Over the last several years, analyses of ever-accumulating human genome sequence data have made it possible to understand the relationships within and between populations. Evaluating population structure and historical demography can be approximated by a complex web of connections, i.e., a network. This is because, as human populations emerged from Africa, they experienced repeated periods of divergence when they were physically separated followed by convergence (or admixture) when they came back together. Admixture, once considered to be a fairly new aspect of human evolution (owing to the development of intercontinental travel), is increasingly recognized as a common feature that has repeatedly occurred throughout our evolution (Jeong et al., 2019; Lipson et al., 2018; Lorente-Galdos et al., 2019; Reich et al., 2009; Sikora et al., 2019).

This reticulate model of evolution is in stark contrast with the strictly bifurcating perspective held by traditional evolutionary biologists who modeled evolutionary history as a tree—a framework that implicitly forbade the interaction of isolated and differentiated branches. Historically, evolution was visualized as a tree where branches became more and more differentiated over time. Early popularization of this interpretation can be seen from Ernst Haeckel’s “Tree of life” where he describes universal common descent of all organisms (Fig. 1A and B). This view has now evolved and adapted as new data have become available following which the field is moving toward more complex schemes that can accommodate hybridization (Fig. 1C). Contrary to a tree model where a node can only have one parent, current evolutionary biologists are using different network-based frameworks to include recent and ancient admixture to assess population history.

Different network architectures can be used to answer specific questions about the histories of populations. For the purposes of this review, we will be focusing primarily on Directed Acyclic Graphs (DAGs) with a few examples of undirected graphs. DAGs are good proxies for modeling relationships between different populations as they can reliably represent relationships between different groups in a temporal fashion. As it is impossible for a population to interact with an ancestral population directly—they can only interact with descendants of an ancestral population in the same temporal space—these relationships can be represented faithfully using DAGs. Fig. 2 shows the use of DAGs in representing population histories. U represents an ancestral population that splits into two populations groups U' and U'' by time T1 as a result of isolation. By time T2, U' splits into two different populations A' and B', while U'' changes into D' simply as a result of the passage of time. During the same temporal slice, we see that B' and D' admix to form population C' in proportions \( \alpha \) and 1 – \( \alpha \), respectively. At time T3, C' results in population C, while A' becomes A, an un-admixed community of B' becomes B and a part of D' becomes D. It should be noted that individuals from population B' could not have interacted with individuals from either U' or U'' because they exist in different periods of time.

Networks and trees are currently used to answer the following questions:

1. Inferring population closeness—Evaluating which populations in a given set are more similar.
2. Inferring admixture—Assessing whether a particular population descended from multiple ancestral populations.
3. Inferring demography history—Exploring how different populations interact and split to form modern populations.
4. Inferring population structure—Identifying population membership and relationships based on individual-level data.

The first two approaches in the list above are often inferred using population phylogenies or population trees. These population phylogenies are models where populations are assumed to be related in a tree-like fashion. The branch lengths in these trees corresponds to the extent of genetic differentiation. Three different tree-based statistics to evaluate population closeness were first described by Reich et al. (Reich et al., 2009) and later refined and expanded upon by Patterson et al. (Patterson et al., 2012). Collectively, these methods are called f-statistics. Several authors have commented and helped expand upon the interpretations of
Fig. 1  Different views of evolution. (A) A traditional view of evolution which emphasizes a universal common descent for all living organisms (Ernst Haeckel). (B) A simple bifurcating tree. (C) A reticulate tree with admixture.

Fig. 2  Schematic showing population divergence and admixture.
the statistics, most notably in (Peter, 2016; Harris and DeGiorgio, 2017). These statistics are calculated under population phylogeny models—which refers to a null model where populations are related in a tree-like fashion.

**Inferring Population Closeness**

In the original description by Reich et al. (Reich et al., 2009), a distinction is made between empirical quantities called $F_2$, $F_3$, and $F_4$, while the overall statistics are denoted by $f_2$, $f_3$, and $f_4$, respectively. The empirical measurements ($F_2$, $F_3$, and $F_4$) can be understood as branch lengths for two, three, and four populations, respectively for a specific topology calculated for a single biallelic marker. These are then averaged over multiple markers to get different $f$-statistics. The branch lengths can be visualized as shown in Fig. 3.

**Genetic Drift Calculation**

The $F_2$ statistic can be used to measure the magnitude of genetic drift that has occurred between two populations. We can define genetic drift simply as a change in allele frequency of polymorphisms in the two populations being considered. For a given biallelic genetic marker, $F_2$ is defined as:

$$F_2(P_1, P_2) = F_2(p_1, p_2) = E[(p_1 - p_2)^2]$$

where, $p_1$ and $p_2$ are the allele frequencies of a single biallelic marker in populations $P_1$ and $P_2$, respectively. The final $F_2$ statistic can be computed by averaging the $F_2$ values at hundreds of thousands of biallelic polymorphisms across the genome. Since $F_2$ is interpreted as branch length, there is an additivity property implicit in the definition.

$$F_2(P_1, P_2) = F_2(P_1, P_0) + F_2(P_2, P_0)$$

In most modern population genomic analyses, several populations (on a scale of tens to hundreds) are tested to understand and infer closeness and admixture. A simple way of achieving this is to use the $f_2$ statistic as a score of dissimilarity. All pairwise $f_2$ statistics can be computed to obtain a dissimilarity matrix following which a best-fit tree can be obtained. However, the process of obtaining a best-fit tree can be challenging, especially as the number of populations and branches increases. To counter this, the $F_3$ and $F_4$ tests provide convenient alternatives that provide simpler tests of closeness that are restricted to trees of size 3 and 4 (for $F_3$ and $F_4$, respectively).

**Identifying Related Populations**

$F_3$ can be seen as a metric that evaluates the shared portion of the branch from population $P_1$ to $P_3$ and population $P_2$ and $P_3$ (Fig. 3B). Formally, $F_3$ is defined as:

$$F_3(P_3; P_1; P_2) = F_3(p_3; p_1; p_2) = E[(p_3 - p_1)(p_3 - p_2)]$$

where, $p_1$, $p_2$, and $p_3$ are the allele frequencies of a single biallelic marker in populations $P_1$, $P_2$, and $P_3$, respectively. $F_3$ can also be expressed in terms of $F_2$:

$$F_3(P_3; P_1; P_2) = \frac{1}{2}(F_2(P_3, P_1) + F_2(P_3, P_2) - F_2(P_1, P_2))$$

The equation above can be extended to interpret $F_2$ as any distance metric which can then be used in the different interpretations of $F_3$.

A simple application of $f_3$ is to identify the most closely related population from a given set for an unknown population $P_X$. This was demonstrated in (Raghavan et al., 2014), where the authors used the length of the common branch to identify the most closely related extant population for a new sample discovered in Mal’ta, South-Central Siberia. As can be seen in Fig. 3C, if $f_3$ is calculated for all populations in a given set while keeping the outgroup ($P_2$) and unknown population ($P_1$) fixed while cycling through the test populations ($P_3$ in the figure), the largest $f_3$ value for $F_3$(Outgroup; Unknown population, Test population) will help
identify the closest test population for the unknown population. This is also called “outgroup $f_3$.” This statistic is used in recent literature to calculate shared genetic drift (Sikora et al., 2019; Tambets et al., 2018; Yang et al., 2017). In a recent publication by Sikora et al. (2019), the relationship of three ancient samples and their genetic relatedness to modern-day populations. The statistic points toward a close relatedness between ancient Paleo-Siberians with present-day populations of the Itelmen, Koryaks, and Chukchis, along with Native Americans.

Inferring Admixture

The usual goal for employing $f_3$ is to test whether population $P_3$ is admixed. For this interpretation, the null hypothesis is that $f_3$ is a positive number—the underlying hypothesis being that the data have been generated from a tree that has positive edge lengths. If $f_3$ is negative, the null hypothesis is rejected and is seen as evidence for admixture. The null model is structured like Fig. 3C. This test is often called the ‘three-population test’ in literature. Several publications use $f_3$ statistics to support their claim of detecting admixture events (Brucato et al., 2018; Haber et al., 2019; Wang et al., 2019). Recently, Haber et al. (2019) use the $f_3$ statistic to assert that present-day Lebanese Muslims were a result of admixture between Medieval Lebanese, African, and Central/East Asians.

A natural extension of the $f$-statistics we’ve seen so far is $F_4$, which can be formalized as:

$$F_4(P_1, P_2; P_3, P_4) = F_4(p_1, p_2; p_3, p_4) = E[(p_1 - p_2)(p_3 - p_4)]$$

where, $p_1$, $p_2$, $p_3$, and $p_4$ are the allele frequencies of a single biallelic marker in populations $P_1$, $P_2$, $P_3$, and $P_4$, respectively. Like $F_3$, $F_4$ can also be written in terms of $F_2$:

$$F_4(p_1, p_2; p_3, p_4) = \frac{1}{2} (F_2(P_1, P_4) + F_2(P_2, P_3) - F_2(P_1, P_3) - F_2(P_2, P_4))$$

The $F_4$ statistic can be used to evaluate if there is a shared path between 4 different populations, thereby testing whether an unrooted tree with two distinct clusters—($P_1$, $P_3$) and ($P_2$, $P_4$) in Fig. 3D. If there is no overlap in the drift between members of the two clusters, the $F_4$ statistic will be 0 (as in the case of $F_4(P_1, P_3; P_2, P_4)$), indicating that they do not share a recent or significant population history.

The $F_4$ statistic is a powerful tool for inferring admixture, especially when coupled with the $f_3$ statistic. On identifying a significantly non-zero value for a given topology, common ancestry for the two clusters can be inferred without knowing the direction of admixture. Questions about the direction of admixture can be directly addressed by employing the $f_3$ statistic. Many recent publications have used the $f_3$ statistic to understand the demographic history of populations (Lorente-Galdos et al., 2019; Haber et al., 2019; Wang et al., 2019).

Inferring Introgression

For one of the last tree-based methods covered here, we talk about Patterson’s D-statistic or the ABBA-BABA test (referred to as the D-statistic going forward) first described in (Green et al., 2010) to test introgression between modern humans and Neanderthals. This test is a powerful test for a deviation from a strict bifurcating evolutionary history.

Simply put, two closely related populations are expected to share almost the same amount of derived alleles with a third similar population, i.e., if populations $P_1$ and $P_2$ are closely related and $P_3$ is a similar population, then the number of derived alleles shared by $P_1$ and $P_3$ but not in $P_2$ and the number of derived alleles common to $P_2$ and $P_3$ but not $P_1$ be approximately the same. However, if there was any introgression between $P_3$ and $P_1$ it would lead to an asymmetry in the shared derived alleles. This expectation forms the basis of the D-statistic. To get information about ancestral and derived alleles, a fourth population $P_4$—which is a common outgroup to all three populations under consideration—is included. This is represented in Fig. 4. The test is formally defined as:

$$D = \frac{\text{Count}_{\text{ABBA}} - \text{Count}_{\text{BABA}}}{\text{Count}_{\text{ABBA}} + \text{Count}_{\text{BABA}}}$$

where ABBA refers to a condition where the derived allele is shared by populations $P_2$ and $P_4$, while BABA refers to a situation where the derived allele is shared by populations $P_1$ and $P_3$. The D-statistic can range from $-1$ in a case of extreme introgression.
between P₂ and P₃ to 1 in case of introgression between P₁ and P₃. A value of 0 indicates no introgression. This simple yet powerful approach is often used to detect signatures of introgression (Racimo et al., 2015; Mondal et al., 2016; Feldman et al., 2019). A recent publication by Feldman et al. (Feldman et al., 2019) uses the D-statistic to understand the genetic affinity of Anatolian hunter-gatherers with other European hunter-gatherer populations to find that the non-basal Eurasian ancestry of ancient Anatolians was derived from a gene pool related to a largely homogeneous gene pool of Later European hunter-gatherers referred to as the “Villabruna cluster.”

It should be noted that even though the methods discussed above use tree-based phylogenies to model population histories, the test only work because of the reticulate and complex nature of human evolutionary history. The null hypothesis for each of these tests is that our evolutionary history is tree-like.

### Inferring Demography History

From the different tests we’ve seen so far, it is safe to assert that population groups exchange genes by the process of admixture and simple bifurcating trees are an incorrect representation of the history of a population. To address this, TreeMix (Pickrell and Pritchard, 2012) is an algorithm that accounts for population splits and gene flow while inferring population history. It estimates a maximum likelihood graph based on allele frequencies in sampled populations. The tool optimizes migration weights and branch lengths first, and then searches for optimal graphs.

In the original publication describing TreeMix, the authors demonstrate the tool with two examples – one with human populations and another using dog genomes. For studying human migrations, they used data for 55 modern and archaic human populations from the HGDP (Harvard HGDP-CEPH genotypes) (Cavalli-Sforza, 2005). In doing so, they found some unexpected inferences: (1) that about 16% of Cambodian ancestry can be traced back to a population that is equally related to both Europeans and other East Asians, and (2) an inferred admixture event between the Mozabite (a Berber population from Northern Africa) and southern European populations. They further explored these surprising findings and were able to confirm their findings with the Cambodian population and were able to conclude that the present-day Cambodian population was founded by an admixture event involving southeast Asian populations related to the Dai (Southern China) and a Eurasian population that are only distantly related to present-day populations. However, their findings with the Berber group was not consistent across independent runs of TreeMix. Following this, they interpret these results as pointing to complex patterns of gene flow between northern Africa, southern Europe, and the Middle East.

The group behind TreeMix also used 82 dog breeds or wild canids. Their results are consistent with the known history and genetic bottlenecks known in the establishment of certain dog breeds. One example of this is an inference that bull mastiffs are a Europe, and the Middle East.

### Inferring Population Structure

In a situation where group membership needs to be evaluated for different individuals, their genomic data can be used to unambiguously cluster them into coherent groups whose membership is dictated by genetic features. This approach is a marked departure from the other model-based approaches discussed in this text since it does not rely on an underlying model (and its associated assumptions) to investigate population structure. There are some examples in the literature that use this model-free approach to infer population structure.

An algorithm called NetView (Greenbaum et al., 2016) for evaluating population structure using an undirected network of pairwise genetic similarity of all sampled individuals. This densely connected network is then partitioned using community-detection algorithms. These communities (or densely connected subgraphs) can be equated with population structure.

The algorithm can be equated with other model-free approaches like Principal Component Analysis (PCA) in that it uses genetic distance (or similarity) to model distances between individuals. However, the network-based algorithm facilitates community-detection in a quantitative fashion—something that is not afforded to researchers using PCA without performing additional analyses.

Using NetView, its authors were able to cluster individuals sampled from global populations into different clusters using varying thresholds. Using a low edge-removal threshold, the authors were able to separate African individuals from non-African individuals. On using a medium edge-removal threshold, they were able to distinguish African, Indo-European, and East Asian populations, and upon using a high edge-removal threshold, they were able to distinguish populations from Africa, Europe, India, China, Japan, and Mexico. For readers familiar with PCA analysis of global human populations, these results will be...
intuitive—however, this network-based method partitions the data without the need for manual/clustering-based partitioning following a traditional PCA.

Another way of using networks to model relationships between individuals is by using Identity-by-Descent (IBD) as a similarity metric. This method uses shared haplotypes as a similarity metric compared to absolute similarity used in NetView. This allows for the detection of closer familial structures and pedigrees in the data. This was first proposed and demonstrated in (Gusev et al., 2012). Haplotype-sharing networks were also employed for detecting population structure is demonstrated in a study published by AncestryDNA (Han et al., 2017).

In conclusion, it can be asserted that simple non-reticulate, bifurcating trees are inadequate for modeling evolutionary relationships and that more complex network-based models are needed to understand the deeply convoluted history of a species. The literature discussed here does exactly that and has been seminal in improving our understanding of human evolutionary history.

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