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GENOMICS PAPER

Time-Series Sequences for Evolutionary Inferences

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Introduction

The application of genomics to evolutionary biology has provided unprecedented power and resolution to investigate processes like mutation (Nachman and Crowell 2000; Rozhok and Degregori 2019), genetic drift (Whitney and Garland 2010; Funk et al. 2016), gene flow (Gallego-García et al. 2019; LaCava et al. 2021), and natural selection (Brauer et al. 2016; Martins et al. 2018). Evolutionary genomics has historically used contemporary or single-timepoint samples to study microevolutionary processes that most often depend on idealized model assumptions (e.g., Wright–Fisher model) (Butlin 2008; Hoban et al. 2016). For example, genome scanning methods aim to detect natural selection by assuming that the impact of selection can be discerned from the effects of neutral evolutionary processes on genetic differentiation (Hoban et al. 2016). However, different demographic scenarios can also produce signatures of selection, leading to false positives (Lotterhos and Whitlock 2015; Haasl and Payseur 2016). Similarly, when using measures of genetic differentiation to estimate gene flow, most models assume that population size is large enough that genetic drift is negligible—that is, not driving neutral divergence between populations (Whitlock and McCauley 1999; Ma et al. 2015).

Overall, violating model assumptions (e.g., neglecting the role of drift) can lead to erroneous results that compound into incorrect inferences (Waples 1998; Strasburg and Rieseberg 2010). On one hand, simulation and methodological research may attempt to remove or account for some of these assumptions (Bradburd et al. 2013; Lotterhos and Whitlock 2015; Ma et al. 2015; Haasl and Payseur 2016). On the other hand, to model processes and predict changes over time, some researchers have employed space-for-time substitutions, which assume that the drivers of biotic change such as changes in allele frequency, species abundance, and community structure between segregated populations (spatially or environmentally) also drive biotic changes across time (Pickett 1989; Fitzpatrick and Keller 2015). Space-for-time substitutions often either use a "different aged sites" or "spatiotemporal turnover" framework (Wogan and Wang 2018). In the "different aged sites" framework, researchers infer rates of evolutionary change using populations of different ages (i.e., different colonization times) or populations that have experienced an environmental condition for different lengths of time as a substitute for longitudinal samples. For example, Terekhanova et al. (2014) used freshwater lakes of different ages to quantify the strength of positive selection on alleles related to adaptation to freshwater environments in the threespine stickleback, *Gasterosteus aculeatus*. Terekhanova et al. (2014) used populations of different ages as time-series snapshots to investigate the temporal dynamics of local adaptation. Under a "spatiotemporal turnover" framework, populations along an environmental gradient (e.g., temperature, urbanization) are used to isolate the impact of abiotic variables on population differences (e.g., allele frequencies, population size) to predict population responses to environmental change through time (Wogan and Wang 2018). Bay et al. (2018)used genomic data from the yellow warbler, *Setophaga petechia*, to quantify the association between genomic variation and climate before applying these associations in a "spatiotempo-

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ral turnover" framework to predict potential population declines with future climate change. Both Terekhanova et al. (2014) and Bay et al. (2018) used single-timepoint samples to make inferences about temporal changes in allele frequencies or populations, albeit with different approaches. The key similarity is the use of contemporary samples from populations that are spatially segregated with different conditions or histories to make inferences about biotic changes across time.

Researchers often use space-for-time substitutions to investigate the drivers of change over time. While Wogan and Wang (2018) identified multiple scenarios in which the substitution was valid, they acknowledged scenarios where the method was inappropriate, highlighting the need for further work validating its application. Furthermore, direct conversions between space (e.g., distance, environments) and time, which are critical for quantifying the rate of evolutionary response, are system-specific (Bergland et al. 2014) due to differences between taxa like generation time or population size. Temporal genomics is an attractive alternative to space-for-time substitutions that applies time-series sequences to evolutionary biology using data derived from either sampling at multiple timepoints (Vega et al. 2017; Mei et al. 2020), historical samples (Tracy and Jamieson 2011; Chattopadhyay et al. 2019), or fossils (Mitchell et al. 2016; van der Valk et al. 2021). As we will discuss below, time-series genomic data from either repeated sampling events or preserved specimens combined with contemporary sampling can also be used to disentangle the impact of multiple evolutionary forces (Buffalo and Coop 2019; Buffalo and Coop 2020).

The application of time-series genetic data is not restricted to fundamental evolutionary questions (Waters et al. 2015; Vega et al. 2017; von Seth et al. 2021). Long-standing conservation programs have used temporal analyses of genetic data to inform their conservation programs (Wasko et al. 2004; Fisch et al. 2013) and evaluate the efficacy of actionable interventions (La Haye et al. 2017; Jensen et al. 2018). For example, Kotzé et al. (2019) compared genetic samples of Cape mountain zebra, *Equus zebra zebra,* from African nature reserves collected between 1999 and 2001 with modern samples collected in 2016 to identify decreases in genetic diversity and population structure that could result in inbreeding depression and a reduction of adaptive evolutionary potential. Similarly, Glover et al. (2012) analyzed 3,049 samples of Atlantic salmon, *Salmo salar*, collected between 1970 and 2010 from 21 Norwegian populations and demonstrated that historic population structure was preserved despite large quantities of farm-raised salmon escaping into the wild, each year. These results indicated that escapees experience low to moderate reproductive success, providing critical information regarding the impact of commercial salmon producers on the genetic variation of wild populations. Well-established conservation programs are therefore an excellent source of long-term temporal samples that enable improved conservation efforts.

Unfortunately, the economic and time constraints of scientific research hinder carrying out longitudinal studies in natural populations that are not already the focus of long-term research. However, natural history collections may offer alternative sources of historical samples that can be used strategically to develop temporal genetic and genomic studies (Lopez et al. 2020; Card et al. 2021). Thanks to recent advances in high-throughput sequencing and DNA extraction protocols, it is possible to assemble genomic data from degraded (Bi et al. 2013; Gamba et al. 2016; Tsai et al. 2020) and formalin-fixed tissues through nondestructive sampling (Tin et al. 2014; Hykin et al. 2015; Ruane and Austin 2017; Nakahama 2021), enabling researchers to harness natural history collections while preserving specimens for future research (Ruane 2021). In the current issue, Cohen et al. (2022) use samples from museum specimens and contemporary samples from wild populations to show that Colorado potato beetles, *Leptinotarsa decemlineata*, underwent a population expansion in the 19th century, and genome scans suggest that agricultural field populations have experienced strong selection on known insecticide resistance genes. von Thaden et al. (2021) compared modern samples collected through a long-term monitoring program and historical museum specimens of European wildcat, *Felis silvestris*, and demonstrated that the metapopulation structure previously attributed to postglacial differentiation was better explained by a human-induced bottleneck followed by re-expansion from isolated refugia. Overall, natural history collections enable researchers to detect recent evolutionary responses without having to establish long-term studies on existing natural populations.

Natural history collections, advancing methodologies, and lower sequencing costs have propelled the field of temporal genomics. The field is diverse, with both applied (Wasko et al. 2004; Vega et al. 2017; Kotzé et al. 2019) and fundamental (Bergland et al. 2014; Machugh et al. 2017) research focused on short (Kawecki et al. 2012; Lenski et al. 2015; Durland et al. 2021) and long (Brunel et al. 2020; Der Sarkissian et al. 2020) timescales. On longer timescales, ancient DNA (aDNA) research focuses on processes that act over thousands (Machugh et al. 2017; Yang et al. 2020) to millions (van der Valk et al. 2021) of years. Loog et al. (2020) used contemporary and ancient samples of the grey wolf, *Canis lupus*, spanning 50,000 years to trace modern wolf ancestry back to an expansion from Beringia

(the land bridge spanning Asia and North America). On a shorter timescale, Perry et al. (2022), this issue, quantify gene expression in brown bears, *Ursus arctos*, in different tissue types to investigate differences in gene isoform expression among seasons of the hibernation cycle. By sampling repeatedly within a year, the authors uncovered specific changes in gene expression that relate to hibernation phenotypes, providing insights into the molecular mechanisms that regulate complex animal behaviors. Furthermore, temporal methodologies can use samples across development to better understand changes in gene expression throughout an organism's life. In this issue, Oomen et al. (2022) used experimental tanks of Atlantic cod (*Gadus morhua*) larvae at different temperatures and sampled them throughout development to examine the temporal dynamics of gene expression under thermal stress. Through this special issue, we aim to highlight the diversity of temporal genomic research while promoting the extension of time-series methodologies to other "omics" approaches (e.g., metabolomics, proteomics, epigenomics). This introduction provides a brief overview of the application of temporal genomics to study microevolution, and its implementation within conservation. We also contextualize the studies within this special issue to inspire future research with time-series molecular data.

Quantifying evolutionary forces

Stochastic evolutionary forces (mutation, gene flow, and genetic drift) and natural selection can be distinguished based on whether deterministic shifts in population allele frequencies occur. While mutation is not random (Martincorena et al. 2012; Sung et al. 2012), it is often considered neutral because mutations do not necessarily have an immediate fitness benefit. Similarly, gene flow is not random either. A host of features, including individual performance, dispersal capacity, and behavior, can impact an individual's dispersal probability (Edelaar and Bolnick 2012). Compared to mutation, and gene flow, genetic drift, the change in allele frequencies across generations driven by chance events independent of phenotype, is random (Wright 1931; Wright 1984). Conversely, natural selection acts on heritable phenotypic variation across generations to drive population trait values toward an environmentally dependent optimum, often resulting in local adaptation. Hence, natural selection drives deterministic shifts that increase the frequency of alleles that confer high fitness in that particular environment. The relative strength of these processes is both species- and population-specific, driven by a combination of genomic, demographic, and environmental factors. Directly quantifying the strength of these evolutionary forces with sequence data requires time-series genomic data because single-timepoint studies cannot estimate the rate of allele frequency change outside of idealized population genetic models. Temporal genomics enables researchers to directly estimate rates of evolutionary change by comparing allele frequencies across time (Kelson et al. 2020; Buffalo and Coop 2019; van der Valk et al. 2019; 2020). Furthermore, different evolutionary processes can generate similar genomic patterns (Barber et al. 2011; Simões et al. 2016; Wolf and Ellegren 2017) that are indistinguishable without repeated sampling events (Buffalo and Coop 2019; Chen et al. 2019; Buffalo and Coop 2020). For example, areas within the genome with high genetic divergence are often referred to as speciation genes and often assumed to be driven by disruptive selection; however, similar patterns of divergence could be attributed to background selection on sites in linkage disequilibrium (Wolf and Ellegren 2016). Similarly, high rates of gene flow from adjacent populations can increase the frequency of alleles at a particular locus and be misattributed to selection (Chen et al. 2019). Therefore, temporal genomics can provide unprecedented insights into the relative contribution of each evolutionary force while disentangling their simultaneous impacts.

Mutation

Only mutation is capable of generating new genetic variation, and the rate at which it occurs varies significantly across taxa, cell types, life stages, and regions of the genome (Martincorena et al. 2012; Sung et al. 2012). Somatic mutations occur within non-gametic cells, are not inherited, and thus do not contribute to the population genetic variability. Germline mutations occur within gametes and can be inherited, resulting in genetic variability that can persist across generations. Therefore, the direct quantification of mutation rates can be arduous as it requires comprehensive sampling across many generations. Researchers have tried to circumvent time-series sequence data extracted from individuals to infer mutation rates (Eyre-Walker and Keightley 1999; Kumar and Subramanian 2002) utilizing, for example, cell cultures (Milholland et al. 2017) that may not be representative of natural reproduction. However, direct quantification of mutation rates requires time-series genomic data from individuals to accurately measure the introduction and potential fixation of mutations across generations.

Experimental evolution has capitalized on model organisms (e.g., *Drosophila melanogaster*, *Escherichia coli*, *Saccharomyces cerevisiae*) with short generation times (20 min–7 days) to quantify mutation rates by repeatedly sampling populations under controlled laboratory conditions (Haag-Liautard et al. 2008; Wielgoss et al.

2011; Zhu et al. 2014; Ramiro et al. 2020). The application of temporal genomics to such experimental evolution studies enables researchers to detect mutations and explore their evolutionary consequences (Cutter 2005; Segrè et al. 2006; Khan et al. 2011). Furthermore, experimental evolution with repeated genomic sampling is instrumental in the study of environmental drivers (e.g., temperature) of changes in mutation rate (Waldvogel and Pfenninger 2012) and heterogeneity of mutation rates across the genome (Lang and Murray 2011; Raynes and Sniegowski 2014). One limitation of time-series genomics in studying mutation rates within natural populations is the ability to identify mutations with confidence. Researchers can use experimental evolution to sequence ancestral populations with high resolution and compare them to subsequent generations to detect novel mutations. However, detecting novel mutations in the wild requires intensive genetic sampling of the focal and neighboring populations to ensure that newly detected alleles are truly new mutations rather than undetected standing genetic variation or private alleles from adjacent populations.

One unparalleled example of the application of temporal genomics in the wild is the case of the COVID-19 pandemic. The advancement and accessibility of high-throughput sequencing techniques have been instrumental in the surveillance and management of the pandemic: tracking the spread of the virus and identifying new mutations and variants of concern, which, in turn, has facilitated the development of clinical tests and predicting the efficacy of vaccines against viral variants (Knyazev et al. 2022). Furthermore, the democratization of viral sequencing has offered free access to millions of sequences worldwide (by May 2022, 10,851,095 h-CoV-19 genomes had been submitted to the GSAID database https://www.gisaid.org/). Analysis of these sequences have enabled scientists to witness the temporal dynamics of viral genome evolution in real-time, even within a single host, and how these changes affect the transcriptome, translatome, and immune response (Pathak et al. 2022).

Gene flow

Gene flow can increase genetic variation in a population by introducing novel or previously lost alleles from adjacent populations (Consuegra et al. 2005; Frankham 2015). High levels of gene flow, on the other hand, can decrease genetic variation across populations through the loss of private alleles and genetic homogenization (Baillie et al. 2016). Among other things, the magnitude of gene flow depends on the geographical distance and environmental differences between populations (Hand et al. 2015; Sacks et al. 2016). Observed patterns of gene flow could be the product of historic or contemporary environmental conditions; however, single-timepoint samples are not able to discern between the two as the modern genetic structure could be driven by events at either timescale. Furthermore, inferences into the abiotic drivers of gene flow are further complicated by the fact that population genetic structure requires time to reach equilibrium after a change in gene flow patterns (Cushman et al. 2006; Anderson and Meikle 2010; Anderson et al. 2010; García et al. 2021); genetic equilibrium is the population genetic structure that is stable at the current pattern of gene flow (e.g., magnitude, directionality). Landguth et al. (2010) simulated different gene flow patterns (e.g., nearest-neighbor dispersal, long-distance dispersal, panmixia) to determine how many generations it takes to detect the genetic signal of a landscape barrier. They showed significant lag time between barrier establishment and any quantifiable genetic effects using F_{ST}, a metric that took >200 generations to reach 50% of its equilibrium value. This could indicate that F_{ST} performs poorly as a metric to measure how recent landscape change affects population connectivity (Landguth et al. 2010). On the contrary, they showed relatively short lag times (1–15 generations) between barrier establishment and the emergence of population genetic structure measured by other metrics like Dps or G'_{ST}. After a barrier disappears, its genetic effects may still be detected after 100 generations (Landguth et al. 2010). Therefore, longitudinal sampling is useful to assess whether populations are at equilibrium and, if gene flow changes between timepoints, whether the change is due to an environmental shift or adjusting to a new equilibrium.

Gene flow estimates based only on single-timepoint samples may fail to detect rapid changes in connectivity patterns, but temporal genetic and genomic studies can identify changes in historical connectivity across a range of timescales (Therkildsen et al. 2013; Holmes 2015; Pascual et al. 2016; Anderson et al. 2020; Mamoozadeh et al. 2020). In this issue, Byerly et al. (2022) use contemporary and historic samples to explore potential changes in historic and contemporary connectivity and diversity between two populations of roseate terns, *Sterna dougallii*. They show that both populations were historically and contemporarily isolated and have not suffered significant changes in their genetic diversity, suggesting temporal stability and that recent environmental shifts are not responsible for limiting gene flow between populations. Conversely, in the alpine chipmunk, *Tamias alpinus*, Bi et al. (2013) observed a reduction in gene flow between populations over time, indicating temporal variability in gene flow, potentially induced by climate change (Bi et al. 2013). Without temporal data, it would be challenging to

determine the relative roles of historical events vs. recent landscape or environmental changes on genetic connectivity. Furthermore, some studies incorporate historical and contemporary data from abiotic factors (e.g., paleoclimatic, Landsat, land-use change) into analyses of gene flow, which can better resolve how specific environmental factors affect population genetic structure. For example, Kelson et al. (2020) showed that the permeability of partial barriers to migration in river populations of rainbow trout, *Oncorhynchus mykiss*, varied temporally as a result of interannual differences in precipitation and high elevation streamflow. Hence, employing time-series genomic data enables researchers to disentangle historical and contemporary drivers of population genetic connectivity and the temporal stability of population structure.

Genetic drift

In contrast to evolutionary forces that generate genetic variation, genetic drift decreases genetic diversity within populations and increases variation between them through stochastic changes in allele frequencies. Given that the strength of genetic drift increases as population size declines (Wright 1931; Wright 1984) and strong drift rapidly erodes genetic diversity (Harper et al. 2006), researchers use patterns of genetic diversity to infer demographic histories and the processes driving population changes. Most current methods rely on the coalescent theory, which posits that the probability that two alleles coalesce or occur in the same individual is inversely proportional to population size and directly proportional to time (in generations). Coalescent models enable researchers to estimate the time to the most recent common ancestor and changes in population size through time (Marchi et al. 2021). However, in many species, genetic diversity is weakly correlated with current population size (Leffler et al. 2012; Prado-Martinez et al. 2013; Díez-del-Molino et al. 2018). Current methods, coalescent-based or otherwise, using contemporary genetic diversity may not always accurately infer changes in population size because multiple demographic histories could drive similar observed patterns thereby limiting the power to quantify environmental drivers of demographic change. Being unable to identify the potential causes of population size declines restricts our ability to predict changes in genetic diversity, and the amount of standing genetic variation is critical to the ability of species to respond to changing selective pressures (Barrett and Schluter 2008; Willi and Hoffmann 2009; Razgour et al. 2019).

Time-series sequence data provides researchers the opportunity to directly compare allele frequencies before and after an environmentally driven demographic shift and quantify the associated change in genetic diversity, or the severity of a bottleneck, thereby giving a proxy for the strength of genetic drift (van der Valk et al. 2019; Sánchez-Barreiro et al. 2021). Temporal genomics along with abiotic data can isolate the environmental factors, either natural or anthropogenic, that drove the demographic change and genetic drift. For example, Hoeck et al. (2010) used historical and recent samples of Galápagos mockingbird species, *Mimus* spp., across islands of varying sizes to directly quantify the impact of patch size on genetic diversity. They found that smaller islands with smaller populations experienced more substantial genetic drift and sharper decreases in genetic diversity even though diversity across the archipelago remained stable. Time-series genomic data enables researchers to discern between potential demographic histories by calibrating models with multiple data points throughout time. Additionally, the temporal genomic framework enables researchers to develop and test the efficacy of single-timepoint methodologies at inferring the demographic history of a population. In this issue, Reid and Pinsky (2022) combine forward genetic simulations with coalescent simulations to compare the accuracy with which temporal and single-timepoint methods can reconstruct demographic changes. They find that temporal and some single-timepoint methods can accurately estimate demographic history, thereby providing critical methodological considerations for researchers investigating recent changes in population size. Globally, population genetic diversity has decreased by ∼6% since the industrial revolution (Leigh et al. 2019). Thus, identifying specific factors driving this loss of genetic variation is critical to mitigating localized extinction and predicting future adaptive potential (Ørsted et al. 2019). In this context, temporal genomics with appropriate environmental data offers more accurate insights into the abiotic drivers of population declines and the relative role of genetic drift in predicting the future of declining populations.

Natural selection

Depending on its type (e.g., positive, diversifying, fluctuating), natural selection can decrease or increase genetic variation in a population. The effects of natural selection can be disrupted by genetic drift as drift drives stochastic changes in allele frequencies regardless of fitness consequences. Because the strength of genetic drift is inversely proportional to population size, drift will be more effective at disrupting natural selection in small populations. In other words, selection can drive more rapid shifts in phenotypic traits in large populations where stochastic mortality (i.e., allele loss) does

not have as great an impact on population allelic composition (Eyre-Walker 2006; Messer and Petrov 2013). Individuals are continuously exposed to changing environmental conditions that alter the selective landscape. However, detecting selection can be challenging as current methods depend on comparing genetic differentiation at candidate genomic variants against a background of putatively neutral loci, even though allele frequencies at these neutral loci can be impacted by gene flow and genetic drift. While detecting selection is essential, quantifying the strength of selection is critical to estimating the relative impact of environmental drivers on population genomic composition. Assessing the strength of selection has been historically restricted to phenotypic studies because quantifying selection requires repeated sampling to track changes over time (Kingsolver et al. 2001; Ousterhout et al. 2018). Therefore, temporal genomic data can move genomics from identifying bouts of selection to quantifying its strength, similar to temporal phenotypic studies.

Rather than comparing population allele frequencies against a putatively neutral background, temporal genomic data offer greater resolution by quantifying allele frequency changes across time to detect loci under selection (Buffalo and Coop 2019; Buffalo and Coop 2020). Time-series genomic data enable researchers to directly estimate the strength of selection on specific loci by measuring the magnitude of allele frequency change (Bertram 2021; Gompert et al. 2021). In this issue, Pfenninger and Foucault (2022) integrated time-series environmental, phenotypic, and genomic data across 3 years to identify signals of positive selection in natural populations of harlequin flies, *Chironomus riparius*. They detected strong positive selection and identified multiple independent allele clusters that segregated temporally and that were strongly correlated with environmental fluctuations. These results suggest that harlequin fly populations can rapidly respond to environmental change through polygenic adaptation. While Pfenninger and Foucault (2022) demonstrate that recurrent abiotic changes can drive temporally heterogeneous selection, rare and dramatic environmental perturbations can also result in intense selection pressures (Lee et al. 2017; Donihue et al. 2018). Using temporal data from wild populations of green anole lizards, *Anolis carolinensis*, along a north–south transect, Campbell-Staton et al. (2017) demonstrated that an extreme winter weather event drove rapid allele frequency shifts accompanied by changes in gene expression and thermal tolerance. Time-series sequence data further enable researchers to identify the effect of fluctuating selection on population allele frequencies that otherwise would be undetected using single-timepoint data (Bergland et al. 2014; Durland et al. 2021). Therefore, temporal genomics offers a robust framework to detect specific loci under selection, quantify the strength selection, and evaluate the genomic mechanisms responsible for phenotypic change, while investigating the consequences of temporally heterogeneous selection landscapes.

Disentangling interactions

Multiple neutral and selective processes operate simultaneously. For example, the combination of high gene flow with spatially heterogeneous selective pressures can maintain genetic variation in a population (McDonald and Yeaman 2018). The magnitude of gene flow also mitigates the impact of genetic drift, with high migration rates counteracting the effects of drift (Blanquart et al. 2012). Interactions between evolutionary forces can further influence genomic architecture; for example, the balance between selection and gene flow can influence the effect size of loci on trait values and linkage between adaptive alleles (Yeaman and Whitlock 2011). In addition to simultaneous interactions, past processes dictate current evolutionary responses through changes in baseline allele frequencies that shift the distribution of phenotypes upon which contemporary evolutionary forces act. Decreases in genetic diversity due to genetic drift that may limit adaptive responses to future climatic shifts are typical examples of this interconnectedness across time (Bay et al. 2018 ; Pacific et al. 2015). Similarly, strong selection can fix specific alleles that may become detrimental in the future if the environment changes (Zimova et al. 2016; Tillotson et al. 2019). High levels of gene flow, on the contrary, can increase genetic diversity while attenuating rates of local adaptation and population genetic divergence in the short term (Frankham 2015; Baillie et al. 2016); however, increases in genetic diversity could preserve a population's ability to respond adaptively to the future stressors. Temporal genomics offers a generalizable framework that, rather than comparing loci across the genome or relying on the coalescent model, accounts for previous evolutionary forces by analyzing the difference between past and current allele frequencies.

When available, time-series genomic data can disentangle the impact of evolutionary forces on allele frequency change with greater precision than single-timepoint studies. Chen et al. (2019) used an extensive pedigree analysis along with a genomic time series to tease apart the relative roles of gene flow and selection on allele frequencies in wild populations of the Florida scrub Jay, *Aphelocoma coerulescens*. Without temporal data, the large genetic contributions from immigrants would have been misattributed to selection. Similarly,

Gompert et al. (2021) used repeat genomic sampling to reveal that even limited gene flow can increase genetic diversity in largely isolated patches of *Lycaeides* butterflies. Evolutionary forces also can act upon each other. Mutation rate may be under variable selection as a factor of genome size and the strength of genetic drift (Lynch 2010). Sprouffske et al. (2018) tested how mutation rate may affect adaptive evolution using strains of *E. coli* with different mutation rates to evaluate their ability to adapt (i.e., populations grow) to novel chemical environments. While genetic diversity increased in populations consisting of strains with high mutation rates, this diversity only facilitated local adaptation when mutation rates were moderate, suggesting that high mutation rates stifle local adaptation to novel environments. Similarly, Wielgoss et al. (2011) demonstrated that selection can drive an increase in mutability until mutation rates reach a rate at which natural selection can no longer purge detrimental alleles at a rate fast enough to maintain the population. Therefore, temporal genomics can disentangle evolutionary forces and investigate complex interactions between them.

Conservation

The utility of genetics in conservation biology (i.e., conservation genetics) has been long recognized (Hedrick and Miller 1992; Frankham 1995; Hedrick 2001), with studies commonly focused on estimating wildlife management units (Balakrishnan et al. 2003; Rutkowski et al. 2017), inbreeding (Krakowski et al. 2003; Parra-Olea et al. 2012), and genetic connectivity (Meffe and Vrijenhoek 1988; Unfried et al. 2013). Established conservation programs offer long-term data and unique and detailed insights into temporal patterns of population genetic structure and the impact of conservation decisions on managed populations. In the past decade, conservation genomics has complemented conservation genetics and increased our ability to infer the impact of neutral processes (e.g., gene flow, genetic drift) on allele frequencies (Campbell et al. 2015; Steane et al. 2015) while measuring adaptive genetic variation (Vandersteen Tymchuk et al. 2010; Oh et al. 2019). Because of the historical cost of obtaining genomic data, genetic data are commonly used in temporal studies (Lancaster et al. 2006; Draheim et al. 2018). Temporal conservation genetic studies with controlled sampling regimes often use putatively neutral microsatellite markers. Per locus, microsatellites are more informative than binary single-nucleotide polymorphisms (SNPs), but the larger number of analyzed genomic markers allows for more resolution than microsatellites (Zimmerman et al. 2020; Hauser et al. 2021). Nevertheless, similar inferences can be drawn from microsatellites and SNPs (Lemopoulos et al. 2019; Hauser et al. 2021), and under certain circumstances, microsatellite data may identify phylogenetic clades (Mesak et al. 2014) and reconstruct parentage relationships (Hauser et al. 2021) better than SNPs. Microsatellites can be highly polymorphic, allowing parentage relationships to be assigned with higher accuracy compared to biallelic SNPs. In this issue, Gray et al. (2022) use microsatellite data from the Floreana tortoise, *Chelonoidis niger*, captive breeding program established in 2011 to evaluate program efficacy and provide recommendations for other captive breeding programs. They demonstrated that breeding success is biased toward females with low levels of inbreeding, and that relatedness between individuals of a breeding pair did not impact breeding success. Further, this study highlights that employing microsatellite data can be more informative to some temporal biological questions than genomic data (Gray et al. 2022).

Conservation plans often depend on precise estimates of demography and connectivity, but as discussed above (see "gene flow and genetic drift" sections) estimates based solely on single timepoints have limitations. Furthermore, applying genomics to conservation programs is challenging (Shafer et al. 2015). For example, genetic diversity is commonly used as a proxy for (effective) population size even though there is no established correlation between measures of genetic diversity and Red list status according to the International Union for Conservation of Nature (IUCN) (Díez-del-Molino et al. 2018). Long-standing conservation programs can capitalize on repeated tissue sampling and use population monitoring to validate temporal methodologies. Indeed, temporal genetic and genomic studies commonly harness samples or data from conservation programs (Chen et al. 2016; Jensen et al. 2018) or charismatic species (Sánchez-Barreiro et al. 2021; Viluma et al. 2022). For instance, Aguillon et al. (2017) explored the genomic consequences of sex-specific limited dispersal in a population of Florida scrub jay, *A. coerulescens*, at Archbold Biological Station in Venus, Florida that had been monitored for decades (Woolfenden and Fitzpatrick 1984; Schoech et al. 1991). Similarly, the grey wolf, *Canis lupus*, has been the focus of conservation efforts in Scandinavia since the 1980s (Flagstad et al. 2003; Seddon et al. 2005; Bensch et al. 2006; Kardos et al. 2017) and the extensive sampling efforts conducted on this charismatic mammal enabled Viluma et al. (2022) to directly quantify the temporal pattern of genomic erosion due to inbreeding and drift. Sánchez-Barreiro et al. (2021) used samples spanning almost two centuries (1845–2012) of two subspecies of the white rhinoceros, *Ceratotherium simum simum* and *C. simum cottoni*, to quantify the genomic conse-

quences of their rapidly declining populations. Importantly, their direct quantification of genetic erosion provides critical inferences for conservation managers attempting to preserve species' genetic diversity (Díez-del-Molino et al. 2018; Sánchez-Barreiro et al. 2021).

Furthermore, time-series genomic data can serve to better evaluate the efficacy of conservation actions by directly quantifying the impact of management decisions on the genetic diversity of endangered species or populations (Chen et al. 2016). As conservation genomics leverages long-term demographic, pedigree, or genetic data from species conservation programs, we would like to stress the importance of open data, collaborative, and citizen science for the advancement of temporal genomics. In this context, long-term investments and efforts to increase communication and collaborations among scientists, scientific collections, and conservation programs would help to alleviate the financial and time constraints of these studies (Card et al. 2021; Nakahama 2021; Ruane 2021; Poo et al. 2022).

Conclusions

Genomic studies based on single time point samples provide critical insights into microevolutionary processes but come with their own limitations (Funk et al. 2016; Martins et al. 2018; Rozhok and Degregori 2019; LaCava et al. 2021). Time-series genomic data, on the other hand, offer greater resolution and certainty about complex interactions between evolutionary forces. Temporal genomics further enables researchers to disentangle the impact of evolutionary forces on population allele frequencies by circumventing assumptions from idealized population genetic models such as genetic equilibrium and neutral genomic backgrounds (Buffalo and Coop 2019; Buffalo and Coop 2020). Hence, temporal genomic can be used to provide inferences with fewer caveats and greater certainty. Already, numerous temporal genomic studies have significantly advanced our understanding of mutation (Raynes and Sniegowski 2014; Ramiro et al. 2020), gene flow (Pascual et al. 2016; Anderson et al. 2020), genetic drift (van der Valk et al. 2019; Sánchez-Barreiro et al. 2021), and natural selection (Bertram 2021; Gompert et al. 2021). Increased affordability of high-throughput sequencing and methodological advances to isolate DNA from museum specimens (Bi et al. 2013; Hykin et al. 2015) are giving more researchers the opportunity to adopt temporal approaches in their studies. Temporal genomics has the potential to rapidly improve our understanding of microevolutionary processes and their temporal dynamics while providing conservation managers with robust methods to guide and evaluate their conservation strategies. Noteworthily, temporal analyses are not restricted to genomic (or transcriptomic) data. In this issue, Snead and Clarke (2022) highlight the importance of integrating multiple types of 'omics approaches (i.e., genomics, transcriptomics, epigenomics, proteomics, and metabolomics) within temporal frameworks to provide a more holistic investigation of evolutionary processes. Therefore, regardless of sequence type, temporal analyses permit researchers to directly quantify molecular changes to investigate fundamental topics within evolutionary biology while opening new avenues and expanding the application of genomics to real-world problems.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

No data or analyses were included within this work.

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