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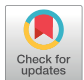

Wilson and Sarich (1969): The birth of a molecular evolution research paradigm

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1. Introduction

Science sometimes progresses through the emergence of a research paradigm leveraging an innovative experimental technique to tackle age-old questions in unexpected, theoretically clever, and ultimately correct manners. Wilson and Sarich's classic 1969 paper made just this sort of progress by integrating several kinds of molecular evidence of protein differences among humans, apes, Old World monkeys, and New World monkeys—especially evidence gathered using the novel immunological technique of micro-complement fixation (MC′F) (1). Their achievement was twofold: i) to effectively pinpoint a relatively early divergence of humans and African apes approximately 4 to 5 Mya and ii) to provide proof of concept of the power and validity of an "evolutionary clock" approach. Both results were surprising: Standard morphological and paleontological approaches had placed the human–African ape divergence at 15 to 30 Mya, while emphasizing the irregularity of character change in evolution.

Allan C. Wilson and Vincent M. Sarich's research paradigm at University of California, Berkeley, elegantly combined immunological protocol, evolutionary theory, and statistical reasoning to address deep human evolution. Although ref. 1 stands as perhaps the pinnacle article and the clearest synthetic exposition of this revolutionary molecular evolution paradigm as it was emerging in the late 1960s, the paper should be understood as channeling an interrelated set of prior articles to which Wilson and Sarich now added one more strand of evidence: amino acid differences among human, chimpanzee, gorilla, rhesus monkey, and horse hemoglobins. Thus, understanding Wilson and Sarich's contributions requires digging deeper than 1969.

In what follows, we first comment on five prior articles of interest. We then briefly cover three key practices central to Wilson and Sarich's research program: the experimental MC′F protocol (and associated index of dissimilarity measure), the regularity test, and the mathematics of divergence time estimation. The core conceptual contribution of their research paradigm—the molecular or evolutionary clock—is subsequently presented from two points of view, a bottom–up protein point of view and a top–down tree point of view. Finally, in light of these discussions, we briefly explore the sections of ref. 1 and gesture toward the paper's broader impact.

2. Prior Work of Wilson and Sarich's Molecular Evolution Immunological Paradigm

Leading up to Wilson and Sarich's classic 1969 PNAS paper, five articles in this research paradigm had been published (2–6). They can be summarized as follows (with a keyword as well as an essential quotation from each):

In 1964, Wilson et al. (2) summarized techniques (e.g., temperature stability and electrophoretic mobility) for measuring cross-species differences of enzymes and other proteins, especially the quantitative, immunological micro-complement fixation (MC′F) technique and their newly defined index of dissimilarity (ID)—the latter two as used in particular for albumin, hemoglobin, and lactic dehydrogenase. (MC′F technique; "Immunological comparisons of enzymes [including the "very sensitive" MC′F method], although not so fast as catalytic, electrophoretic, and temperature-stability comparisons, can be done much faster than determinations of amino acid sequences, amino acid compositions, or fingerprint comparisons" p. 1262.)

In 1966, Sarich and Wilson (3) presented extensive ID data, gathered with the MC′F technique, for at least 21 species of primates—humans and other great apes, gibbons, Old World monkeys, and New World monkeys—but without explicit attention to phylogenetic or temporal analysis. (Index of dissimilarity measure; "These data are also in qualitative agreement with those obtained with different immunological techniques" p. 1565.)

In 1967, Sarich and Wilson (4) explored the logic and statistical reasoning of how to use index of dissimilarity data to test for the regularity of evolution: Different modern species of different lineages of a consensus primate tree had each experienced similar amounts of evolutionary change in albumin proteins compared to a calibrating modern outgroup species. (Regularity test; "Results have been obtained which suggest that during the approximately 45 My that have elapsed since apes, man, Old World monkeys and New World monkeys last shared a common ancestor, the various lineages leading to the modern species have experienced [a] similar amount of albumin evolution" p. 147.)

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Also in 1967, Sarich and Wilson (5) offered a conceptually clear and mathematically cogent discussion of the use of catarrhine (humans, great apes, gibbons, and Old World monkeys) albumin index of dissimilarity data to date the split between humans and our nearest living relatives, gorillas or chimpanzees, at approximately 5 My. (Divergence time estimation; "The calibration of that [evolutionary] clock, that is, the elucidation of the relationship between ID and time, would allow us to calculate the time of divergence between apes and man" p. 1202.)

In 1968, Sarich (6) analyzed the advantages of the immunological approach on primate albumin evolution—summarizing its results with some of the figures and tables found in the other articles—commenting also briefly on both the basic logarithmic formula of time-scale derivation and on the implications of these empirical results for certain specific debates in human evolution. (Motivation; "The molecule should be antigenic and large enough to provide a statistically reliable sample of differences between the forms to be studied" p. 97.)[*]

The main points and keywords of these five prior articles articulate together nicely. These publications are parts of a whole: Wilson and Sarich's broad research paradigm. We refer to them throughout our perspective.

3. Three Key Practices

Three practices of Wilson and Sarich are worth focusing on because of their importance to their research paradigm: the MC'F experimental protocol (and associated ID measure), the regularity test, and the mathematics of divergence time estimation.

3.1. The MC'F Experimental Protocol, and Associated ID Measure. In order to systematically assess many pairwise differences between multiple species, a sensitive method for comparing species in a quantitative and reliable manner would be helpful. One such powerful and versatile early method in the history of molecular evolution was MC'F (7).

First, the "principle of the method" summary statement in a clear publication by Allan C. Wilson and coauthors which focused exclusively and directly on the technique in a cross-taxa context is worth quoting in full:

> Complement is a series of sequentially acting components found in vertebrate serum. Complement fixation techniques make use of two properties of complement. One is the ability of complement to bind irreversibly to antigen-antibody complexes. The second is the ability to lyse sensitized red blood cells. Experimentally, if complement is added to antigen (Ag) and antibody (Ab) in solution under suitable conditions, it will become fixed within the three-dimensional latticework of Ag—Ab complexes as they are formed (reaction 1). After an appropriate time of incubation, sensitized red blood cells (SRBC) are added, and any complement (C') not fixed by the Ag–Ab complexes is available to lyse the cells (reaction 2).
>
> 1. Ag + Ab + C' → AgAbC' aggregate + residual C'
> 2. Residual C' + SRBC → Lysed cells
>
> To determine the amount of lysis, unlysed cells are removed by centrifugation and the concentration of hemoglobin in the supernatant fluid is measured spectrophotometrically. Complement fixation is thus inversely proportional to the hemoglobin concentration [(8) p. 398].

Now, regarding the ID measure, Champion and coauthors observed that "MC'F is most typically used in this laboratory [the Wilson laboratory at UC Berkeley] to measure the degree of immunological difference between a reference protein from one species of organism and the corresponding protein from another species" [(8) p. 406]. The founding paper which had first mentioned the use of MC'F in a cross-taxa context had also first defined the ID measure:

> As a measure of cross reactivity, we ... obtain a value for the ratio E/O, where E is the antiserum concentration required for 50% complement fixation with the heterologous antigen and O is the antiserum concentration required for 50% complement fixation with the homologous antigen. This value can be viewed as an immunological index of dissimilarity [ID measure] between a homologous and a heterologous antigen [(2) p. 1263].

Here is how the MC'F technique had been deployed in Wilson and Sarich's research program to assess indices of dissimilarity across primate species: Rabbits were immunized with what we could call "donor" albumins from particular primate species, including *Homo sapiens*, *Pan troglodytes*, *Macaca mulatta*, and *Cebus capucinus*—that is, humans, chimpanzees, rhesus [Old World] monkeys, and Colombian white-faced capuchin [New World] monkeys. Note that each rabbit was immunized with albumin from only one primate species. Antiserum with that primate species's antibodies was then isolated from the rabbit. That antiserum was subsequently reacted to and complemented with the isolated, as it were, "target" albumin (the "antigen" in this case) taken directly from different primate species, including the four from which antiserum was itself made. "Homologous antigen" means that the antiserum is from the same species as the target albumin, and "heterologous antigen" means that they are from different species. The value of the necessary concentration of the latter divided by the necessary concentration of the former is the ID measure, for a particular direction of cross-reactivity. Or, alternatively and equivalently, ID is "the factor by which the antiserum concentration must be raised to give a reaction with a heterologous albumin equal to that given by the homologous one"—moreover, "the larger the ID, the weaker the cross-reaction" [(4) p. 144]. The ID measure is always equal to (when the donor and target albumin are from the same species) or greater than (when the donor and target albumin are from different species) unity.[†]

[*] Incidentally, ref. 6 was "an abridged and slightly altered version of an unpublished doctoral dissertation presented to the University of California, Berkeley, June 1967" (p. 94). While many of the basic ideas of the research program are captured in this publication, they are sharpened and deepened in the other articles.

[†] Note also that ID was referred to as both "index of dissimilarity" and "immunological distance" in ref. 3 p. 1564, ref. 4 p. 144, and ref. 5 p. 1200. However, this synonymization "practice" was "later discontinued" when ID was referred to as only as the "index of dissimilarity" while "immunological distance" became defined as $100*\log_{10}$ID [(8) p. 408].

Table 1. Three Systematic Comparisons of ID Values of Primate Lineages

Systematic comparison	Approximate, robust ID value	Source
1. Lemurs and lorises (and tarsids*) vs. New World monkeys, apes†, and Old World monkeys (The split with X as common ancestor in Fig. 1)	ID ≈ 10	(3) Table 3, p. 1565, (4) Table 1, p. 145, (6) Table 6–2, p. 105.
2. New World monkeys vs. apes and Old World monkeys (The split with Y as common ancestor in Fig. 1)	ID ≈ 4	(4) Table 2 and Table 3, pp. 145, 146, (6) Table 6–3 and Table 6–4, pp. 106, 107.
3. Old World monkeys vs. apes (The split with Z as common ancestor in Fig. 1)	ID ≈ 2.23	(4) Table 4 and Table 5, pp. 146, 147, (6) Table 6–5 and Table 6–6, pp. 108.

Remarkably, actual ID values of multiple species were robustly similar for each of the three comparisons of primate outgroup vs. ingroup lineages. Both cross-reactivity directions of antisera are averaged for systematic comparisons (2) and (3). As we wished to take means across various ape species, we focused on the indicated tables from Wilson and Sarich's publications. Even so, (3) Table 3, p. 1565 and (6) Table 6–1, p. 102, which focus on the ID values only of antiserum to *Homo sapiens* albumin of, respectively, 21 and 27 primate species chosen fairly evenly across primate superfamilies, families, and subfamilies give results highly consonant with our table. (The publications erroneously listed *Tupaia glis*, the common treeshrew of southeast Asia, as a Prosimii.) N.b. Wilson and Sarich's immunological analyses left the *Pan troglodytes—Gorilla gorilla—Homo sapiens* relation unresolved: antiserum to *Homo sapiens* albumin (i.e., human donor albumin) was slightly more similar (i.e., lower ID) to the gorilla than to the chimpanzee, but antiserum to *Pan* albumin (i.e., chimpanzee donor albumin) was slightly more similar (i.e., lower ID) to *Homo sapiens* than to the gorilla (all IDs here ≤ 1.17), cf. (5) Table 1, p. 1201.

*Wilson and Sarich used the contemporaneous taxonomic category of Prosimii, which was considered a suborder of primates. However, it is a paraphyletic group, as tarsids (infraorder Tarsiiformes) share a more recent common ancestor with monkeys and apes (Simiiformes)—i.e., these two infraorders form the suborder Haplorrhini—than they do with lorises and lemurs (infraorder Lemuriformes, suborder Strepsirrhini) (14–16). Thus, we here distinguish tarsids from lemurs and lorises and only portray Lemuriformes as the furthest outgroup in Fig. 1, even if the ID values overlap, and the temporal difference between the two distinct sets of common ancestors while uncertain is probably small (17–19). Moreover, especially the ID values for this row vary slightly depending on which table is deployed.

†To be clear, we use "ape" synonymously with Hominoidea; "great ape" leaves out gibbons; "African ape" leaves out orangutans.

3.2. The Regularity Test. If a given outgroup differs, systematically, by the same amount from the various lineages (or equivalently, ingroup tree paths), then evolutionary change has occurred regularly along the branches. Wilson and Sarich's regularity test examines whether the index of dissimilarity was roughly the same between, on the one hand, the extant species of a given outgroup and, on the other hand, the extant species of two or more lineages of the ingroup. Note that this does not actually have to be the case—different lineages could have exhibited significantly different IDs compared to the relevant outgroup, thereby indicating different evolutionary rates along distinct branches.

Wilson and Sarich's regularity test was held for primate evolution (Table 1). What could possibly explain this? The same amount of time—logically and geometrically speaking—has passed from the outgroup tip to the tips of the distinct primate lineages of the comparative monophyletic ingroup (Fig. 1). That the same amount of difference had also been empirically measured by Wilson and Sarich—for many different species along the primate tree—could be best explained by postulating regular protein changes along every branch or lineage. This robust theoretical inference to a constantly and gradually ticking molecular clock also allowed one further inference: divergence time estimation.

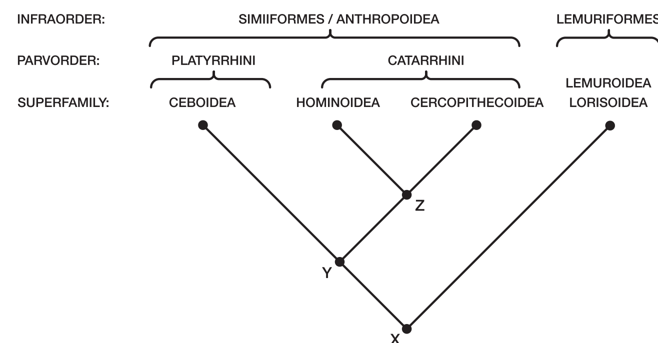

Fig. 1. Basic Primate Phylogeny. We have left out Tarsiidea (tarsids) from the figure because they do not form a monophyletic group with Lemuroidea and Lorisoidea (lemurs and lorises) even though Wilson and Sarich had grouped these three together as Prosimii and labeled the outermost branch as such. (See above note for further details.) There is no parvorder under Lemuriformes. Image credit: Adapted from refs. 4 and 6; redrawn by Rasmus Grønfeldt Winther and Mats Wedin (illustrator).

3.3. The Mathematics of Divergence Time Estimation. Wilson and Sarich found experimental evidence for a basic mathematical function relating the ID measure and divergence time [(5) p. 1202, (6) p. 111, cf. (1) p. 1091]. That function is:

$$\log_{10}\mathrm{ID} = kT, \tag{1}$$

where the L.H.S. is the common logarithm of the index of dissimilarity (ID) between a pair of species, T is the time of divergence between the two lineages of which the species are the leaves, and "the proportionality constant k … describes the rate at which the particular set of molecules being studied evolves and certainly cannot be assumed to be the same for each set" [(6) p. 112]. This basic evolutionary clock equation was of course a simplification and idealization, involving empirical curve-fitting, but it was among the earliest explicit mathematical formulations of the molecular protein clock.‡

4. The Evolutionary Clock: Two Points of View

Now that the basic framework of Wilson and Sarich's molecular evolution immunological research paradigm is in place, let us turn to the conceptual core of the paradigm: The molecular or evolutionary clock (Figs. 2 and 3). It will help to contrast two points of view implicit in Wilson and Sarich's overarching clock picture: The "bottom–up protein" point of view and the "top–down tree" point of view.

Let us first consider evolutionary change from the point of view of a protein changing regularly along different branches of the tree of life [cf. (1) pp. 1090–1091, notes 19

‡Cf. (20) for contemporaneous, consonant empirical results in amphibia, but without the equation. More recent work commenting on logarithmic scales of difference exists (21, 22).

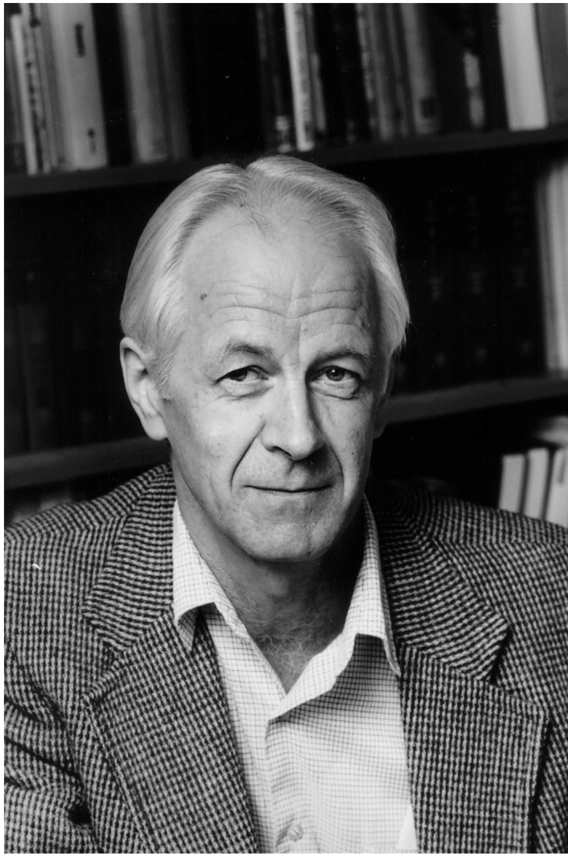

Fig. 2. Allan C. Wilson. Image credit: Jane Scherr (photographer).

and 25, pp. 1092–1093, (5) p. 1200, (6) pp. 95–98]. The basic picture of the bottom–up protein point of view is this:

1. An ancestral protein molecule exists in a species or lineage;
2. The species splits into two new species, leading to isolation and independent evolution of the protein in the two new lineages;
3. Genetic changes/mutations of the now homologous proteins in the two lineages build up regularly over time;
4. Splitting of one or both lineages happens again, with the leaf or tip species (and its protein) of one or both lineages now each serving as a common ancestor for two new species—i.e., recursion back to Step 1.

The needle eye for going from a single protein to two homologous proteins is the splitting event involving a common ancestor. This point of view highlights the evolutionary fate of the regularly changing protein as it transforms from a single, mutating protein in a given species lineage to two homologous, independently evolving proteins in two lineages, to two more homologous, independently evolving proteins whenever any single lineage splits.

The second basic picture of the evolutionary clock is the top–down tree point of view. Starting with a consensus tree [e.g., Fig. 1 adapted from (4) Fig. 2, p. 144, (6) Fig. 6–4, p. 105], we follow these steps:

1. Clarify the various nodes of common ancestry along the consensus tree;
2. Empirically quantify the ID (index of dissimilarity) of the protein of interest along different tree paths, in particular from the extant tip or leaf of a given outgroup [e.g., Lemuroidea and Lorisoidea (lemurs and lorises)] to each of the extant leaves of the distinct lineages [e.g., Ceboidea (New World monkeys), Hominoidea, and Cercopithecoidea (Old World monkeys) in Fig. 1] of the relevant monophyletic group (e.g., Simiiformes/Anthropoidea in Fig. 1);
3. Use the regularity test to evaluate whether the changes along different branches as quantified from Step 2 are indeed similar.
4. Because each node of the tree is a single species of common ancestry, a neat tree structure is given [i.e., no hybridization; homologous proteins up the entire tree; and possibility of one–ingroup–lineage–becomes–an–outgroup recursive comparison], and steps 2 and 3 can be repeated (*N*–1) times—where *N* is the number of common ancestry nodes—"zooming in" to a nested subbranch each time (e.g., Ceboidea now compared to Hominoidea and Cercopithecoidea).

As we saw when discussing the regularity test in section 3, Wilson and Sarich found robustly similar IDs across different branches of the consensus primate tree (Fig. 1). Juxtaposing the abstract, imaginative bottom–up protein point of view and the concrete, methodological top–down tree point of view hopefully helps illuminate the core of

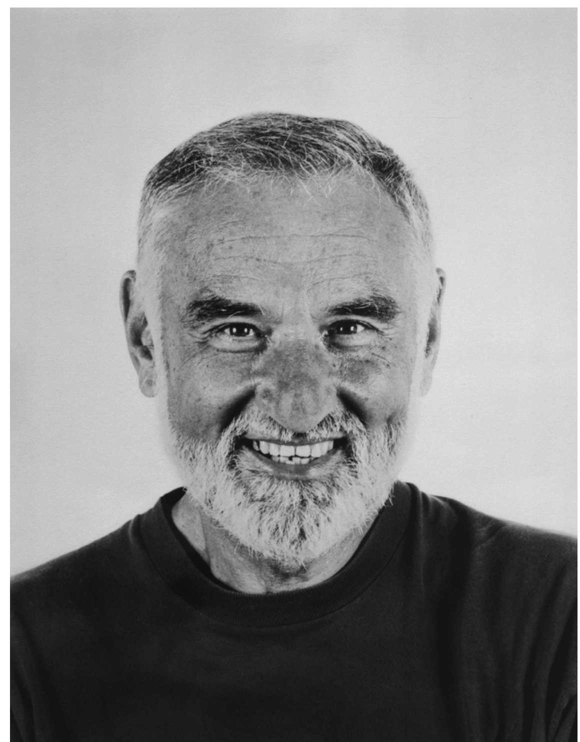

Fig. 3. Vincent M. Sarich. Image credit: University of California (Berkeley, CA).

Wilson and Sarich's research program: the molecular or evolutionary clock.

5. Wilson and Sarich (1969) Structure

With this framework in hand, let us delve into the sections of ref. 1, the publication which synthesized Wilson and Sarich's research paradigm.

5.1. "Introduction". The introduction presents a figure contrasting two "alternative hypotheses" about the phylogeny of humans with respect to African apes and Old World monkeys. Fig. 1*A* places the divergence of all three groups—a trichotomous split—at 30 Mya, while Fig. 1*B* places humans' split with Old World monkeys at 30 Mya and the human–African ape split at approximately 5 Mya. The authors argue that primatologists such as Adolph Hans Schultz and Louis Leakey accepted an old divergence time, back to 30 Mya—i.e., Schultz and Leakey accepted something like Fig. 1*A* rather than Fig. 1*B*. (To be fair, it is unlikely that either Schultz or Leakey would have accepted a trichotomous event.) For instance, Leakey had argued that *Kenyapithecus africanus* was from the early Miocene, which is some 16 to 23 Mya (9). At the time, potassium–argon (K–Ar) dating was emerging as a standard geochronological method for fossil dating. Wilson and Sarich observe that "Molecular biology now offers new methods of estimating both degree of relationship and time of divergence among living species, thereby helping to circumvent the problems caused by few fossils and uncertain anatomical conclusions" [(1) p. 1088]. The "new methods" here, of course, include the practices discussed in Section 3 above. In light of their tempered critique of paleontological and morphological approaches to primate and human evolution, regarding especially the dating of splits, it is worthwhile pointing out that the top–down tree point of view of Section 4 above is a consensus tree built primarily around morphological data, i.e., "nonmolecular evidence" [(4) p. 144, (6) p. 105], as even Wilson and Sarich admit (cf. Section 2 above).[§]

5.2. "Albumin and Transferrin," "DNA Hybridization," and "Hemoglobin". In the next three brief sections, Wilson and Sarich synthesize "quantitative comparisons of human macromolecules with those of African apes and Old World monkeys" [(1) Fig. 2, p. 1089] for four kinds of macromolecules: albumin (their published work) and transferrin (their unpublished work) serum proteins; DNA hybridization (10); and hemoglobin (their novel analysis, deploying extant data). We have already discussed the MC'F technique, which they used on albumin and transferrin, in Section 3.1. DNA hybridization as reported in ref. 10 had showed, in Wilson and Sarich's evaluation, that human and chimpanzee DNA differed "slightly (9%)," while human and rhesus monkey DNA exhibited "a relatively large difference (34%)," in their competitive ability "for binding to unfragmented human DNA embedded in agar" [(1) p. 1089; cf. the first column of (10) Table 4, p. 125]. In the hemoglobin section, Wilson and Sarich collate hemoglobin primary amino acid sequence information from humans, chimpanzees, gorillas, rhesus monkeys, and horses, tabulating the "number of amino acid differences" and "mutational distance" for nine pairwise comparisons of these five species (they left out the chimpanzee–gorilla comparison). Mutational distance is "the minimum number of base [nucleotide] substitutions required to account for the observed amino acid substitutions," calculated using the method of, for instance, ref. 11 [(1) p. 1090].

5.3. "Evolutionary Relevance". In this section, the authors return to Fig. 1, noting that given "the consistent molecular similarity" between humans and African apes, Fig. 1*A* would be consistent with the evidence "only if molecular evolution had been retarded in the ape and human lineages relative to that in the monkey lineage." However, Fig. 1*B* "would be indicated if molecular evolution had proceeded at approximately the same rate in all three lineages" [(1) p. 1090]. How are such rates to be inferred? As we saw in Section 3.2 above, the regularity test permits testing for the similarity of rates of evolutionary change across lineages.

5.4. "Regularity Test". This section starts by noting that their previous work on albumin had already satisfied the regularity test while "there are insufficient data to apply a regularity test in the transferrin and DNA cases" [(1) p. 1090]. However, they now have a new source of data: hemoglobin evolution. In this section and in notes 19 and 25, Wilson and Sarich present arguments for how to apply the regularity test to this data. Perhaps the easiest way to see how the regularity test was satisfied, and recalling Step 2 of the top–down tree point of view of the evolutionary clock of Section 4 above, Wilson and Sarich's Table 1 [(1) p. 1090] shows the mutational distance between horses versus humans, chimpanzees, gorillas, and rhesus monkeys as, respectively, 52, 52, 54, and 52. According to this data, which uses horses as an outgroup, evolutionary change occurs regularly in primate evolution: "the hemoglobins of monkeys on the one hand, and those of the apes and man on the other, have changed to about the same extent since these species last shared a common ancestor." By testing for the regularity of hemoglobin evolution in this way, they show that there is a protein clock regularly ticking on different branches of the primate consensus tree.[¶] They conclude this section with a segue to the "exciting possibility" of the use of proteins as "evolutionary clocks" [(1) pp. 1090–1091].

5.5. "The Clock Approach". The takeaway of this section is a robust African ape–human divergence time estimate of approximately 5 My, calculated in two ways. First, Wilson and Sarich use Eq. **1**, explicitly referring to their calculation in ref. 5 p. 1202. In that earlier article (cf. Section 2 above), they had calibrated k by arguing for an approximate date of 30 My for the Old World monkey vs. ape split. Their note 21 (p. 1203) stands as a thoughtful, reasoned argument for such a date, based on broadly shared assumptions about taxonomy and gradual evolution. The common logarithm of an ID value of 2.23 between Old World monkeys and great apes (Table 1 above) is 0.3483, so that $k \approx 0.012$. Now, while the calculation is sensitive to the exact ID value chosen for the immunological difference between African apes and humans, (5) states an ID value of 1.13 (the average

§For instance, in a note to the caption of its Fig. 2, p. 144, (4) cites (23).

¶They also give credit to other authors, such as Zuckerkandl and Pauling (24), who "have already recognized that protein molecules often appear to have evolved in a regular fashion with respect to time" [(1) p. 1091, cf. (25, 26)].

of just the African apes and human IDs of their Table 1, p. 1201 is actually closer to 1.08), which, again following Eq. **1** above, gives a T of 4.42 My.

In this context, it is odd that Wilson and Sarich state that the albumin of rhesus monkeys is six times as different from African apes and humans as the albumin of humans and our closest relatives are from each other [(1) p. 1091]. In reality, the factor is approximately 2 [inference from Table 1 above, (3) Table 3, p. 1565, (6) Tables 6–1, 6–5, and 6–6, pp. 102, 108]. But there is an interesting reason for this error: the power—alternatively, the cognitive bias—of linear thinking over logarithmic imagination. It is true, from the calculations of ref. 5 that the ratio of the date of the Old World monkeys vs. apes split to the date of the African apes vs. humans split is approximately six. However, this does not mean that the ID value has to be six times as large. Rather, some rudimentary calculations involving logarithms and antilogarithms—assuming an ID value for the latter split of 1.13—show that the ID value between the two branches of the former split has to be 1.84 times as large, as is actually roughly the case (ID value = 2.23, Table 1 above, which is 1.97 times as large).

Now, the second way Wilson and Sarich calculate an African ape–human divergence time estimation was to use the aforementioned hemoglobin data. According to these data, humans have a mutational distance of 0 with chimpanzees and a mutational distance of 2 with gorillas [(1) Table 1, p. 1090]. Given evidence and arguments from other researchers, "the average rate of evolutionary change among mammalian hemoglobins is only one amino acid replacement per approximately 3.5 My" [(1) p. 1091]. Thus, the mutational distances among African apes and humans are consistent with a divergence time of approximately 5 My. Indeed, ref. 1 concludes with a qualitative discussion of model fitting, arguing statistically that an African ape–human split of roughly 5 My fits the data many orders of magnitude better than a divergence time of 15 to 30 My: "a divergence time of 4 to 5 million years is highly probable according to the protein clock approach" [(1) p. 1092].

6. Conclusions

In this PNAS Classics perspective piece, we have presented Wilson and Sarich's revolutionary research program in a holistic manner. We felt it necessary both to explore the full set of relevant publications, by the coauthors, appearing prior to ref. 1 (Section 2 above) and to present the basic components of the paradigm (Section 3 above). As an aid to an intuitive understanding of the molecular or evolutionary clock, which is the conceptual core of their molecular evolution paradigm, we presented the clock from both bottom–up protein and top–down tree points of view (Section 4 above). Using these resources, we then turned to a section-by-section exposition of Wilson and Sarich's classic 1969 *PNAS* paper.

Molecular evolution emerged in the 1960s. Another contemporaneous key research paradigm was Lewontin and Hubby's work on genetic variation in populations of *Drosophila* using gel electrophoresis.[#] Just as Richard Lewontin collaborated with and trained many population geneticists focusing on the structure and evolution of genetic variation in natural and laboratory populations of *Drosophila*, so Allan C. Wilson collaborated with and trained many evolutionary biologists focusing on deep primate and human evolution. There is little doubt about the influence and importance of Wilson's school of science, which found its first expression in the molecular evolution immunological paradigm whose origins and motivations we have here explored. Grounded in Wilson and Sarich's early work, this school also produced the founding papers on ancient DNA (12)[‖] and "mitochondrial Eve" (13), and it provided empirical validity to Motoo Kimura's Neutral Theory of evolution and Sir John Kingman's coalescent theory. Finally, of course, the theory of the molecular clock itself continues evolving.[**] All of this, however, is a story for another occasion.[††]

[#]See refs. 27 and 28, which are placed in a broader context in refs. 29 and 30.

[‖]The last substantive sentence of ref. 12 is worth reproducing here: "If the long-term survival of DNA proves to be a general phenomenon, several fields including palaeontology, evolutionary biology, archaeology and forensic science may benefit" (p. 284).

[**]See refs. 29, 31, and 32.

[††]A documentary on Wilson can be viewed via NZ On Screen (33).

1. A. C. Wilson, V. M. Sarich, A molecular time scale for human evolution. *Proc. Natl. Acad. Sci. U.S.A.* **63**, 1088–1093 (1969).
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