Hominid Phylogeny and Immunology: A Critical Appraisal

Abstract. *Although there seems to be a regular relation of protein change versus time via an exponential equation of the form* $ID = e^{kt}$*, nevertheless, examination of the data indicates that such an exponential fit may be premature. Two other models,* $ID = kt + b$ *and* $ID = bt^k + c$ *represent better fits. Without better data none of the models appears convincing.*

Substantiation of phylogenetic relationships has generally relied upon morphological criteria. Attempts have been made to correlate protein changes with evolution (*1*) as indicated by the discovery of seemingly regular changes in protein structure over time (*2, 3*). An outcome of this research has been to relate changes in protein structure to an absolute time scale (*4*). Especially intriguing is the attempt by Sarich to show that the African apes separated from the line leading to man at about 5 million years ago (*5*). This date is considerably more recent than most of those that have been proposed and thus raises questions about the methods by which it was derived.

The immunological approach used by Sarich is based on the amount of cross-reaction obtained between purified antiserum and the antigen of the various species under consideration. This cross-reaction is measured by the technique of micro-complement fixation. For the same concentration of antiserum, the strength of this cross-reactivity seems to decrease as the phylogenetic distance of the heterologous species being tested increases. By increasing the antiserum concentration, it is possible to obtain a cross-reaction equal to that given by the homologous species. The factor by which the antiserum concentration must be raised in order for the heterologous species to give a cross-reaction equal to that of the homologous species is called the index of dissimilarity or ID (*6*). The ID value of the homologous species is 1.0 by definition. As the time of divergence from a common ancestor increases the ID value also appears to increase. Thus if it is known how this ID value varies with time, a "biological time clock" can be constructed.

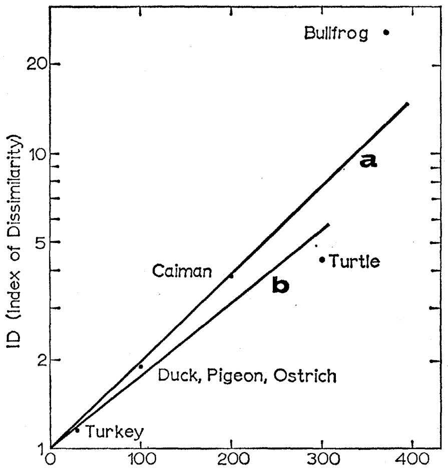

Fig. 1. Semilog plot of index of dissimilarity (ID) versus time for seven species with chicken as standard ($t = 0$; $\mathrm{ID} = 1.0$). Plot (*a*) represents a regression line through all data. Plot (*b*) represents a regression line that excludes the bullfrog. Least-squares fit: (*a*) $\log \mathrm{ID} = 0.00297\ t$; (*b*) $\log \mathrm{ID} = 0.00248\ t$. ID data is from Wilson *et al.* (*6*), and times of separation are approximations suggested by Sarich (*4*).

Wilson *et al.* (*6*), using the chicken as the homologous species, have measured the ID values of six proteins for several species. For seven of these (turkey, duck, pigeon, ostrich, caiman, turtle, and bullfrog), the approximate time of branching from the line leading to the chicken can be determined from the fossil record.

In order to determine the relation between ID values and time, Sarich constructed a graph using the data of Wilson *et al.* (*6*) on ID values and the dates derived from the fossil record and concluded that an equation of the form $\mathrm{ID} = e^{kt}$ provided the best fit to the points of this graph (*4*). He assumed that this exponential relationship between ID and time also holds true for the primates. Sarich then measured the ID values within the primate order, using a single protein, albumin, and man as the homologous species. Finally, by using the average ID value of 2.3 and the single presumed date of 30 million years ago for the divergence of the Cercopithecoidea from the line leading to man, the constant k in the above equation was determined. Utilizing the constant k and the ID value of 1.13 for the time (t) of separation of the African apes from the line leading to man, Sarich then calculated a date of 5 million years (*5*).

Since this date is at variance with most of the current estimates of the time of divergence (*7, 8*), we thought it worth while to examine the data used to formulate the exponential model. As the time of separation of the lines leading to man and the Old World monkeys is not precisely known, any value derived from the paleontological record is approximate. The effect of changing this date will be discussed below. There is also a certain amount of variation in the ID values that would affect the predicted date of man's separation from the African apes (*9*). But much more serious is whether the data allow us to conclude that an exponential relationship represents the best fit. As will be shown, the particular equation being used to estimate the times of separation is critical.

The data from which the exponential relation between ID and time was determined by Sarich (*10*) are shown in Fig. 1. The ID values are those from Wilson *et al.* (*6*), and the dates are those given by Sarich (*4*). The ID value of the bullfrog lies well off the regression line (*a*) and elimination of this point gives a new regression line (*b*) with a considerably different slope. Since the variance of the ID value of the bullfrog is quite high ($s^2 = 135.0$) in comparison with that of the turkey ($s^2 = 0.04$); duck, pigeon, and ostrich ($s^2 = 0.89$); caiman ($s^2 = 0.15$); and the turtle ($s^2 = 0.33$), we suspect that the single ID value of the bullfrog is misleading.

If the exponential equation correctly represents the relation between ID and time over the interval of 0 to 370 million years, this relation should not change over the interval of 0 to 300 million years. Consequently, finding whether or not an exponential equation best fits the data, after omission of the ID value for the bullfrog, is a test of the validity of the exponential model.

Where the nature of the function relating ID values to time is unknown, simple functions, such as:

$$\mathrm{ID} = kt + b \qquad (1)$$

$$\mathrm{ID} = be^{kt} \qquad (2)$$

and

$$\mathrm{ID} = bt^k + c \qquad (3)$$

whose plots on linear, semilog, and log scales respectively give straight lines, are generally tried as first approximations. Further, since in this case the equations must also satisfy the condition of $\mathrm{ID} = 1.0$ when $t = 0$, these relations simplify to:

$$\mathrm{ID} = kt + 1.0 \qquad (4)$$

$$\mathrm{ID} = e^{kt} \qquad (5)$$

and

$$\mathrm{ID} = bt^k + 1.0 \qquad (6)$$

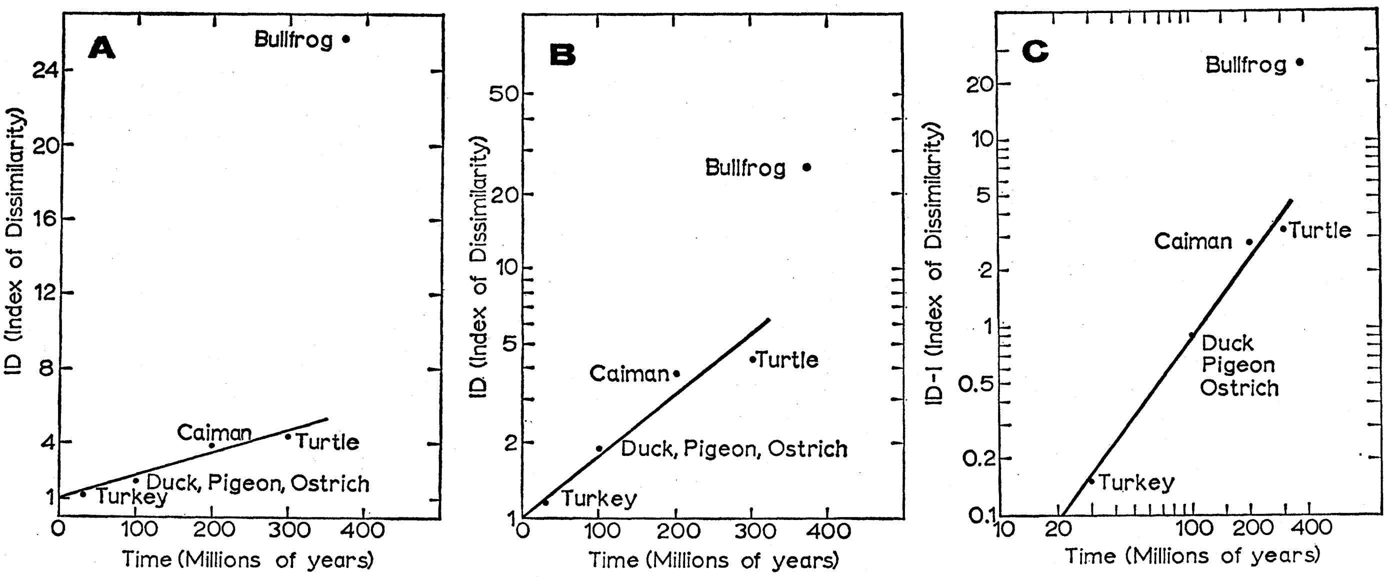

Fig. 2. Plots of index of dissimilarity (ID) versus time of separation for seven species with chicken as standard ($t = 0$; ID $= 1.0$). Regression lines (A, B, C) calculated with bullfrog excluded. Linear plot (A), least-squares fit: ID $= 0.0115\ t + 1.0$. Semilog plot (B), least-squares fit: ID $= 0.00248\ t$. Log plot (C), least-squares fit: log (ID $-$ 1) $= 1.40$ log $t - 2.85$. ID data from Wilson *et al.* (*6*) and approximate times of separation from Sarich (*4*).

Figure 2 shows straight lines fitted by least squares to the data of the three plots. The visual impression that the linear equation (Eq. 4) seems to give the best fit and the exponential equation (Eq. 5) the worst fit is confirmed by noting that the variances in the three cases are: linear, $s^2 = 0.13$; semilog, $s^2 = 0.64$; and log, $s^2 = 0.23$ (*11*). The removal of the bullfrog from consideration effectively changes the presumed exponential relation of ID versus time, and we may conclude that the model suggested by Sarich is not supported by the empirical data.

If we look at other published results giving ID values and approximate dates of divergence from common ancestors, the results are similar. Salthe and Kaplan have also indicated that an exponential equation fits their data (*12*), but this is based on two values and hence can also be fitted exactly by the power function (Eq. 6) (*13*). The data for the primates do not support the exponential relation either.

Two approximate dates are known for the divergence of the primates from the line leading to man: namely, that of the Prosimii at about 60 million years ago and that of the Cercopithecoidea around 30 to 45 million years ago (*5, 14*). As Fig. 3 indicates, an exponential equation gives a reasonable fit to these values only if a date of 30 million years for the divergence of the Cercopithecoidea is assumed (for example, if a date of 40 million years is used, the semilog plot becomes decidedly nonlinear), a difficulty observed by Sarich (*5*). The power function will give an exact fit regardless of the date assigned to the Cercopithecoidea.

In addition, the choice of model (linear, exponential, or power function) used to approximate the relation of ID to time drastically affects the predicted date of man's divergence from the African apes. This can be demonstrated by fitting a regression line to the ID values of 2.3 and 10 and dates of 30 million and 60 million for the Cercopithecoidea and Prosimii, respectively. This yields the following values for man's separation from the African apes: 1.1 million years by the linear model, 3.8 million years by the exponential model (*15*), and 13 million years by the power function model. If 45 million years ago is used as the time of divergence of the Cercopithecoidea, these figures become 1.3 million, 4.4 million, and 31 million, respectively. Thus, there is a considerable time range possible if the date for the divergence of the Cercopithecoidea is allowed to vary. Whereas the linear and the exponential models shift only slightly, the power function model varies from 13 million to 31 million years, covering the range of reasonable estimates of the time of separation of man from the African apes.

Finally, if we assume a date of 37 million years (the mean of 30 and 45) for the divergence of the Cercopithecoidea, the power function model gives the following times of separation: Prosimii, 60 million; Ceboidea, 47 million; Cercopithecoidea, 37 million; *Hylobates*, 25 million; *Pongo*, 24 million; *Pan* and *Gorilla*, 21 million [from ID values given by Sarich (*4*) in each case]. This time scale is consistent, within reasonable limits, with published estimates of the time of divergence of the major primate taxa from the line leading to man.

Of the three models under consideration, the evidence supports the superiority of the power function. However, the present data do not justify the power function any more than it justifies the exponential model. Indeed, there may be other reasons for acceptance or rejection of one or the other

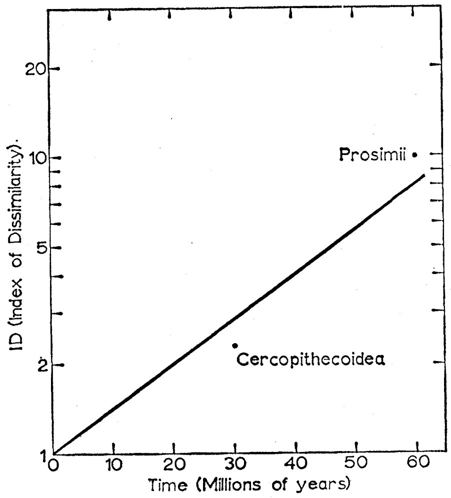

Fig. 3. Semilog plot of index of dissimilarity (ID) versus time for the primates using man as standard ($t = 0$; ID $= 1.0$). Least-squares fit: log ID $= 0.0151\ t$. ID and time of separation data from Sarich (*4, 5*).

of these equations, but these data are simply not sufficient for indicating which, if any, of the three possible models is valid.

While we could reject the linear model in this case as it gives incorrect dates for the divergence of man from the African apes, this is not the case for the other two models. One model agrees with conventional estimates for the primate time scale and the other, while giving debatable results, cannot be rejected out of hand. We thus conclude that no one of these models without better evidence can be assumed valid. Mathematical models of this type must be considered questionable if based on such scanty data.

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References and Notes

1. M. Florkin, *A Molecular Approach to Phylogeny* (Elsevier, New York, 1966), pp. 157–164; M. Goodman, in *Classification and Human Evolution*, S. L. Washburn, Ed. (Aldine, Chicago, 1963), p. 204; C. A. Williams, Jr., in *Evolutionary and Genetic Biology of Primates*, J. Buettner-Janusch, Ed. (Academic Press, New York, 1963), pp. 25–27.
2. A. S. Hafleigh and C. A. Williams, Jr., *Science* **151**, 1530 (1966).
3. A. C. Wilson, N. O. Kaplan, L. Levine, A. Pesce, M. Reichlin, W. C. Allison, *Fed. Proc.* **23**, 1264 (1964).
4. V. M. Sarich, in *Human Evolution*, S. L. Washburn and P. C. Jay, Eds. (Holt, Rinehart and Winston, New York, 1968), pp. 102–112.
5. ——— and A. C. Wilson, *Science* **158**, 1202 (1967).
6. A. C. Wilson, N. O. Kaplan, L. Levine, A. Pesce, M. Reichlin, W. C. Allison, *ibid.*, p. 1262.
7. See for exam ➡ D. Pilbeam, *Nature* **219**, 1335 (1968); ——— and E. L. Simons, *Sci. Amer.* **212**, 55 (1964).
8. G. G. Simpson, personal communication, as quoted in A. S. Hafleigh and C. A. Williams, Jr. (*2*).
9. The variation in ID values tends to increase as the phylogenetic distance increases. The data from Wilson *et al.* (*6*) for the bullfrog indicates a range of 14 to 40 ($s^2 = 135.0$). Sarich's data (*4*) is also variable, with a range of 8.6 to 18.0 for the Prosimii ($s^2 = 12.4$).
10. Our ID value for the duck, pigeon, and ostrich, obtained from Wilson *et al.*, differs from the value indicated by Sarich (*4*). However, the discrepancy is minor.
11. In each case, s^2 was computed from observed ID values and predicted ID values derived from each of the equations.
12. S. N. Salthe and N. O. Kaplan, *Evolution* **20**, 607 (1966).
13. The power function provides a perfect fit to two points since two points plotted on log paper can be fitted exactly by a straight line.
14. Sarich has opted for a more recent divergence (30 million years) (*5*) than generally accepted, partly on the basis of scanty fossil data. Simpson, however, representing the viewpoint of a relatively early divergence, has suggested a range of 35-45 million years (*8*).
15. Sarich bases his 3.5 million and 5 million years for the divergence of man from the African apes on a single point in each case (*4, 5*), whereas the proper statistical procedure is to fit a regression line to both of these points (and going through the origin), which gives a date of 3.8 million years for this divergence.
16. We thank D. R. Ackerman, B. J. Williams, and P. S. Miller for review of the manuscript.

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Thoracic Temperature Stabilization by Blood Circulation in a Free-Flying Moth

Abstract. *The sphinx moth,* Manduca sexta, *maintains its thoracic temperature within a degree of 42°C while in free flight over a range of air temperatures from about 17° to 32°C. Tying off the dorsal vessel abolishes temperature control. Moths with tied off vessels overheat and then stop flying at air temperatures of about 23°C. However, flight at this temperature is possible when the thoracic scales are removed. The mechanism of temperature control involves transfer of the heat produced in the thorax to the blood pumped from the dorsal vessel, and the subsequent dissipation of this heat when the blood returns to the relatively cool abdomen.*

Most members of the family Sphingidae are fast-flying, narrow-winged, nocturnal moths which feed on nectar while they hover. They range in weight from about 200 mg to 4 g and occur from the equator to boreal regions.

The adults of the tobacco hornworm *Manduca sexta* used in this study weighed from 1.8 to 2.6 g, which is in the same range as the smallest hummingbirds. Like other sphingids, *M. sexta* undergoes a warm-up involving "wing-whirring" before flight (*1*), a process analogous to shivering that results in the elevation of thoracic temperature by the production of endogenous heat. Flight is not initiated until thoracic temperature reaches approximately 38°C. High thoracic temperature is apparently necessary for controlled hovering, which may require a nearly "maximum" effort (*2*). At low ambient temperature the heat produced during flight is readily lost to the environment, but at high ambient temperatures this endogenous heat can result in excessive elevation of thoracic temperature unless rates of heat loss are increased. I now report that circulation of blood affords a mechanism for the control of temperature in the thorax during free flight at a variety of ambient temperatures.

The laboratory population used in this study was reared from eggs obtained in the western Mojave desert between Lancaster and China Lake, California, where the animals are active from early June to September. The larvae were reared on tobacco leaves through the 4th larval instar, and on a prepared diet of wheat germ, agar, and additives (*3*) during the last larval instar.

Thoracic temperatures were obtained from moths that had undergone warm-up and had flown for 2 minutes in a controlled-temperature room with an interior volume of 5.0 m^3. Thoracic temperatures were measured by inserting a sharpened, 30-gauge copper-constantan thermocouple into the center of the thorax, and they were registered on a recording potentiometer within 5 seconds of the time of capture of the flying moth. Unless otherwise specified, each moth was measured only once.

After 2 minutes of flight, mean thoracic temperatures were between 41° and 42°C over a range of ambient temperatures from 20° to 30°C (Fig. 1). Below 20°C thoracic temperatures were lower, and at 12.5°C they averaged 37.7°C. At 12°C or less many moths did not arouse when stimulated. At an ambient temperature of 35°C, moths often had to be prodded to keep them flying, and thoracic temperatures increased to a mean of 43.3°C. The moths were reluctant to fly continuously at ambient temperatures above 35°C, although they could fly for about 1 minute even at 41°C.

If the amount of work performed during flight is independent of ambient temperatures, these data suggest that at ambient temperatures above 20°C the moths can increase the rate of heat loss so that thoracic temperature remains essentially uniform.

The temperatures toward the posterior end of the abdomen of *Manduca sexta* during flight were only slightly above ambient. Thus the abdomen is a potential site of heat loss if the heat produced in the thorax during flight can be transferred to it by the circulatory system. The dorsal vessel is well developed in sphingids and is visible through the cuticle in all seg-