confidence limits about the mean ranged from 0.431 to 0.577. According to Kitchell and MacLeod, these "results indicate statistically significant ($P < 0.05$) bottom-heavy asymmetry only for clades of $CG < 0.431$ and top-heavy asymmetry only for clades of $CG > 0.577$." Noting that our value of 0.482 for the mean CG of Cambro-Ordovician clades falls within their 95% range, they infer that 0.482 "is not significantly different from the expected shape of randomly branching (symmetrical) clades."

But it is incorrect to compare our *mean* value of 0.482 to the 95% confidence intervals for *individual* clades. To compare our mean to the expectation of their simulation model (which is also a mean) requires a test for comparing *means*, such as the standard t test. Correctly comparing our mean CG of 0.482 to Kitchell and MacLeod's simulated mean of 0.503 using a t test, we find that our mean for Cambro-Ordovician taxa *is* statistically different from the mean for their randomly branching clades ($P < 0.001$) (*5*).

We agree that identification of evolutionary patterns rests on methods of statistical inference. When the relevant statistical tests are made, time's arrow emerges as a fact of life (*6*).

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2. J. A. Kitchell and N. MacLeod, *ibid.* **240**, 1190 (1988).
3. $P = 0.0038$, $P = 0.0161$, and $P = 0.0124$ for regression analyses based on three different measures of bottom-heaviness. Details of the analyses are presented in (*1*).
4. Data on families within orders are from J. J. Sepkoski, Jr., *Milwaukee Pub. Mus. Cont. Biol. Geol.* **51** (1982). For Cambro-Ordovician clades, $n = 47$, mean $CG = 0.484$ and SD of $CGs = 0.083$. For post-Ordovician clades, $n = 11$, mean $CG = 0.560$, and SD of $CGs = 0.109$.
5. P value is from a t test comparing the mean CG of Cambro-Ordovician genera within families discussed in (*1*), to the mean CG of simulated clades discussed in (*2*). For Cambro-Ordovician genera within families, $n = 353$, mean $CG = 0.482$, and SD of $CGs = 0.079$. For simulated clades, $n = 1000$, mean $CG = 0.503$, and SD of $CGs = 0.033$; data from table 1 in (*2*). We also used a t test to compare the mean CG of 47 Cambro-Ordovician families within orders [data source cited in (*4*)] to the mean CG of 1000 simulated clades discussed in (*2*) and discovered that here, too, $P < 0.001$, despite the smaller sample size. For Cambro-Ordovician families within orders, $n = 47$, mean $CG = 0.484$, and SD of $CGs = 0.083$. For simulated clades, $n = 1000$, mean $CG = 0.503$, and SD of $CGs = 0.033$; data from table 1 in (*2*).
6. David Backus assisted with the tabulation of the data.

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Response: Norman L. Gilinsky *et al.* are making an argument where there is none. We did not question the *statistical* results of the t tests as reported in their 1987 article (*1*), nor would we question the *statistical* results of the t tests presented in this comment. The t test is a standard statistical test for assessing differences between means. However, whether or not this statistical difference is meaningful depends on the question posed. With the use of the t test, it has been shown that the widths of hairs from the left side of a sheep are statistically different from the widths of hairs from the right side of the same sheep. Similarly, although it can be shown that the mean height of class A basketball teams is greater than the mean height of class B basketball teams, it would be erroneous to conclude that the shorter team was short. In this case, "short" is a nominal category whose definition derives from some other expectation.

The purpose of our work on clade shape (*2*) was to provide *expectations of nominal categories*. Three categories of clade shapes had been previously recognized (*1, 3*): the asymmetrical categories of "bottom heavy" and "top heavy" and the intermediate "symmetrical" category. Idealized diagrams of each clade shape category were given in figure 1 of Gould *et al.* (*1*). These three patterns are the outcome of distinct processes. In the symmetrical case, the probabilities of speciation and extinction are equiprobable over time, whereas, in the asymmetrical cases, the probabilities of speciation and extinction are inequiprobable over the clade's duration.

A common error is to assume that the distribution of a novel statistic is known. In the case of the center of gravity (CG) statistic, however, the relevant distributions were unknown. Figure 1 of Gould *et al.* (*1*) limits the symmetrical clade shape category derived from an equiprobable process to $CG = 0.5$, defining clades of $CG < 0.5$ to the category of bottom heavy and clades of $CG > 0.5$ to the category of top heavy. What was needed, and what our work provides, are distributions for the CG statistic of individual clade shape. Our work generates probability distributions of the CG statistic for individual clades in which the generative process is equiprobable with respect to speciation and extinction (the symmetrical assumption). Using these distributions, we addressed the following question: Is the Cambro-Ordovician group, defined on the basis of time of origin, a "bottom-heavy" group (meaning a group whose individual members are characteristically bottom heavy) and the post-Ordovician group, similarly defined on the basis of time, a "symmetrical" group (whose individual member clades are characteristically symmetrical)? The question follows from the assertion in Gould *et al.* (*1*) that the "Cambro-Ordovician clades show a significant tendency to bottom heaviness . . . whereas clades arising later are temporally symmetrical."

Our original contention stands. The probability distributions we generated show that the equiprobable process gives rise [even for the most conservative cases; see (*4*)] to CG statistics for individual clades whose 95% confidence intervals include values ranging from 0.388 to 0.571. One can now determine the nominal category of individual clades. On the basis of the reported mean CG and standard deviation, approximately 74% of the individual clades placed by Gould *et al.* within the Cambro-Ordovician group are not bottom heavy (*5*). Instead, like the post-Ordovician group, these clades predominantly belong to the symmetrical (or top heavy) nominal category. Because the symmetrical nominal category dominates both the early and later arising groups, it is not easy to determine whether one is holding a chart of clade diversity diagrams "upside down or right side up." A test more appropriate to the question would involve assigning individual clades to nominal categories and testing for serial randomness of these nominal categories.

[We are perplexed by the statement by Gilinsky *et al.* that one should not compare empirical data, such as that from the fossil record, to expectations from theoretical models. The comparison of sets of numbers with theoretical models is one of the principal activities of statistics. Also, we did not mention the results of the regression analysis in Gould *et al.* (*1*, figure 3) because we determined that the statistical significance of this result was not robust to the removal of a single (extreme) datum. When we excluded this single observation (highest CG), analysis of variance accepted the null hypothesis of zero slope, that is, no linear trend.]

Our contribution is a positive one: we have provided the probability distributions from the relevant equiprobable generative process, thereby enabling individual clade shapes to be placed into nominal (process) categories. Because the two groups selected by Gould *et al.* were each dominated by individual clades belonging to the *same* nominal categories and not to different nominal categories, as stated, we rejected their conclusion. Our work, however, provides a basis for objectively evaluating the question of differences and similarities in the shapes (and processes) of clade diversification histories. It may be that the empirical record, when analyzed according to the ways we have suggested, will support a claim of temporal directionality. We would naturally be delighted by either outcome.

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4. The results displayed in table 1 of (*2*) represent cases most biased against bottom-heaviness, that is, most biased toward symmetrical clades of *CG* = 0.5, and tightly constrained standard deviations; "all clades" simulations of table 1 are dominated by single-lineage clades whose *CG* statistic is 0.5 by definition. (Minimum *CG* for case 5 is 0.379 and not 0.505, which is the mean.)
5. We assumed a normal distribution, on the basis of the decision of Gould *et al.* (*1*) to utilize the *t* test.
6. We thank W. Ericson and T. Kramer for discussion.

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Possible Role of Carbamates in Neurotoxicity and Neurotransmitter Inactivation

John H. Weiss and Dennis W. Choi (*1*) report that the neurotoxicity of β-*N*-methylamino-L-alanine (BMAA) requires bicarbonate and suggest a "noncovalent interaction" of bicarbonate with the secondary amino group of BMAA to explain that finding. Carbon dioxide, present in the bicarbonate solutions used by Weiss and Choi, reacts rapidly with the unprotonated form of primary and secondary amines to form carbamates (*2*): $RNH_2 + CO_2 = RNHCO_2^- + H^+$ and similarly for secondary amines. Carbamates have well-established physiological roles: for example, carbamates formed at the amino terminus of hemoglogin ("carbamino hemoglobin") account for a significant fraction of the carbon dioxide transported from tissues to the atmosphere (*3*). This well-known covalent interaction may make it unnecessary to invoke a "noncovalent interaction" of BMAA and bicarbonate.

Because carbamate formation involves the nonprotonated form of amines, only a fraction of a percent of most amino acids (which have amino groups with pK_a's over 9) is in the carbamate form at physiological pH and P_{CO_2}. Amines with lower pK_a's (such as peptides), however, can have substantial proportions of carbamate. BMAA is a diamine, and might be expected to have one particularly low amino pK_a. For example, ethylamine has a pK_a of 10.63, while its diamino counterpart, ethylenediamine, has a pK_a for the first deprotonation of only 6.85 (*4*). A substantial proportion of BMAA may be in the carbamate form under the conditions studied by Weiss and Choi.

The results of Weiss and Choi thus have an implication for the physiology of the nervous system that is even more intriguing than simply another mechanism of neurotoxicity: they may provide a clue to an "inactivating mechanism . . . inherent in the nature of the transmitter itself," suggested by Werman (*5*) as a mechanism for terminating neurotransmitter action. Because the readily reversible formation of carbamates occurs only on unprotonated amines, the stability of carbamates depends strongly on the pH. Thus differences in pH between synaptic vesicles and the extracellular environment could provide an inactivating mechanism. For example, suppose that the carbamate but not the unmodified form of an amine were a neurotransmitter. If this amine were stored in a synaptic vesicle at a high pH, a significant proportion of it could be stored as a carbamate. The few microseconds required for diffusion across the narrow synaptic cleft mean that significant amounts of the carbamate should reach postsynaptic receptors after release. But in the extracellular environment, more acidic than inside the vesicle, the carbamate would decompose over a course of milliseconds [the decomposition rate is about $200\ s^{-1}$ for the carbamate of glycylglycine at 5°C (*3* and references therein)], producing inactivation without enzymatic assistance. BMAA might represent an example of a "failure" of this inactivation mechanism, either because of a high potency of its carbamate form or because it has a high proportion of carbamate under physiological conditions.

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5. R. Werman, *Comp. Biochem. Physiol.* **18**, 745 (1966).

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Response: We thank Edmund A. Mroz for his suggestion (also made by William Jencks at Brandeis University) that an interaction between the beta-amino group of BMAA and bicarbonate/CO_2 could take place covalently to form a carbamate, rather than noncovalently as we had originally proposed (*1*). We agree that such a rapid and reversible covalent interaction could also attractively account for our basic observation that BMAA activates glutamate receptors only in the presence of a bicarbonate cofactor. Further study would be required to distinguish between these covalent and noncovalent alternatives. Independent of the exact nature of the interaction between BMAA and bicarbonate, this interaction could permit certain other compounds, not themselves structurally recognizable as glutamate agonists, to serve as glutamate agonists—either as neurotoxins or, as Mroz suggests, excitatory neurotransmitters. We have now examined two other compounds structurally related to BMAA, 2,3-diaminopropionate and 2,4-diaminobutyrate, and have found that the neurotoxicity of both of these compounds can be substantially increased by adding bicarbonate to the medium.

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