From desert to deluge in the Mediterranean

Judith A. McKenzie

A time when the Mediterranean became cut off from, then reconnected to, the Atlantic has been securely dated. An approach known as astrochronology lies behind this achievement.

Some time between five and six million years ago, the Mediterranean Sea became isolated from the Atlantic Ocean. In consequence some areas dried out — hence the title of Kenneth Hsü's book *The Mediterranean was a Desert*[1] — and large salty lakes recharged by rivers flowing through deep canyons replaced the previously marine basins. During this time, the remaining bodies of water were either too salty or not salty enough for normal marine fauna to flourish. This was the so-called Messinian salinity crisis. Because Messinian sediments are essentially devoid of marine fossils this dramatic geological event has been difficult to date or place accurately in the global stratigraphic record, meaning in turn that its causes and environmental impact have been unclear.

Beginning in 1833 with Charles Lyell's formal subdivision of geological strata into relative periods, based on the percentage of fossil shells representing living specimens found in the respective formations[2], marine fossils in sedimentary sequences have been the fundamental tool used to help impose chronological order on the stratigraphic record. Without marine fossils, the precise correlation of the Messinian salinity crisis with the global stratigraphic timescale has been an elusive goal. The significance of Messinian events can be judged from the estimate that as much as 6% of the ocean's salt was transformed into giant deposits left on the Mediterranean sea floor by evaporation[3].

Now, the application of the relatively new stratigraphic technique of astrochronology, as reported by Krijgsman *et al.* on page 652 of this issue[4], has enabled accurate dating of these fossil-poor sediments. Alterations in Earth's obliquity and distance from the Sun occur in regular cycles, and the resulting changes in climate are reflected in the contents of sedimentary deposits. Astrochronology involves time-series analyses of a sedimentary sequence as related to these astronomically forced cycles. When supported by biochronology and magnetostratigraphy, the approach allows for an unprecedented precision in the definition of geological time, and consequently for an equally high-precision worldwide correlation of the Messinian events with other sedimentary sequences deposited during this period of Earth's history.

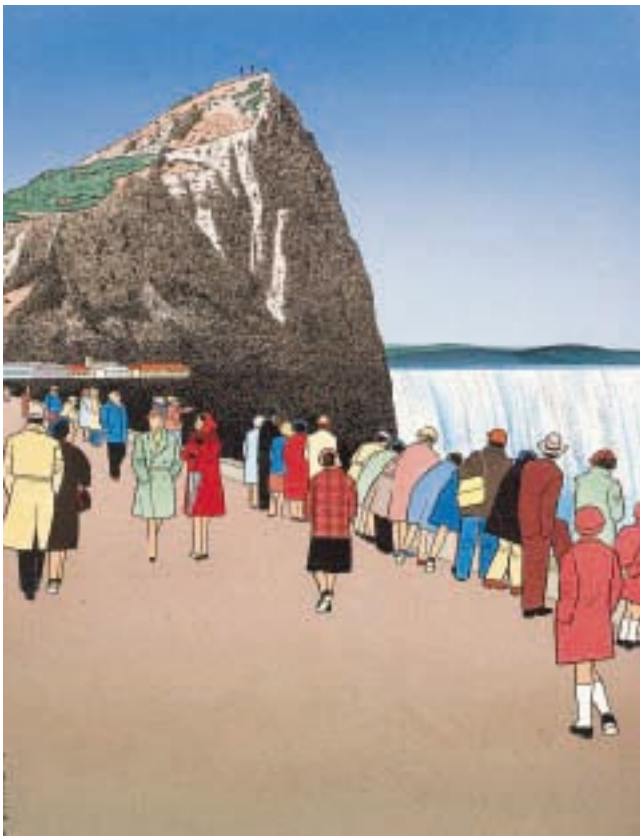

Figure 1 **"The Filling of the Mediterranean Sea" by Guy Billout. This whimsical cartoon, first published in *The Atlantic Monthly* and mixing modern promenaders at the Rock of Gibraltar with an event that happened 5.33 million years ago, depicts the catastrophic flood that ended the Messinian salinity crisis when Atlantic waters re-entered the Mediterranean.**

In the 1950s, geologists working in southern Europe were already beginning to recognize the immensity of the Messinian salinity crisis because of the unusual stratigraphic positioning of the evaporitic deposits, which are sandwiched between deep-water marine sediments, composed of clay and calcium carbonate, known as marls. In contrast to the salt minerals of the evaporites, which were precipitated from hypersaline waters, these marls represent deposition under normal open-marine conditions. The upper transition from continental evaporites to deep-sea sediments is particularly abrupt and can be explained only as the result of a huge flood in which Atlantic water poured across the Gibraltar arc and into the Mediterranean basin (Fig. 1).

This transition to full marine conditions was designated as the boundary between the Miocene and Pliocene epochs — which, dated at 5.33 million years (Myr) ago, is a major marker in the geological calendar[5]. The evaporitic deposits of the 'Gessoso-solfifera' formation in Sicily were taken as denoting the Messinian stage of geological time[6]. But defining these stratigraphic units or stratotype localities in Sicily, or for that matter anywhere else in the Mediterranean, violated long-held stratigraphic principles, because a continuous marine sequence straddling the Miocene–Pliocene boundary exists nowhere in the region. A stratigraphic controversy was thus born[7].

That controversy, however, has not been limited to the problems of stratigraphic correlation. With the initiation of scientific ocean drilling, two expeditions of the Deep Sea Drilling Program (DSDP) in the Mediterranean showed that the Messinian evaporitic deposits were not restricted to the shallow marginal basins, where geologists had studied land sections. Astonishingly, they were widespread in all of the deeper Mediterranean basins, as imaged by basin-wide seismic profiles and sampled by drilling[8,9].

With the publication of these exciting results, debate over the Messinian salinity crisis erupted into a full-blown scientific fracas, with opposing camps developing

radically contrasting views and models about the environmental conditions surrounding the evaporite deposition[7]. Now, almost 30 years after the first DSDP discovery, the combatants may be able to reach an accord. Krijgsman and colleagues' application[4] of astrochronology permits a new reading of the complex stratigraphic record produced by the Messinian events.

In my view, this work resolves several issues. First, as I have mentioned, the method sidesteps the need for marine fossils to date the Messinian sequences. Second, the new chronology shows that the beginning and end of the Messinian salinity crisis were synchronous throughout the Mediterranean; Krijgsman *et al.* date the beginning at 5.96 Myr and the end at 5.33 Myr ago. The causes and consequences of events can thus be distinguished. It is now clear that a tectonic closure in the general region of the modern Straits of Gibraltar, possibly augmented by a fall in sea level, led to the synchronous, basin-wide onset of evaporative conditions. The initial evaporative draw-down of water in the Mediterranean would have been gradual, with the development of both shallow-water and deep-water evaporitic sequences depending on the size and shape of individual basins. Many of the arguments put forward by the proponents of the various models can be accommodated within this picture.

Regardless of that, the massive flood that permanently ended evaporative conditions in the Mediterranean must have been caused by a dramatic rise in sea level. Such a rise would undoubtedly have had consequences elsewhere in the world. One example may have been the major flooding of the Bahamas Platform at around the Miocene–Pliocene boundary, which led to a huge 'back-stepping' of the reef system to a higher, more central location on the platform[10].

But what can explain the large erosional surfaces and deep canyons cut into the sea floor that are observed in parts of the Mediterranean[8,9]? The extraordinary existence of very deep, dried-out basins during the Messinian remains an essential element of any such explanation, and our understanding will have a large gap in it as long as the complete sedimentary sequences in the deepest Mediterranean basins remain unexplored. It is only in the very deepest basins that sedimentation could have continued uninterrupted during the salinity crisis. Securing the necessary samples to fill in the missing period between 5.59 and 5.50 Myr ago will require advanced drilling technology to penetrate through the thick evaporite sequences to determine what lies below. Until this final 'Messinian gap' is closed, the giant salt deposits of the Messinian salinity crisis will remain a theme for speculation and argument. □

Judith A. McKenzie is at the Geological Institute, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland.
e-mail: sediment@erdw.ethz.ch

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Immunology

Developing B-cell theories

David G. Schatz

Mark Twain once wrote, "The reports of my death are greatly exaggerated". Perhaps the same can now be said for some aspects of 'clonal selection' in the adaptive immune response[1,2]. According to this theory, B and T cells have a diverse array of antigen receptors before they encounter antigens (bacteria or viruses, for example). Those cells whose receptors recognize antigen are then selected to proliferate and differentiate into effector and memory cells.

The possibility that antigen-specific interactions trigger cells to produce a new array of receptors is specifically forbidden by the clonal-selection theory. Yet a series of surprising studies has implied that, in mature B cells, assembly of antigen-receptor genes can be reactivated in response to antigen. Now, reports by Yu *et al.*[3] (on page 682 of this issue) and Monroe *et al.*[4] (in *Immunity*) describe a powerful new tool that should allow a more sophisticated analysis of B- and T-cell development, and indicate how the previous findings might be reconciled with the clonal-selection theory.

The development of B and T cells is organized around *V*(*D*)*J* recombination — the assembly of immunoglobulin and T-cell receptor (TCR) genes from component *V* (variable), *D* (diversity) and *J* (joining) gene segments. Two periods of *V*(*D*)*J* recombination, at the pro- and pre-lymphocyte stages of development, lead to expression of the B-cell receptor (BCR) and TCR on the surface of immature B and T cells, respectively (Fig. 1, overleaf). The essential, lymphocyte-specific components of the *V*(*D*)*J* recombination machinery are encoded by the recombination-activating genes, *RAG1* and *RAG2*. These genes are expressed in pro- and pre-lymphocytes, coincident with the two periods of *V*(*D*)*J* recombination. But if immature B cells in the bone marrow recognize a self antigen, they can maintain expression of *RAG1* and *RAG2*, and alter their BCR through a process known as receptor editing[5,6].

The RAG proteins initiate recombination by recognizing and cleaving chromosomal DNA at specific recombination sites. Mature B and T cells do not express the genes that encode these proteins — or so it was thought, until *RAG* messenger RNA transcripts, protein and *V*(*D*)*J* recombination activity were detected in B-lineage cells in the spleen, lymph nodes and tonsils[7–13]. The two basic aspects of these findings were distinguished by their kinetics. First, within one to two days, cultured B cells from the spleen or lymph nodes could be stimulated (by cytokines[9,11], or by interaction with antigens[13]) to perform *de novo* immunoglobulin-gene recombination, replacing one BCR with another. Second, *RAG*-expressing B cells and evidence of recombination could be found late in the immune response (after 12–16 days) in splenic germinal centres[7,10] — sites where mature B cells are activated by antigen and T cells to differentiate and secrete immunoglobulins. These results supported the conclusion that mature B cells can be induced by antigen to re-express *RAG1* and *RAG2*, and, by immunoglobulin-gene recombination, to generate new BCR specificities. With them, a central pillar of the clonal-selection theory seemed to collapse.

Much of this argument relied on the inference that expression of *RAG1* and *RAG2* is reactivated in mature B cells after having been turned off. But this had, in fact, never been demonstrated, because methods for detecting and quantifying expression of *RAG1* and *RAG2* in individual, live cells were not available. Yu *et al.*[3] and Monroe *et al.*[4] have now created strains of mice in which a green fluorescent protein (GFP) is a marker for cells that express *RAG2* (and, by inference, *RAG1*, because the two genes seem to be coordinately expressed).

Yu *et al.* used a large reporter transgene consisting of the *RAG* chromosomal region with GFP coding sequences substituted for those of *RAG2*. Monroe *et al.* created 'knock-in' mice, in which the endogenous *RAG2* locus directs expression of a RAG2–GFP fusion protein. Both approaches yield GFP expression in the expected subsets of developing B and T cells, but each has a limitation. Although the GFP protein encoded by Yu and colleagues' transgene is highly expressed, it seems to be more stable than endogenous RAG2 protein. The result is some 'green' cells containing little or no