Speciation and Stasis in Marine Ostracoda: Climatic Modulation of Evolution

Abstract. *Morphologic and paleozoogeographic analysis of Cenozoic marine Ostracoda from the Atlantic, Caribbean, and Pacific indicates that climatic change modulates evolution by disrupting long-term stasis and catalyzing speciation during sustained, unidirectional climatic transitions and, conversely, by maintaining morphologic stasis during rapid, high-frequency climatic oscillations. In the middle Pliocene, 4 to 3 million years ago, at least six new species of* Puriana *suddenly appeared as the Isthmus of Panama closed, changing oceanographic circulation and global climate. Since then morphologic stasis has characterized ancestral and descendant species during many glacial-interglacial cycles. The frequency and duration of climatic events have more impact on ostracode evolution than the magnitude of climatic changes.*

Knowledge of the evolution of organisms and their environment is an important yet poorly understood aspect of evolution (*1*). In particular the role of climate change in cladogenesis, the speciation process, and in the maintenance of within-lineage stasis has not been systematically investigated despite rapidly increasing knowledge of the earth's paleoclimatic history (*2*). An ideal time to assess climate's role in evolution is the last 5.0 million years because (i) extant taxa whose modern biology is known can be studied as fossils, (ii) accurate age-dating permits stratigraphic analyses at various levels of time resolution, and (iii) paleoclimatic history is well known. Ostracodes, which are small (length, 0.5 to 2.0 mm) bivalved Crustacea, may be useful in evolutionary study because temporal changes in features such as the positions of the eye, adductor muscles, sensory pores, and even individual cells can be mapped in sequential fossil samples with confidence as to homologous relations (*3*). Adults are easily distinguished from juveniles, sexual dimorphism is evident in the carapace of many taxa, and ecophenotypic variation can be identified in many groups, so that secular morphologic changes can be attributed to genomic changes more confidently than with other fossilized groups (*4*).

The formation of the Isthmus of Panama 4.0 to 3.0 million years ago changed ocean circulation and the earth's climate by ending the low latitude surface connection between the Atlantic and Pacific and increasing the northeastward flow of warm Gulf Stream water along eastern North America (*2, 5*). A warm ocean next to cool continents in high latitudes amplified the effects of Milankovitch orbital variations (eccentricity, 100,000 years; obliquity, 41,000 years; precession, 23,000 years) leading to the growth of ice sheets on continents and high-amplitude Pleistocene-type glacial-interglacial cycles now known from deep-sea, island and continental margin, and lacustrine geologic records (*6*).

Morphologic and paleozoogeographic study of the marine ostracode genus *Puriana* from more than 40 formations and numerous modern samples (*7*) from the eastern Pacific, Caribbean, Gulf of Mexico, and western Atlantic indicates a period of cladogenesis during the climatic transition about 4.0 to 3.0 million years ago, but directionless evolutionary stasis within lineages during the frequent and rapid Milankovitch climatic cycles of the last 3.0 million years. Female left valves of *Puriana* species (*8*) were measured for length, anterior and posterior height, length and height from margin to muscle platform, distance of eye to muscle platform, distance of posteroventral margin to posteroventral tubercle, length of posterior ridge, surface ornament, number of dorsomedian ridges, number of segments in median ridge, and posteroventral surface. Data were analyzed by canonical discriminant analysis (*9*).

Puriana is endemic to tropical and subtropical climatic zones of the eastern Pacific, western Atlantic, Caribbean, and the Gulf of Mexico (*7*). Because poor zoogeographic coverage has inhibited the use of fossils for speciation studies, an effort was made to obtain material from as much as its zoogeographic and stratigraphic range as possible. In Fig. 1A the first two canonical axes reveal a diversification about 3.5 million years ago represented by the appearance of at least six new species in the Pliocene (*mesacostalis, convoluta, carolinensis, pacifica, floridana*, and perhaps *gatunensis*). Although the precise location and duration of each speciation event has not yet been determined, all species first appear about 3.5 to 3.0 million years ago. Diversification of *Puriana* and other temperate and subtropical marine ostracode genera (*Muellerina, Bensonocythere, Microcytherura, Cytherura, Paracytheridea, Proteoconcha*, and *Hulingsina*) is causally linked with a climatic transition about 3.5 to 3.0 million years ago (*10*), suggesting that, rather than causing a mass extinction as has been suggested for bivalves (*11*), this event actually disrupted long-term stasis, creating opportunities for rapid speciation. Heterogeneous environments caused by irregular oceanographic changes would have provided diversified habitats along the coast, possibly isolating small populations. Figure 1A also shows that since speciation occurred, ancestral (*rugipunctata* and aff. *elongorugata*) species and their descendants have coexisted, in some cases sympatrically. For five species that occur abundantly (*convoluta, carolinensis, mesacostalis, floridana*, and *rugipunctata*), discrete phenotypes were maintained for more than 3.0 million years, as shown by clustering of different-aged specimens of each species.

Because morphological stasis is such an important phenomenon for understanding evolutionary mechanisms, a more detailed analysis was performed on *P. mesacostalis* and *P. floridana* to assess the relation between morphology and rapid, cyclic climatic change. Figure 1B shows the first two canonical variates of an analysis on 212 specimens of Pliocene to Holocene *P. mesacostalis* and *P. floridana*. Morphologic variability was examined at five stratigraphic levels (*12*): level 1 is a sample (10^2 to 10^3 years); level 2 is a formation—that is, the interglacial part of a 100,000-year cycle, 10^4 to 5×10^4 years; level 3 is two formations spanning one and a half cycles or 10^5 to 2×10^5 years; level 4 is 1.5 million years; and level 5 is the species' entire stratigraphic range of 3.5 million years. Specimens of *P. mesacostalis* from the Core Creek Sand and Flanner Beach formations in North Carolina (dated at 70,000 to about 220,000 years) are outlined in Fig. 1B by three nearly concentric ovals representing levels 1 through 3. The amount of morphologic variability, as shown by the scatter of points, increases only slightly as specimens from coarser stratigraphic sampling levels are included in the ovals. Total within-sample variability representing 10^2 to 10^3 years is only slightly less than variability over 10^5 to 2×10^5 years. *Puriana mesacostalis* shows no secular trends in its morphology over this time interval that might be evident from a lack of concentricity of the ovals—stasis is directionless. Yet high-amplitude environmental fluctuations occurred during this time that could have catalyzed speciation or caused extinc-

tion. Despite a drop in sea level of 100 to 130 m between 180,000 and 140,000 years ago that eliminated much of the continental shelf as available habitat and decreased water temperatures, populations from the last two interglacials are morphologically similar. Morphologic stasis characterizes most shallow marine ostracodes from the western Atlantic that were subjected to these climatic changes, suggesting a pattern predicted by the model of punctuated equilibrium (*13*).

Over longer periods of time at stratigraphic levels 4 and 5, *P. floridana* and *P. mesacostalis* form two loose clusters, but some middle (circles in Fig. 1B) and early Pleistocene specimens of both species plot highly on the second axis. Why these populations differ in morphology is not clear but may be related to the warmer climates in the early Pleistocene (*14*). Whatever the cause, no new species split from either *P. floridana* or *P. mesacostalis* over their entire stratigraphic range and, with the exception of increased intraspecific variability in early Pleistocene forms, these two closely related species remained distinct for about 3.5 million years.

Because there are few case studies of cladogenesis and stasis, especially those linked with paleoclimatic change, critics of the punctuated equilibrium model have stated that the fossil record provides sampling resolution of a maximum of 5×10^5 years and concluded that gradual change is more common than abrupt transitions and stasis (*15*). With comprehensive paleozoogeographic and stratigraphic coverage for a single extant genus and high resolution sampling intervals, the *Puriana* data fulfill most criteria needed to study evolutionary trends in fossil lineages. Further, the strong correspondence between living and fossil species in ostracodes allays concern (*15*) that fossil species do not correspond to modern species. Most morphologic change in *Puriana* is confined to cladogenesis, the speciation event, while stasis persists through extended periods of repeated climatic and habitat change. The duration of speciation (<300,000 to 500,000 years) was rapid relative to the species' entire range (3.5 million years) (Fig. 2). The pattern of brief, intense change alternating with long-term stability is explained by either the punctuated equilibrium model (*13*) or the shifting balance theory (*16*), but the relative rarity of speciation events in *Puriana* over 5 million years and the persistence of ancestral species after cladogenesis favor the former model.

Because of increasing evidence for rapid speciation mechanisms, some investigators (*17*) have downplayed the importance of geography, traditionally considered the primary factor in allopatric models of speciation (*13, 18*). Valentine and Jablonski (*19*) postulated that over longer periods of geologic time, vicariant and clinal modes of speciation operate in nonplanktotrophic species living on linear shelves. Geographic barriers such as land masses or bodies of deep water did not play a role in the radiation of *Puriana* about 3.5 million years ago,

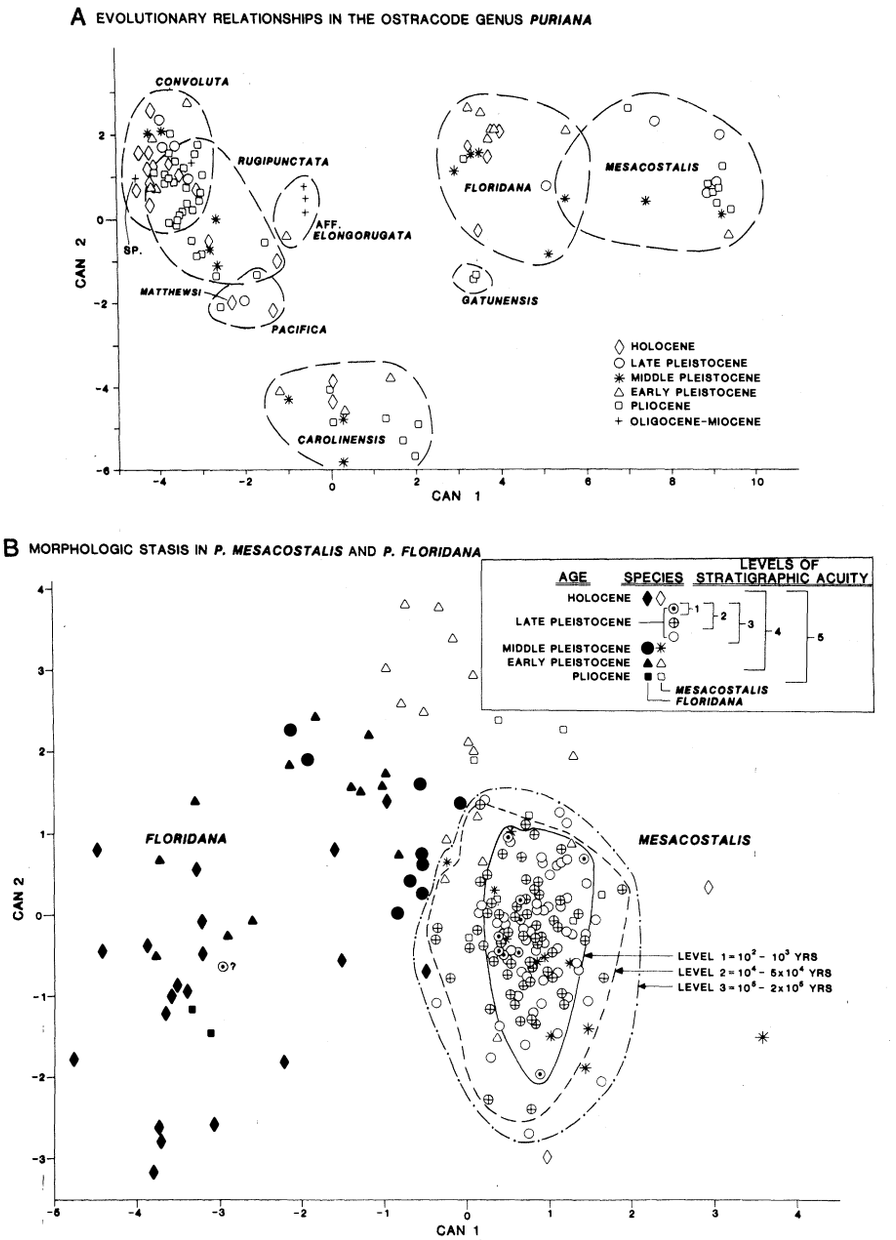

Fig. 1. (A) Plot of 114 specimens on first two canonical (CAN) variates for canonical discriminant analysis of nine species of *Puriana*. Axes 1 and 2 account for 73.5 and 14.2 percent of the variance, respectively. Symbols indicate age of specimens. Specimens of different ages for each species generally cluster distinctly (except closely related *P. convoluta* and *P. rugipunctata*) indicating that the morphologic integrity of species persists for about 3.0 million years. (B) Plot of 212 specimens on first two canonical variates for canonical discriminant analysis of *P. mesacostalis* (open symbols) and *P. floridana* (closed symbols). Axes 1 and 2 account for 66.0 and 26.4 percent of the variance, respectively. Most specimens are from the Atlantic coastal plain of Virginia, North and South Carolina, and Florida. Three ovals outlining levels of stratigraphic acuity enclose specimens of *P. mesacostalis* from one sample (level 1, 10^2 to 10^3 years), one formation, the Core Creek Sand (level 2, 10^4 to 5×10^4 years), and two formations (level 3, 10^5 to 2×10^5 years). The larger the oval, the greater the morphologic variability. The near concentricity and similar size of the ovals indicate morphologic stability over 200,000 years of climate change.

but other environmental isolating mechanisms related to habitat heterogeneity caused by the unpredictable shifts in climatic and oceanographic conditions were probably disruptive factors providing opportunities to speciate. Speciation may have been sympatric, parapatric, or allopatric but seems to have occurred within the zoogeographic range of the ancestral species. Sympatric speciation may be common in organisms without planktotrophic larvae such as ostracodes. Although clinal speciation cannot be conclusively demonstrated for *Puriana*, this mechanism can explain the observed patterns of speciation in *Puriana* and other temperature-sensitive ostracode genera living on north-south shelves (*19*). In any case, environmental perturbations sustained over tens of thousands of generations were apparently the key to the establishment of reproductive isolation in *Puriana*.

The maintenance of morphologic stability and species integrity over long periods of time has been attributed to stabilizing selection (*20*), gene flow (*21*), and developmental constraints (*22*). Whether stabilizing selection weeded out unfavorable phenotypes of *P. mesacostalis* during glacial-interglacial cycles, thus accounting for its morphologic stability, is unclear. Selection pressure would probably have increased as sea level dropped 100 m, eliminating most of the genus's habitat; it would then relax during subsequent transgressions. Yet documented environmental changes were so frequent and rapid that it is doubtful that relatively small populations surviving glacial episodes of low sea level would maintain morphologic stability through stabilizing selection. Rather, long-term phenotypic stability in *Puriana* suggests developmental constraints as limits to the range of phenotypic expression.

The correlation of stasis with periods of cyclic climatic change indicates that marine ostracode species can tolerate cyclic, predictable sea-level oscillations such that some populations withstand severely diminished habitat resources but recolonize quickly when habitat becomes available during the next sea level rise. The paleontologic record indicates that many organisms prosper during geologically rapid, potentially adverse environmental changes (*23*). Possibly, Milankovitch cycles or other cycles of higher frequency cause environmental changes that are too frequent to allow reproductive isolation to develop in ostracodes and perhaps other groups. As shown in Fig. 2, genomic reorganization, proposed by Carson (*24*) as the second step in speciation, does not have enough time to occur during cyclic climatic change before the original environmental conditions are restored upon completion of the cycle. Potential speciation events, in effect, are aborted. Conversely, the Pliocene long-term climatic transition allowed genetic reorganization and the establishment of reproductive isolation. This contrast between cladogenesis during long-term unidirectional climatic change and stasis during rapid, periodic climatic change suggests that climate, in concert with endogenous genetic activity (*25*), modulates evolution. The geologic record, particularly fossil sequences from climatically induced cyclic sedimentary sequences in the Phanerozoic, provides not only the pattern of paleontologic change but a record of extrinsic environmental changes. When examined together they provide clues on how environment and genome interact through the phenotype.

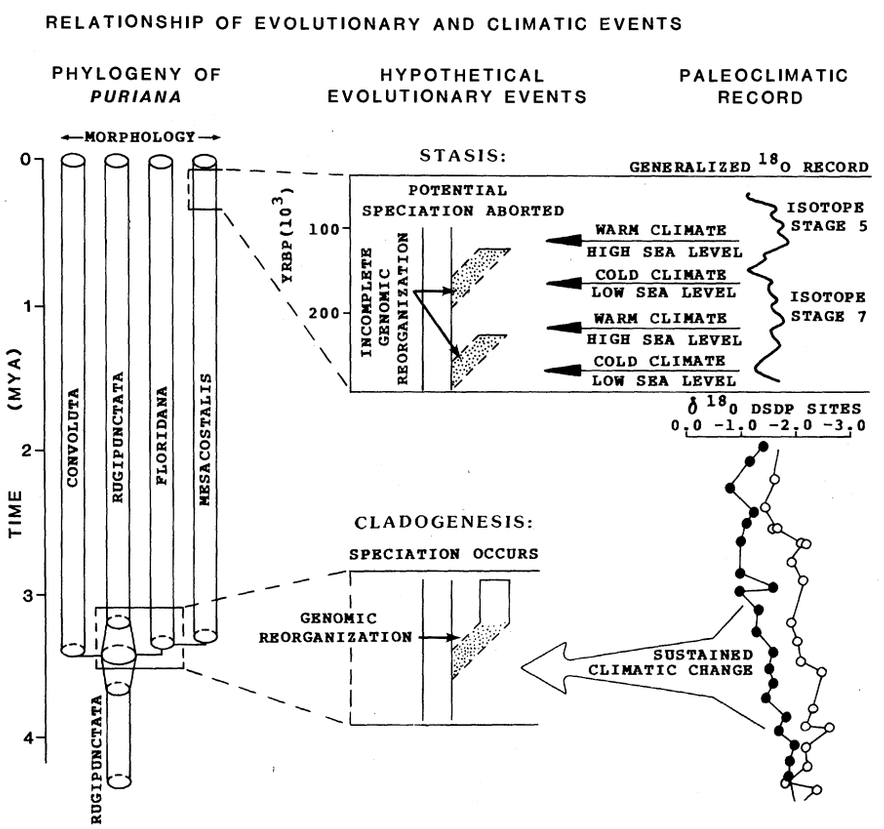

Fig. 2. Summary of phylogeny of four species of *Puriana*, hypothetical evolutionary events, and paleoclimatic record. A major climatic transition between 4.0 and 3.0 million years ago is shown in oxygen isotope data from Keigwin in (*5*); closed circles, Caribbean Deep Sea Drilling Project (DSDP) site 502; open circles, DSDP site 503. Increasing $\delta^{18}O$ in Caribbean core signifies more restricted circulation and closure of Isthmus of Panama. See (*2, 5*) and Cronin *et al.* in (*7*) for discussion. Late Pleistocene generalized isotope curve from Fairbanks and Matthews in (*6*). Isotope stages 5 and 7 are the last two interglacial periods and correspond to Core Creek Sand and Flanner Beach Formations, which yielded *Puriana* specimens.

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Bowdon, Thomonde, Gurabo, Charcol Azul; and (v) Pacific: San Ignacio, Imperial, Cueva de Angostura, unnamed units on Tres Marias Islands and Baja California. T. M. Cronin *et al.* [*Palaeogeogr. Palaeoclimatol. Palaeoecol.* **47**, 21 (1984)] summarize age data for many Atlantic formations. See W. Bold [in *Sixth International Ostracode Symposium*, H. Löffler and D. Danielopol Eds. (Junk, The Hague, 1977), pp. 175–186] on occurrences of *Puriana*. Most fossil material was collected by T.M.C., but other samples were provided by W. Blow, W. A. van den Bold, A. L. Carreno, J. E. Hazel, K.-H. Paik, J. T. Smith, T. R. Waller. *Puriana* samples from modern continental shelves off the eastern United States, Hispañola, Veracruz, Campeche Banks, Belize, Gulf of California, Texas, Baja, Mexico, and California were examined. Specimens were provided by R. H. Benson, M. Kontrovitz, P. R. Krutak, F. M. Swain, J. W. Teeter, and P. C. Valentine. For detailed locality maps see Cronin (*10*).

8. These species were studied: *carolinensis* Hazel, 1983; *convoluta* Teeter, 1975; *elongorugata* Howe, 1936; *floridana* Puri, 1960; *gatunensis* (Coryell and Fields, 1937); *horrida* Benson and Kaesler, 1963; *matthewsi* Teeter, 1975; *mesacostalis* (Edwards, 1944); *pacifica* Benson, 1959; and *rugipunctata* (Ulrich and Bassler, 1904). *Puriana congestocostata* van den Bold, 1963 and *P. fissispinata* Benson and Coleman, 1963 belong in *Coquimba* [W. Bold, *Bull. Am. Paleontol.* **79** (No. 312) (1981). *Puriana* aff. *elongorugata* is an undescribed species. For illustrations of most taxa, see J. E. Hazel, *U.S. Geol. Surv. J. Res.* **5**, 373 (1977); T. M. Cronin and J. E. Hazel, *U.S. Geol. Surv. Prof. Pap. 1125-B* (1980); and J. E. Hazel, *Smithson. Contrib. Paleobiol. 53* (1983), p. 81.
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Direct Imaging of Live Human Platelets by Flash X-ray Microscopy

Abstract. *A 100-nanosecond pulse of long-wavelength x-rays was used to produce high-resolution stop-motion images of living human platelets. Although some aspects of the structure conform to those seen in dehydrated specimens, novel features are apparent. The technique should permit detailed stop-motion examination of the interaction of platelets with their surrounding medium as well as exploration of the phagocytic and secretory activities of a wide variety of other cells.*

Long-wavelength (soft) x-rays may be used with x-ray–sensitive material (x-ray resist) to produce high-resolution images that reflect the photon absorbance patterns of specimens (*1, 2*). The technique, termed contact x-ray microscopy, can provide a bridge between light microscope and electron microscope observations, since the resolution obtainable is 5 to 10 nm and, with a slight loss of resolution, specimens as thick as 10 μm may be studied (*3*). Furthermore, contact images appear to provide unique morphological information not available when light or electrons are used for imaging.

Because of their utility for studying fixed and dried material, soft x-rays have frequently been considered for the imaging of living material (*4, 5*). Exposure times with conventional and synchrotron sources have proved to be too long to prevent natural and radiation-induced motion of the living specimen from blurring the image (*6*). Flash x-ray sources with plasmas as emitters are of sufficient intensity to produce resist images in nanosecond periods (*7, 8*). With this range of exposure times, a specimen may remain alive at the instant of exposure and its image captured before the specimen is destroyed by the exposure. We report here what may be the first soft x-ray image of this type, that of a living human blood platelet, produced with a flash x-ray source that emits a 100-nsec pulse of soft x-rays. The wavelengths used to image the hydrated specimens were between 24 and 43 nm, a region in which the relative absorption of water is low compared to that of protein (*3*). The images reveal details not previously seen in images of fixed or dried platelets.

Human platelets were isolated and resuspended in buffer at a density of 10^9 cells per milliliter (*9*). A 10-μl portion of the suspension was placed on the surface of a Si_3N_4 window 100 nm thick that had been coated with a 1-μm layer of poly-

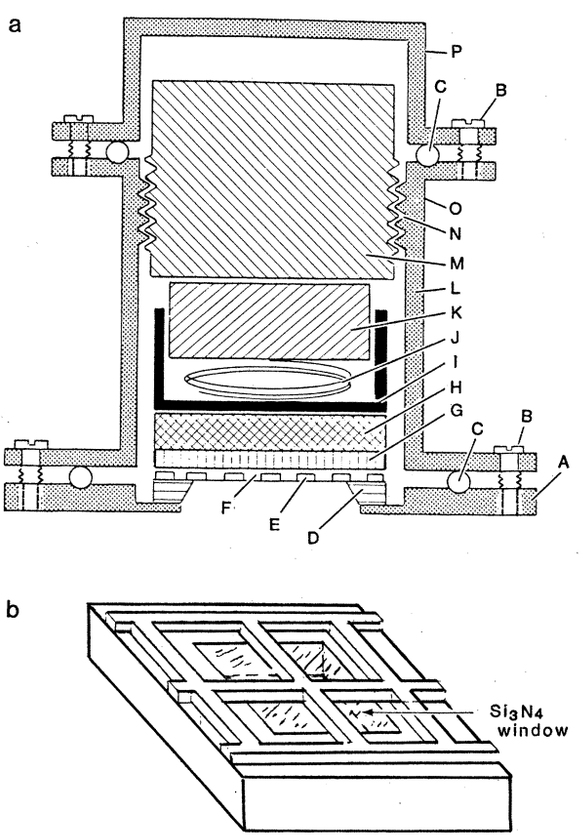

Fig. 1. (a) Diagram of the wet chamber, showing the (A) substrate holder, (D) substrate for vacuum window, (E) resist honeycomb, (F) specimen area, (G) recording resist, (H) substrate for resist, (H to K) spring mechanism to hold resist in position, (B, C, and L) body of wet chamber with O-ring seals, (M and N) threaded spring compressor, and (P) top of chamber. (b) Honeycomb subchamber [enlarged view of (E)].