correlations that may exist between these modifications on disparate portions of the protein. Such correlations may exist, for example, on a subpopulation of the protein that carries a phosphate moiety at two or more sites simultaneously.

In the top-down approach (see the figure, bottom panel), intact protein ions are introduced into the gas phase by ESI and are subsequently fragmented in the mass spectrometer, yielding the molecular masses of both the protein and the fragment ions. If a sufficient number of informative fragment ions are observed, this analysis can provide a complete description of the primary structure of the protein and reveal all of its modifications, as well as any correlations that exist between these modifications. Although the molecular masses of intact proteins have been successfully measured by MALDI– and ESI–mass spectrometry for some time (*3*), it has proved difficult to produce extensive gas-phase fragmentation of intact protein ions, especially from large proteins.

Han *et al.* now demonstrate that they can obtain highly informative fragmentation for proteins with molecular masses extending to more than 200 kD. The authors achieve this remarkable feat by pumping relatively large amounts of energy into the ionized protein throughout the ion injection and collisional dissociation steps, apparently maintaining the protein in an unfolded and conformationally uncollapsed state. In so doing, they considerably improve the prospects for the top-down approach.

Together with the recent introduction of two other highly effective methods for fragmenting large peptides and proteins—electron capture dissociation (*6*) and electron transfer dissociation (*7*)—this critical fragmentation component of the top-down approach now appears within reach. However, other formidable challenges remain to be overcome before the top-down approach can be considered truly robust for proteomics studies, rather than a technique for studying single purified proteins.

One major challenge is the need to separate small quantities of complex mixtures of proteins prior to mass spectrometric fragmentation. The distinctly different physico-chemical properties of different proteins make them difficult to handle as mixtures without incurring overwhelming losses of certain components or rendering the proteins incompatible with ESI-mass spectrometry. This problem has been successfully addressed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), because the association of the detergent SDS with proteins tends to nullify their individual properties. Unfortunately, the presence of ionic detergents, such as SDS, is not compatible with ESI, and this option is therefore not open to top-down proteomic studies. Other possibilities that are compatible with ESI–mass spectrometry include chromatography in agents that keep a wide range of proteins in solution (*8*), separations within the mass spectrometer based on mass (*9*) or ion mobility (*10*), or combinations of these methods.

Equally challenging is the need to separate slightly different forms of the same protein that differ as a result of modifications and in vivo proteolytic processing. Sensitivity is also a major challenge, because effective fragmentation of a high-molecular-mass protein implies that the protein will break up in a very large number of different ways. Thus, the intensity of any given fragment will be weak compared to that from small low-molecular-mass peptides.

Despite these challenges, it seems likely that the bottom-up and top-down approaches will continue to coevolve. Perhaps they will initially meet halfway as a hybrid approach, in which large fragments or whole domains of proteins are analyzed intact. Ultimately, developments such as those described by Han *et al.* should allow us to analyze and describe in detail the complete primary structures of proteins on a proteomic scale.

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EVOLUTION

Fossil Record Reveals Tropics as Cradle and Museum

Charles R. Marshall

Over the past 11 million years, most bivalves that originated in the tropics expanded their ranges out of the tropics, where they now dominate the living extratropical fauna.

Most groups of organisms show a pronounced decrease in biodiversity from the tropics to the poles. Understanding this long-recognized latitudinal biodiversity gradient requires unraveling the evolutionary dynamics behind it. Typically, work has centered on whether the tropics have unusually high origination rates (in which case they are a cradle of biodiversity), or unusually low extinction rates (in which case they represent a museum of biodiversity). On page 102 of this issue, Jablonski *et al.* (*1*) add a new wrinkle to understanding the evolutionary dynamics of latitudinal diversity gradients by showing that much of the diversity of bivalves outside of the tropics is driven by the expansion of the geographic ranges of species that originated in the tropics. Thus, they argue that the tropics are both a cradle of biodiversity and a museum.

Most studies ignore the possible role of migration in latitudinal diversity gradients. Jablonski *et al.* report the first comprehensive analysis of the fossil record to document the patterns of origination, extinction, and migration. With a meticulously standardized taxonomy, they analyzed the fossil record of 163 genera and subgenera of bivalve mollusks that originated since the beginning of the late Miocene, 11 million years ago.

However, using the fossil record is not straightforward. Jablonski *et al.* had to overcome the relatively poor fossil record of the tropics. The lack of outcrop, the deep weathering of tropical rocks, and the dearth of research effort in the tropics have led to the recovery of, at the very least, 25 times as many bivalve fossils from the extratropics as from the tropics [see note 43 in (*1*)]. Thus, even if a

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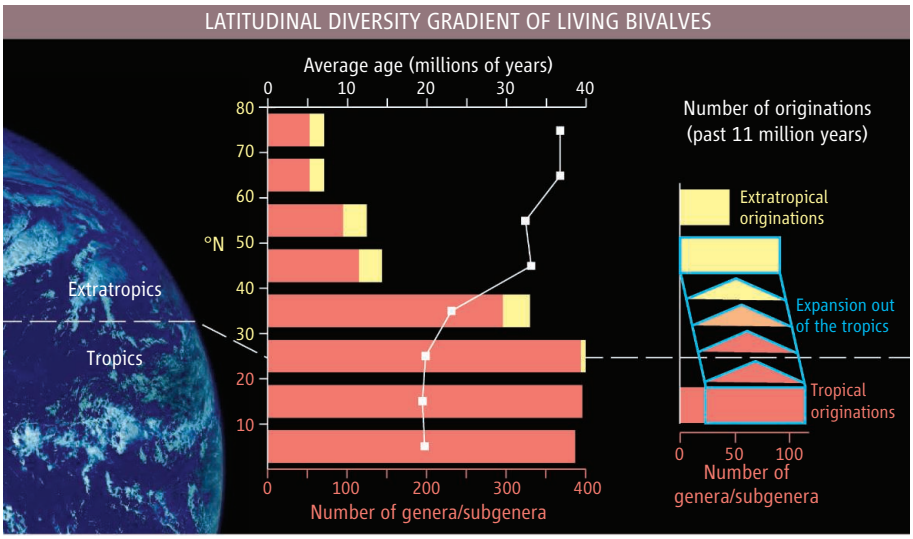

Diversity dynamics. (**Left**) Cosmopolitan and tropical endemic genera and subgenera (red) dominate the latitudinal diversity gradient of living bivalves [Northern Hemisphere shown only (*18*)]. There are relatively few extratropical endemics (beige). In addition, the age of the living genera (white squares) increases toward the pole (*18*), which suggests a higher extinction rate in the tropics. (**Right**) Analysis of the global fossil record (*1*) shows that most of the genera and subgenera that originated in the tropics over the past 11 million years have expanded their range out of the tropics—that is, the tropics are both the cradle and museum of extratropical diversity.

genus originated in the tropics and then expanded into temperate latitudes, the fossil record would most likely record the opposite signal—an extratropical origin, followed by a range expansion into the tropics. Jablonski *et al.* work around this problem by using genera from only the best-preserved families, those that have at least 75% of their living genera represented in the fossil record. Using this subset of taxa, they show that ~80% of genera that originated in the tropics later expanded into the extratropics (see the figure). This represents so many taxa that only about a third of extratropical genera actually had their origins there. Strikingly, there are virtually no cases of the reverse scenario—extratropical origins followed by range expansions into the tropics.

The recognition of this expansion out of the tropics has led Jablonski *et al.*, via a new route, to corroborate an emerging conclusion (*2*–*4*): that the tropics are both a museum and a cradle of biodiversity. From an extratropical perspective, the tropics (at least for bivalves over the past 11 million years) are a cradle, given that so many genera found in the extratropics have their origins in the tropics, and a museum, given that the earliest history of much of the extratropical fauna is tropical.

Importantly, the exact meanings of "cradle" and "museum" depend on when the terms were used historically, and on the data being analyzed. For example, in the first clear discussion of the tropics as a cradle and/or museum, Stebbins (*5*) argued that the tropics were a museum because, in his view, the tropical taxa were primitive in nature. In the modern language of cladistic systematics, where groups are identified on the basis of evolutionary innovations rather than overall similarity, this translates into the claim that the tropics are a museum because they have a disproportionate number of plesiomorphic taxa (those that retain primitive characteristics while lacking evolutionarily derived characteristics).

In contrast, McKenna and Farrell (*4*), using a temporally calibrated molecular phylogeny, identified the tropics as a museum given their finding of an accelerated rate of diversification early in their leaf beetles' history. More typically, the tropics may be characterized as a museum if they have low extinction rates (*5*–*8*) [which is equivalent to Stebbins's (*5*) notion of a museum if the low extinction rates translate into the persistence of plesiomorphic taxa], and as a cradle (*4*) if they have high origination rates (*9*–*15*). Finally, whether a region is a cradle or a museum may depend on the taxonomic level of analysis. For example, if a clade originated extratropically, migrated to the tropics and radiated there, and then if some of those species migrated back into the extratropics, then the extratropics would be the cradle at a higher taxonomic level, whereas the tropics would be the cradle at a lower taxonomic level.

For the bivalves, the tropics' status as museum may depend on the criterion used for identifying museums. For example, Jablonski *et al.* show that the tropics are a museum in the sense that most extratropical taxa have tropical origins. But they also show that for the living genera, the extratropical ones are typically older. This suggests that the extratropics might have lower per-lineage extinction rates. If true, then the tropics would not be a museum according to the extinction rate criterion. To determine which geographic region is a museum by the extinction rate criterion, we need to know the relative extinction rates of tropical and extratropical endemics. Jablonski *et al.* provide an estimate of the number of tropical endemic extinctions, but are unable to disentangle the extinction rate for the extratropical endemics from that for the cosmopolitan taxa (those that are found both tropically and extratropically).

Jablonski *et al.* conclude by arguing that a major extinction in the tropics would have a major effect on the extratropics as well. This raises interesting questions about the effect of tropical invasions in the extratropics. If there were no tropical invasions, would the diversity in the extratropics be greatly reduced or would there simply be a greater number of extratropical originations? If the latter were the case, what is it about the tropical invaders that suppresses the origination of new genera in the extratropics? Perhaps a way of teasing apart this issue is to compare terrestrial systems, in which migrations out of the tropics appear quite limited (*2*, *16*, *17*), with marine systems, in which expansion out of the tropics may be pervasive.

PHOTO CREDIT: NASA

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