 90°$; bottom row). The left and right columns of (C) represent cases where vector lengths are similar ($\Delta L \sim 0$, left column) or different ($\Delta L > 0$, right column). Evolution is highly parallel in the top left box ($\theta \sim 0$ and $\Delta L \sim 0$), and no divergence or convergence is possible. For all other scenarios it is possible to have divergence or convergence for both parallel and nonparallel evolution.

Sidebar 1. Experimental study of parallel evolution

Many convincing studies of (non)parallelism come from selection experiments in laboratory populations (Bailey et al 2015, Graves et al 2017, Lenski 2017, Meyer et al 2010). By limiting variation in as many possible explanatory factors as possible, the design of these experiments permits careful tests of a limited number of mechanisms at a time. A meta-analysis of evolve-and-resequence experiments with bacteria and yeast revealed a positive relationship between population size and the probability of parallel change (Bailey et al 2017). Mutation rate heterogeneity strongly influenced the extent of parallel genetic change during selection in shared environments. Deviations from parallel evolution were therefore partly non-adaptive. An important lesson from these studies is that the likelihood of observing parallel evolution is often dependent on the level of the biological hierarchy that is investigated. Because of many-to-one mapping (see main text), repeatability is typically highest for fitness itself, lower for phenotypes, lower still at the level of the genes, and lowest at the level of individual mutations (Tenaillon et al 2016). There is also growing experimental evidence that frequency dependent ecological interactions can contribute to (non)parallel evolutionary dynamics (Douglas et al 2016, Herron & Doebeli 2013, Josephides & Swain 2017).