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Paleopopulation Genetics

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Abstract

Paleopopulation genetics is a new field that focuses on the population genetics of extinct groups and ancestral populations (i.e., populations ancestral to extant groups). With recent advances in DNA sequencing technologies, we now have unprecedented ability to directly assay genetic variation from fossils. This allows us to address issues, such as past population structure, changes in population size, and evolutionary relationships between taxa, at a much greater resolution than can traditional population genetics studies. In this review, we discuss recent developments in this emerging field as well as prospects for the future.

Keywords

ancient DNA; population history; admixture; natural selection

INTRODUCTION

Traditionally, population genetics has sought to understand how various factors, such as demographic history, genetic drift, and natural selection, interact and influence patterns of genetic variation in extant populations. However, with recent advances in molecular sequencing technologies and sequence data analysis, we now have an unprecedented ability to examine the population genetics of extinct groups and ancestral populations (i.e., populations ancestral to extant groups). In this review, we provide an overview of the rapidly growing field of paleopopulation genetics, starting with a description of methods that utilize only current DNA sequences, followed by a discussion of how the recovery of ancient DNA (aDNA) sequences allows one to address a wider range of historical questions. We focus on analyses of data from mammals because this is where most recent work is concentrated. We also do not attempt to be comprehensive in our review. Rather, we highlight a handful of topics in which the data, the approach, or the population genetic analyses is of particular interest.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ONE GENOME, MANY HISTORIES

The amount and the pattern of genetic variation that we observe today are strongly influenced by the particular evolutionary history of the individuals sampled. Because this history cannot be repeated, it is often difficult to distinguish between competing hypotheses when analyzing sequence data from a single genetic region. Analyses of autosomal (and X-linked) DNA sequences are potentially much more informative because intragenic recombination allows different stretches of sequence to have independent evolutionary histories (23, 34). Takahata (88) was the first to realize that these multiple histories could be exploited to estimate ancestral population parameters. Consider a simple model in which a panmictic (i.e., randomly mating) population splits at time T into two panmictic daughter populations that are completely isolated from each other (Figure 1). If a single sequence is sampled from each descended population, then the amount of time until the two sequences share a common ancestor depends on the split time (T) and the effective population size of the ancestral population (N_a). With data from a single locus, it is not possible to assess the relative contributions of T and N_a to the sequence divergence time. However, with data from multiple independent loci, it is possible to estimate both T and N_a . Takahata and colleagues exploited this observation to develop moment-based (88) and maximum-likelihood (91, 102) estimators of the ancestral population size under a model with no migration and no recombination. They then applied the maximum-likelihood method to estimate N_a in primates and *Drosophila* (48, 89). Estimates of N_a can produce unexpected conclusions, such as the suggestion that past human population sizes were several times larger than estimates of the current effective population size N_e (89).

Although the method described above assumes the presence of many unlinked loci, similar approaches can be used to analyze whole-genome data (47, 53). For example, Li & Durbin used a sequentially Markovian approximation of the coalescent to estimate the history of population size changes from one diploid or two haploid human sequences (47). The potential to estimate the temporal dynamics of population size changes is very appealing, and although their results are sensitive to the particular assumptions made about local mutation and recombination rates, their estimates of N_a from different human samples are very similar for times in excess of 100,000 years ago, consistent with the idea that current human population structure results from events that have occurred recently (i.e., within the past 100,000 years) in human history.

DIVERGENT GENE TREES

It is straightforward to adapt the simple model shown in Figure 1 to a null model of speciation with more than two species. In the special case of three closely related species (e.g., humans, chimpanzees, and gorillas), a comparison of gene trees from different loci can provide information about the size of the population ancestral to two of them (reviewed in 90). Specifically, the genealogical relationships at any particular locus (i.e., the gene tree) may not match the true phylogenetic relationships of the species, and larger ancestral population sizes lead to a greater proportion of gene trees that do not match the species tree. If the species divergence times are known, N_a can then be estimated from the observed proportion of discordant gene trees (58, 101; but see 35). Application of this approach to

orthologous data from humans and great apes has led to estimates of the human-chimpanzee N_a of 50,000–150,000 (11, 63, 79, 83). As before, this estimate is much higher than the standard estimate of $N_e = 10,000$ –15,000 for current human populations. This result shouldn't be taken at face value because population structure in the ancestral population can lead to overestimates of N_a .

The general idea of exploiting the information in the discordance across gene trees has been extended to large contiguous regions (33, 66), whereas the scenario of estimating ancestral population parameters using a single sequence from multiple species has also been explored using maximum-likelihood (97) and Bayesian Markov chain Monte Carlo (MCMC) (103) methodology. Patterson and colleagues analyzed 20 Mb of aligned sequence data from human, chimpanzee, gorilla, and orangutan and found that the proportion of discordant gene trees is much smaller on the X chromosome than on the autosomes (66). They theorized that this might be explained by an initial period of divergence between humans and chimpanzees, followed by a limited amount of hybridization between the two populations. However, other authors have pointed out that simpler alternative explanations for the data exist (2, 70, 95).

DEMOGRAPHIC INFERENCE USING EXTANT DNA SEQUENCE DATA

More generally, many different computational methods have recently been proposed to coestimate both ancient and modern demographic parameters using sequence polymorphism data (i.e., multiple sequences) from multiple populations. Perhaps the most well-known approach uses Bayesian MCMC methodology to estimate migration rates, divergence times, and ancestral population sizes under the IM (isolation-migration) model, which assumes constant migration rates and population sizes and no recombination (30–32, 59). In principle, this IM approach makes full use of the polymorphism data to estimate demographic parameters but at the cost of a high computational burden, which limits the size of the analyzed data set to a few dozen loci and requires unrealistic biological assumptions (e.g., no intragenic recombination within a locus). Other approaches, which address some of these shortcomings while sometimes creating others, include full likelihood methods that assume no migration but that can be applied to whole-genome data (26, 73), composite likelihood methods that assume free recombination between single nucleotide polymorphisms (20, 27, 51, 55), and approximate likelihood methods that utilize either approximate Bayesian computation (65, 93) or composite likelihood (94, 99) methodology to estimate demographic parameters. As whole-genome polymorphism data become more common in the coming years, it is likely that methods that can handle large-scale data sets (e.g., 27, 73, 99) will be more widely used in the future.

ANCIENT ADMIXTURE

One particular demographic scenario that research has focused on is the question of whether evolutionarily distant populations have interbred with one another. In the context of human evolution, researchers have debated what relationship archaic human groups, such as Neandertals and Asian *Homo erectus*, have with modern humans and what contribution (if any) these archaic human groups have made to the modern human gene pool. Theoretical studies suggest that this ancient admixture can be reliably detected from the patterns of

linkage disequilibrium (LD) from many evolutionarily independent loci (96) by identifying diverged putatively archaic haplotypes and running simulations to determine whether they are consistent with patterns of genetic variation expected under models of recent population structure. Analyses of extant human polymorphism data have consistently found evidence for ancient admixture in many human populations (21, 22, 29, 68, 99). This LD-based approach is appealing because it can be applied to populations where no known fossil ancestor has recoverable ancient DNA (e.g., sub-Saharan African populations), but the results are somewhat sensitive to assumptions regarding modern human demographic history. The conclusions though have been generally consistent with analyses that incorporate information from the Neandertal and Denisovan genome projects (results not shown; see below).

STUDIES OF ANCIENT DNA

aDNA broadly defined is DNA obtained from specimens that have been dead for some time and were not collected for the purpose of genetic or genomic studies. We have restricted ourselves to studies that are primarily concerned with population genetic issues, using aDNA to either reveal patterns of genetic variability in past populations, to detect evidence of natural selection, or to infer past demographic events, such as migration, range expansion, and changes in population size. We ignore the many other uses of aDNA, including the inference of past diet, burial practices, species composition of ancient communities, and sexual dimorphism. We also do not discuss purely archaeological or forensic uses of aDNA to identify specimens and determine their place of origin, nor do we review the growing field of aDNA studies of pathogens.

Our restrictions lead us to focus on aDNA studies on vertebrates because the many studies of aDNA from microbes are concerned with species identification and composition and the few studies of aDNA from insects and other invertebrates have not yielded enough sequences to allow population genetic analysis. Even among vertebrates, the bias is strongly toward mammals and birds because more present-day genomes from those groups are available.

The age of specimens in aDNA studies varies from a few years to roughly 100,000 years. Although claims have been made that aDNA has been extracted from specimens older than 100,000 years, sometimes called antediluvian DNA, those claims have not withstood scrutiny and are generally disregarded (64). We review several broad areas in which aDNA has figured prominently. In each area, we discuss the way or ways that aDNA has been used and give a few examples. We focus as much as possible on uses that have led to new insights that would not have been attainable without the availability of aDNA and that have led to new methods of data analysis required by the presence of aDNA.

TEMPORAL SERIES OF ALLELE FREQUENCIES

Temporal series of allele frequencies have been used to estimate selection coefficients and effective population sizes in experimental populations of many species. In natural populations, the variance in the change in allele frequencies between successive samples has been used to estimate the effective population size (100). Times between successive samples

from natural populations are typically too short to allow detection of selection, except in microbial populations. aDNA provides much longer time intervals between samples and hence more opportunity to detect changes driven by selection, in addition to estimating effective population size.

Bollback et al. (4) developed a method to jointly estimate selection coefficients and effective size from a time series of allele frequencies. This method is based on a numerical solution to the forward diffusion equation for selection and genetic drift. Bollback et al. applied their method to the $\Delta 32$ allele of the CCR5 locus, for which historical samples with ages up to 2,900 years before present (ybp) were obtained by Hummel et al. (36). This allele is of considerable biomedical interest because European individuals homozygous for it cannot be infected by HIV-1 (14). $\Delta 32$ has an average frequency of 11% in Europe, with higher frequencies in the north, and is absent or in low frequencies in other populations (14). Stephens et al. (87) concluded that $\Delta 32$ arose by mutation less than 1,000 years ago because it is in strong LD with markers roughly 1 cM away. An increase in frequency to 11% in such a short time requires strong selection (85). However, those conclusions were based on an incorrect genetic map, and LD on a corrected map no longer indicates either a young age or strong selection (80). Hummel et al. (36) found that $\Delta 32$ was at a frequency of 11% 2,900 ybp and showed no significant change in frequency from people buried in 1316, 1350, and 1750–1810 in what is now Germany. One sample from Sicily from 1837 had a lower frequency of 2.6%. Bollback et al. concluded that there is no evidence of selection on $\Delta 32$, but their method could not exclude selection coefficients as large as 0.05.

Ludwig et al. (52) carried out a similar study of alleles at six loci that affect coat color variation in horses. Before 7,000 ybp, all samples were homozygous for the roan genotype, and no mutants creating other colors were found. By 6,000–7,000 ybp, the black allele was detected at 76% frequency. By 2,000 ybp, alleles creating several other colors and a spotting pattern were found in substantial frequency. The Bollback et al. (4) method showed significant evidence of selection at two of the six loci. Further analysis using a related method of inference confirms that conclusion (54).

aDNA studies of individual alleles have also been used to test historical hypotheses. Most adult Europeans and European-Americans can digest lactose because they carry one or two copies of a haplotype at the *LCT* locus that has a frequency greater than 77% (3). That haplotype is at a low frequency in other populations. Bersaglieri et al. (3) showed clear evidence of recent positive selection on this haplotype in Europeans, presumably because of the selective advantage conferred by being able to digest milk and milk products. Nagy et al. (57) tested for the lactase-persistence haplotype in 23 Hungarian individuals from 1,000–1,100 ybp and found it at a frequency of only 11%, even though that haplotype has a frequency of 77% in present-day Hungarians. Nagy et al. concluded that the low frequency of the lactase-persistence haplotype in aDNA confirms the recent Asian origin of early Hungarians. Using even older samples of eight individuals from 7,500–8,000 ybp, Burger et al. (9) found that the lactase-persistence allele was not present. On the basis of this observation, they supported the hypothesis that the lactase-persistence allele did not increase in frequency until milk and milk products became generally available.

In some cases, aDNA evidence complicates what was thought to be a simple story. Two such cases involve Neandertals. The microcephalin locus (*MCPHI*), which is expressed in the brain and can influence brain size, has two deeply diverged haplotypes. The D haplotype of *MCPHI* is at 70% frequency worldwide, with a substantially higher frequency in non-African than African populations (18). There is so little variation among D haplotypes that Evans et al. (18) concluded that it arose only 37,000 years ago and was swept to high frequency by strong positive selection. Furthermore, the deep divergence between the D haplotype and others at the locus suggested that it entered modern human populations from an archaic hominin population, probably Neandertals. Lari et al. (45) and Green et al. (24), however, found no evidence of the D haplotype in several Neandertal individuals. The possibility remains that the D haplotype entered modern humans from another hominin population, but it probably did not enter modern humans from Neandertals.

The *FOXP2* locus tells almost the opposite story. This locus is of importance to the origin of language because mutations in this gene segregate with developmental disorders of speech and language (44). Enard et al. (17) concluded that the derived allele at *FOXP2* was positively selected in modern humans and reached fixation within the past 120,000 years. Yet Krause et al. (39) found that the derived allele is apparently fixed in Neandertals as well and concluded that the derived allele rose to high frequency 300,000 or more years ago, before the separation of lineages leading to modern humans and Neandertals. It remains an open question as to why patterns of LD in humans suggest more recent fixation (13).

POPULATION-LEVEL ANALYSIS

Most studies involving aDNA are concerned with drawing conclusions about populations rather than individual loci. We divide these studies into two groups based on whether they are primarily descriptive or whether they are also concerned with estimating parameters in population genetic models. Until recently, almost all aDNA studies were purely descriptive because the few data available did not allow the application of population genetic methods. It was enough of a technical achievement just to recover and sequence any endogenous DNA from preserved specimens and fossils.

Most studies of aDNA, even recent ones, have been of mitochondrial DNA (mtDNA) for three reasons. First, the higher copy number of mtDNA makes it more easily recovered from ancient specimens. Second, the generally higher mutation rate of vertebrate mtDNA ensures that more haplotype diversity will be seen in mtDNA than in comparable amounts of nuclear DNA. Third, because there is no recombination in animal mtDNA, gene genealogies of mtDNA haplotypes are readily inferred using standard phylogenetic methods.

Although aDNA studies of mtDNA have yielded valuable insights, the intrinsic limitation of mtDNA has to be borne in mind. Although mtDNAs are inherited as a unit and hence can be described by a gene genealogy, it is generally incorrect to assume that an mtDNA gene genealogy is the same as the phylogeny of populations from which the samples were drawn; that is, gene trees are not necessarily the same as population or species trees. When abundant autosomal aDNA has been obtained, conclusions previously drawn from mtDNA are often found to be incorrect. Consequently, conclusions based on mtDNA alone must be regarded

as tentative unless corroborated by other means. The temptation to overinterpret conclusions based on an mtDNA genealogy, no matter how well resolved, should be resisted.

DESCRIPTIVE STUDIES

Many studies have compared levels of mtDNA and Y-chromosome haplotype diversity in ancient populations with modern descendent wild or domesticated populations. For example, in present-day domesticated horses, there is very little diversity of Y-chromosome haplotypes, but Lippold et al. (50) found much greater levels of diversity in fossil horses from up to 45,000 ybp. Often, the discovery of higher levels of genetic diversity in ancient populations has implications for conservation. The colonial rodent, tuco-tuco (*Ctenomys sociabilis*), is endemic to Patagonia and is listed by the International Union for Conservation of Nature as threatened. Chan et al. (10) found substantially higher levels of mtDNA haplotype diversity in aDNA samples from 2,000–10,000 ybp than in present-day samples collected in the same area. Similar results were found by Prost et al. (71) in their study of mtDNA variation in the collared lemming (*Dicrostonyx torquatus*). They found that haplotype diversity was reduced during past episodes of global warming and predicted that a similar loss of diversity would result from the current warming trend. Similar results have been obtained for many modern human populations. For example, Fehren-Schmitz et al. (19) found a substantial reduction in mtDNA and Y-chromosome haplotype diversity in Native Americans, indicating the drastic increase in mortality after contact with Europeans.

Another frequent use of aDNA is to establish the relationship between ancient and contemporary populations. Some of these studies have the goal of establishing the origin of domesticated species. For example, aDNA from aurochs has shown that European cattle were not derived from aurochs in Europe but from those in the Middle East (16), where presumably domestication of cattle from aurochs took place. Larson et al. (46) analyzed aDNA from domesticated pigs and wild boars in Europe. They showed that patterns of mtDNA haplotype diversity are consistent with the arrival of domesticated pigs into Europe from the Middle East along with Neolithic farmers. Later, European wild boars were either domesticated separately or interbred with pigs, resulting in replacement of Middle Eastern mtDNA lineages with those from wild boars.

In rare cases, aDNA can provide insight about the evolution of morphology. The mtDNA of polar bears is derived from one lineage of brown bears (92). Recently, Lindqvist et al. (49) obtained the complete mtDNA sequence from a jawbone dated to 110,000–130,000 ybp and six complete sequences from living polar bears. They found that the ancient sequence joined the mtDNA genealogy just at the branching point between polar bears and brown bears. The authors argued that the mtDNA genealogy suggests that the ancient jawbone is morphologically ancestral to modern polar bears, thus providing unique insight into polar bear evolution. This conclusion assumes that the mtDNA genealogy accurately reflects the population phylogeny, an assumption that will be tested once autosomal sequences are obtained from the same samples.

Using much younger aDNA, Petren et al. (67) examined the provenance of 14 finches collected from the Galapagos Islands by Darwin in 1835 and 22 more specimens collected in

1901. They typed 14 autosomal microsatellite loci and found that two populations of the giant ground finch (*Geospiza magnirostra*) and two populations of the sharp-beaked ground finch (*G. difficilis*) were unrelated to any extant populations.

Many studies of human remains from archeological sites and ancient graves have been carried out with the goal of determining the relationship of the bodies to modern populations. Most such studies are of mtDNA, including recent studies of Native Americans (86), Japanese (37), Chinese (104), Siberians (82), Beothuks (43), and Melanesians (78). The population affinities established in these and others studies will have to be confirmed with analysis of autosomal sequences.

Of particular interest is the role of aDNA in resolving a long-standing controversy about the transition from the Paleolithic to the Neolithic in Europe, which occurred 6,000–10,000 ybp. Paleolithic populations were hunter-gatherers and Neolithic populations were farmers. The question is whether the transition was purely cultural, with populations adopting farming technologies from their neighbors, or demic, with farming populations replacing nonfarmers. If the transition were purely cultural, then modern Europeans would be direct descendants of the Paleolithic populations, but if the transition resulted from the movement of farming populations into Europe, then the Paleolithic populations would have left no modern descendants. Instead, modern Europeans would trace their ancestry primarily to populations from the Middle East. Analysis of present-day populations has not been able to distinguish between the two hypotheses. Analysis of aDNA has favored demic diffusion, but the issue is not completely resolved. Bramanti et al. (5) found a discontinuity between mtDNA haplotypes before and during the Neolithic in central Europe, and Haak et al. (28) found clear affinities between Neolithic mtDNA haplotypes and those currently found in the Middle East. In contrast, Sampietro et al. (81) did not find a similar discontinuity in aDNA samples from the Iberian Peninsula, causing them to suggest a greater role for cultural diffusion. In this case, the caution about overinterpreting evidence based on mtDNA alone is especially germane, and the relative importance of population dispersal and cultural diffusion will not be determined until abundant autosomal aDNA can be obtained from Neolithic and Paleolithic individuals.

ESTIMATING PAST POPULATION SIZES

Earlier, we discussed the work of Li & Durbin (47) to estimate the history of past population size changes from extant sequence data. In principle, the availability of extensive aDNA (and modern DNA) samples should make answering this question much easier. Drummond, Shapiro, and colleagues (15, 84), for example, examined the changes in population size of the Beringian steppe bison using mtDNA sequences from hundreds of extant and fossil bison. Using a Bayesian MCMC model that samples genealogies without migration or recombination, they concluded that bison population sizes increased from 35,000–100,000 years ago but declined sharply over the past approximately 35,000 years, coincident with environmental changes associated with the last glacial maximum (15). However, Axelsson and colleagues (1) pointed out that postmortem damage in aDNA samples can cause spurious inferences of population size changes, which are consistent with the pattern claimed for bison. Postmortem DNA damage generally leads to apparent transitions, and the

pattern of population growth and decline in bison disappears when only transversions are analyzed (see Figure 4a in Reference 1). Rambaut et al. (72) countered that the original pattern of population growth and decline can be recovered if postmortem DNA damage is explicitly modeled, although these conclusions are sensitive to the specific assumptions of the DNA damage process. In any case, their results still do not address the large differences between transitions and transversions observed by Axelsson et al. (1).

ANCIENT POPULATION GENOMICS

Genomic analysis of aDNA has become possible only recently. Unlike studies of one or a few loci, which are generally intended to answer particular questions, genomic studies produce a wealth of data that can answer many questions. The first aDNA genomic studies were by Noonan et al. (61), who obtained approximately 26 Kb of nuclear sequence from two 40,000-year-old cave bears (*Ursus spelaeus*), and by Poinar et al. (69), who obtained 12.8 Mb of nuclear sequence from a woolly mammoth (*Mammuthus primigenius*). Both of these studies employed what they called a metagenomics approach, in which all DNA that could be recovered was sequenced and then endogenous DNA was identified by alignment to a closely related genome—the dog genome for the cave bear sequences and the elephant genome for the mammoth sequences. This metagenomics approach is now routine in more recent aDNA genomic studies.

These first two genomic studies were proof-in-principle that abundant DNA from fossils could be recovered and that endogenous DNA could be distinguished from DNA of bacterial and other origins by purely computational means. The mammoth study showed that woolly mammoths are the sister group to Indian elephants. In a later study, Miller et al. (56) obtained 3.3 Gb from two 20,000-year-old fossils, which allowed them to confirm results from the previous study and infer that the mammoth genome comprised roughly 4.6 Gb.

Several genomic studies of aDNA have been concerned with humans and their close relatives. Rasmussen et al. (75) sequenced DNA from a 4,000-year-old hair sample which was found in permafrost in southwestern Greenland and which is associated with the Saqqaq culture that represents the first human residents of Greenland. Rasmussen et al. obtained an average of 203× coverage. The hair was from a male who was more closely related to three modern populations in northeastern Siberia than to three North American native populations—Amerinds, Na-Dene, and Greenland Inuit. Rasmussen et al. concluded that the Saqqaq individual represented an early wave of the settlement of Greenland that left no modern descendants.

Before genomic methods were available, mtDNA from several Neandertal fossils had been sequenced, first by Krings et al. (42). This initial study found no evidence of admixture between modern humans and Neandertals. The mtDNA of Neandertals was an outgroup to the mtDNA of all modern humans, a conclusion that was confirmed by partial mtDNA sequences from 14 other Neandertal fossils (41) and later by several complete Neandertal mtDNA sequences (6, 40). Although the mtDNA gene trees provided no evidence of admixture, Nordborg (62) cautioned that the data were still compatible with substantial admixture from Neandertals. He noted that the coalescent history of a single locus is so

intrinsically variable that it is difficult to draw conclusions about admixture from an mtDNA gene genealogy alone.

The first autosomal DNA from Neandertals was obtained by Noonan et al. (60) and Green et al. (25) in 2006. Noonan et al. obtained 65 kb of Neandertal autosomal sequence using a targeting method. They concluded that the average time of genetic ancestry (i.e., the average coalescence time) between the human reference and Neandertal was approximately 700,000 years and that, based on a population genetics model, the populations ancestral to Neandertals and modern humans separated approximately 370,000 years ago. Noonan et al. tested for admixture between Neandertals and the ancestors of modern humans by determining whether there was an excess of low frequency alleles in Europeans at sites where the Neandertal carried the derived allele (i.e., the allele not on the chimpanzee reference genome). They estimated the admixture rate to be 0 but showed by simulations that they could not exclude as much as 20% admixture.

Green et al. (25) obtained approximately 1 Mb of Neandertal sequence using metagenomic methods and estimated the average coalescent time between modern humans and Neandertals to be approximately 500,000 years ago, much more recent than the 700,000 years ago time estimated by Noonan et al. Later it was established that the Green et al. (25) data were contaminated with modern human sequences (98), thus accounting for the more recent estimated coalescence time. Improved laboratory and computational filtering techniques have since been developed to detect and drastically reduce the rate of modern human contamination in hominin aDNA studies (7, 8).

In 2010, Green et al. (24) used new sequencing methods to obtain more than 4 Gb of Neandertal sequence primarily from three 40,000-year-old bones from a cave in Vindija, Croatia. Green et al. presented evidence that the contamination rate was less than 1%. With 1.3× coverage, roughly 50% of the genome was covered by at least one sequencing read. Green et al. estimated the date of genetic divergence between Neandertals and modern humans to be 12.7% of the divergence time between chimpanzees and humans, which is consistent with the estimate of Noonan et al. (60). Assuming the latter time is 6.5 million years, the genetic divergence occurred roughly 825,000 ybp. There is so much data that the uncertainty about that estimated population divergence time comes almost entirely from the uncertainty in the human-chimp divergence time.

Green et al. (24) compared the Neandertal genome to those of representatives from five modern human populations, two African (San and Yoruban) and three non-African (French, Han Chinese, and Papua New Guinean). Using a new method based on the calculation of D -statistics, they concluded that the three non-African genomes were approximately 4% more similar to the Neandertal than were the two African genomes. Further analysis led to the conclusion that admixture was predominantly from Neandertals into the ancestors of non-Africans. They inferred that if the admixture was the result of a single pulse of immigration, then 1%–4% of the ancestral population was replaced by Neandertals. They also found independent evidence of admixture with Neandertals from detecting several low-frequency haplotypes in European genomes that are deeply diverged, absent from the Yoruban genome, and present in the Neandertal genome. Simulations showed that admixture was required to

explain the presence of so many haplotypes with those properties. Green et al. also listed 78 sites at which modern humans are fixed for an amino acid that differs from both Neandertal and chimpanzee. It is likely that most or all of those amino acid substitutions were fixed by natural selection because the probability that such a site would be fixed by drift alone is quite small (12).

Soon after the Neandertal genome was published, DNA sequences were recovered from an approximately 50,000-year-old fossil phalanx found in the Denisova Cave in the Altai Mountains in south-central Siberia. The fossil provided no morphological clues to its identity. Its stratigraphic position was puzzling because only much younger Paleolithic artifacts and fossils of modern humans had been found in that cave, and no Neandertal fossils had been found that far east. The complete mtDNA sequence indicated that the Denisova phalanx was from a group quite different from both humans and Neandertals (38). The estimated genetic divergence time between the Denisovan mtDNA and both humans and Neandertals was estimated to be slightly more than one million ypb, compared with the estimated genetic divergence time of 466,000 years for mtDNA of humans and Neandertals. Based on the mtDNA alone, the population represented by the fossil was an outgroup to humans and Neandertals, possibly *Homo erectus*.

The complete nuclear genome of the Denisovan fossil told a different but also surprising story. Reich et al. (76) obtained 1.9× coverage of the nuclear genome. In contrast to the mtDNA gene genealogy, the nuclear genome indicated that the Denisovan fossil was from a sister group of Neandertals. They named the group Denisovans, which has the distinction of being the first hominin group identifiable only from its DNA sequence. Reich et al. also described a molar found in the Denisova cave of approximately the same age as the phalanx. The molar differs morphologically from both Neandertal and modern human molars, and its mtDNA sequence differed by only two bases from the mtDNA sequence of the phalanx. Too little nuclear sequence could be recovered from the molar to allow any analysis.

The Denisovan nuclear sequence showed a surprising relationship to modern human populations. It was significantly more similar to Melanesian populations, represented by two individuals from Papua New Guinea and one from Bougainville, than to several other Eurasian populations. A recently obtained genome sequence of a 100-year-old Australian hair sample has a similar closer relationship to Denisovans (74). Reich et al. (76) concluded that the data were consistent with a minimum of two admixture events, one from Neandertals into the population ancestral to all non-African populations and the second into the population ancestral to Melanesians and Australians. Although this is not the only hypothesis consistent with the data, it is the simplest.

The conclusion that Denisovans admixed only with Melanesians was confirmed by an analysis of many more Asian populations (77). This study, along with that of Rassmussen et al. (74), provides support for the Southern-route hypothesis: The ancestors of modern Melanesians and Australians diverged from the ancestors of other Eurasians and relatively quickly reached Southeast Asia, New Guinea, and Australia, whereas the colonization of the rest of Eurasia proceeded more slowly. It is an open question as to where admixture with Denisovans took place. Southern or Southeastern Asia is a much more likely location than

Siberia but that would require that Denisovans had a large geographic range. At present, there is no fossil or archaeological evidence that indicates the presence of Denisovans anywhere else, but so little is known about Denisovans that it is possible that archaeological evidence of Denisovan populations exists elsewhere but is unrecognized as such.

CONCLUSIONS

Paleopopulation genetics is undergoing a dramatic transition because of the abundance of new data from both extant and extinct species. The new data have led to the development of new statistical methods that allow inference of population histories and selective regimes. At present, most studies have been of humans and their close relatives, but we anticipate comparable growth in analyses of other species as genomic resources become more widely available. In the not too distant past, population genetics suffered from having an excess of theory and very little data for analysis. The situation is rapidly changing and theoreticians are struggling to make sense of all the new data becoming available.

Already paleopopulation genetics has given us a new picture of our history. The history of humans, Neandertals, and Denisovans is much more complex than previously supposed. Additional genomic data from hominin fossils are inevitable and will probably reveal even more complexity in our recent history. Subjects such as population structure within Neandertals, uniquely modern human adaptations, and the evolutionary relationships between Denisovans and other hominins are impossible to address from extant genetic data alone, but may be answerable with aDNA data. Future aDNA studies will also open up additional avenues of research in other species, and progress may be more limited by rare finds of well-preserved fossils with endogenous DNA than it is by any technological, theoretical, or financial limitations.

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Figure 1.

Schematic of a simple isolation model. An ancestral panmictic population of size N_a splits at time T into two panmictic daughter populations that are completely isolated from each other. The distribution of coalescence times between sequences sampled from the separate populations depends on both T and N_a .