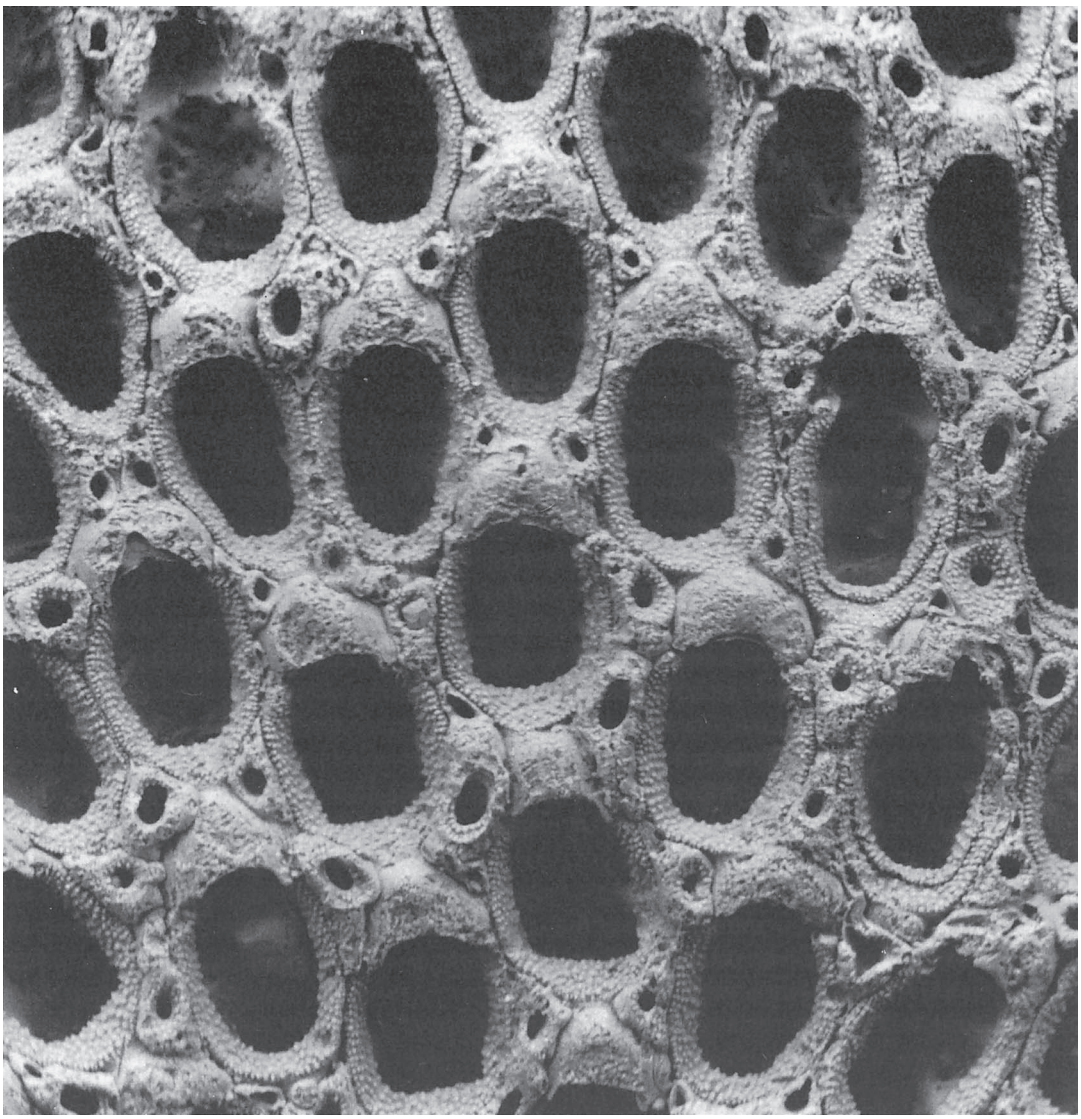
6
INFRASTRUCTURE OF PALAEOBIOLOGY



Scanning electron micrograph of an uncoated cheilostome bryozoan *Akatopora circumsaepta* (Uttley), imaged using back-scattered electrons, from the Pleistocene of Wanganui, New Zealand, × 100.

6.1 Computer Applications in Palaeontology

J. A. KITCHELL

Introduction

Computer techniques enable palaeontological questions to be addressed on a scale unheard of in earlier times. The capacity of the computer to organize and manipulate immense amounts of information is well known. Consequently, this article is *not* about computer applications that merely change the magnitude of analyses but is instead a response to the question 'What *qualitative* changes have resulted from this quantitative leap in computing speed, efficiency, and capability?' The focus will be on 'the new eyes' provided by the computer, emphasizing the ways in which computing techniques enhance our ability to 'see' both problems and data.

The computer as experimental tool

True experiments are not possible within the historical sciences, because history cannot be repeated in novel contexts. Computer modelling serves instead as the experimental tool. Experimentation is made possible by the fact that simulation models, unlike analytical models, have no exact solution. Evolutionary theory and simulation modelling are in this respect analogous. Each simulation run may represent a different evolutionary trial in which differences and novel contexts are introduced by stochastic variables or changing parameter values of deterministic variables. By explicit and systematic manipulations, the palaeontologist is given the power to complete *'If ... then ...'* statements about evolutionary process and the resultant pattern.

Despite the fact that 'scientists are incessantly saying to each other "Let's play around with that" — and modelling is the quintessential way of playing with the way things might work and might be' (Judson 1980), palaeontologists historically have not developed mathematical models. Yet it is well understood that theories, whether explicitly or implicitly, *are* mathematical, even though the impetus of theory formulation is outside mathematics and distinctly empirical. As a result, theories that are made into models use the explicit language of mathematics.

The purpose of mathematical modelling is to capture in specific and explicit terms the essential bits and connections of the theory. The purpose of simulation modelling is to take this process a step further: simulation is an exploratory technique. Simulation modelling explores the consequences of a given set of assumptions. The outcome is created as a logical consequence of the theorized process, to discover the way things would be, if the theory of process were operative. Using the capability of the computer the technique is used to determine whether, given a certain formulation of a process or system, this formulation (i.e. this set of assumptions) *can* produce behaviour similar to that known empirically. Such an approach is necessary to augment and even to develop our limited intuition in dealing with complexity (e.g. solving simultaneous situations) and nonlinearity. Modelling becomes indispensable when it expands the limits of our understanding beyond both intuition and the exploration of what did happen to what *could* happen.

Another important aspect of the computer has been referred to as 'its power to feed a new mathematics *of the eye*' (Gleick 1987). What this means is that images (easily readable graphic output) have increasingly replaced more abstract formulations. Such graphics are also necessitated by the fact that there is no unique solution to many theories (models). The dynamics and range of solutions can now be shown in the form of a 'portfolio' (Fig. 1). Most of the work in palaeontology using simulation modelling has relied on this appeal of graphic imagery. Examples include the behaviours of random processes, the transformation of morphologies, and the features of time series.

In palaeontology, simulation modelling has been used largely in the following cases: (1) to model aspects of randomness, as a branching process, a diffusion process, or a random walk; (2) to model growth and form and the (descriptive) transformation of related morphologies; and (3) to model the behaviour of classical functions (e.g. the exponential and logistic). In each of these cases (except the

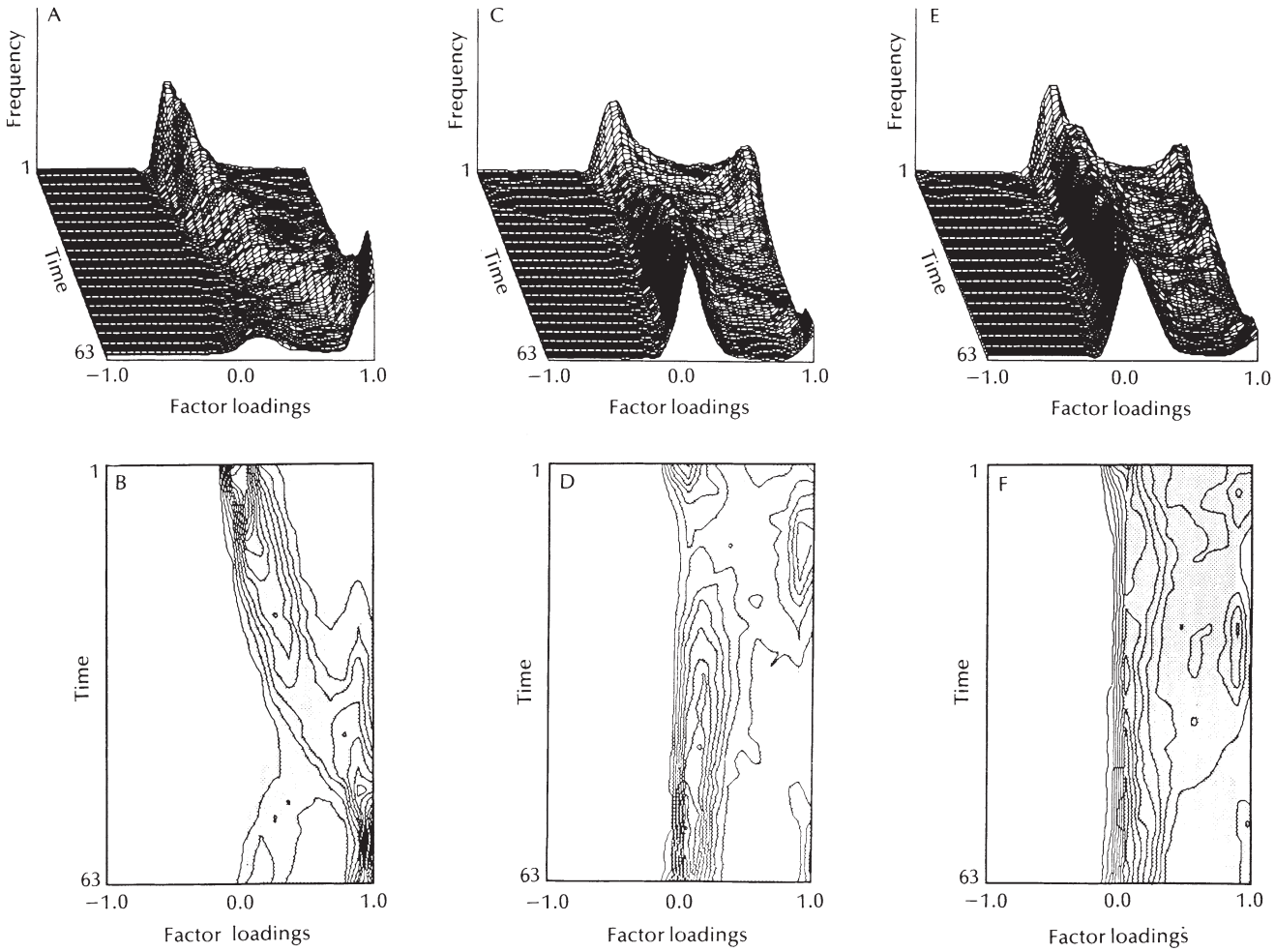


Fig. 1 The approach that yielded these results combines simulation modelling with a statistical analysis dependent on computer solution. The research question requires that the expected distributions of temporal covariation among clades generated by a random process be known. Because there is no analytical solution to the problem, a random branching process was used to generate 45 000 simulated monophyletic clades, where the differences between each clade's history are due to the random elements of the branching algorithm. Each evolutionary 'trial' of 90 such clades, allowed to evolve for 63 time steps (where 90 and 63 were chosen to match the empirical data of number of taxa and stratigraphic stages, respectively), was then subjected to Q-mode factor analysis (to match the method of analysis of the empirical data). The frequency distribution of these 500 factor analyses are shown in A, C, and E which represent Factors I, II, and III, respectively. The stippled areas of B, D, and F represent the corresponding patterns not significantly different from expectations of a random branching process. (After Kitchell and MacLeod 1988.)

coupled logistic) the exploration of behaviour involves only kinetics. Kinetics are more inherently intuitive than dynamics, which incorporates feedback.

A more ambitious undertaking of theory development and simulation exploration involving feedback, nonlinearity, and complexity, is the work of DeAngelis *et al.* (1985) on potential coevolutionary dynamics, a series of studies motivated by (but not confined to) palaeontological questions. What this work has gained is a new intuition to replace the old expectation of linear escalation. In addition, it has shown the salient features of nonlinear dynamics (Fig. 2): how the behaviour of the individual parts are qualitatively different from the behaviour of the whole; and the influence of evolutionary change on itself, where 'playing the game changes the rules'.

Computer-intensive statistical inference

Science is argument focused on the differential credibility of competing hypotheses. Palaeontology, a historical science, must make argument of process

(where the interest generally lies) from evidence of pattern (where the information generally lies). Fortunately, hypotheses of process contain predictions of pattern, and so there can be effective argument provided by historical pattern. Statistics similarly deals with an end product (namely, some observed set of data) and makes arguments, among others, regarding what factors are, and to what extent, causally responsible.

The power of computing is currently changing the field of statistics. In general, the computer has allowed even classical statistical methods to be applied to what would once have been unmanageably large data sets. Palaeontology has benefited from this increased capability; the compilation and analyses of large databases have changed the tenor of arguments, for example, on patterns of diversification (Section 2.7), extinction (Section 2.12.3), rates of phenotypic evolution, and taxonomic turnover (Section 2.11). Palaeontology, however, has been hampered by the limits of classical statistics: the need to make *a priori* assumptions about the form of the probability distributions that are sampled by the data, and the restriction to measures whose theoretical properties are simple enough to have analytical proofs. These limits have been transcended recently by computer recursion techniques that replace analytical solutions with enormous numbers (10^5–10^9) of computations.

Bootstrapping represents such a computer-intensive method, described as the 'substitution of raw computing power for theoretical analysis' (Efron & Gong 1983). Using the traditional approach, one would hypothesize a process (or model) and deduce (or simulate) its behaviour, to compare these outcomes with empirical data. The bootstrapping approach is logically different. Bootstrapping derives its power from the assumption that the empirical sample provides an informative 'glimpse' of the real or underlying process. This empirical sample is resampled with replacement a large number of times, with the statistic(s) of interest calculated for each bootstrapped sample, in order to construct the bootstrapped probability distribution, against which the empirical sample is compared. The bootstrap is especially useful in cases where the probability distribution is unknown, or if the data violate certain (particularly parametric) distributional assumptions. A large number of palaeontological cases fall into these categories.

The bootstrap method has been applied in palaeontology to problems that include estimating confidence limits around phylogenies, assessing patterns of extinction probability and the shape of clade diversity histories, and the significance of differences in rates of evolution. A problematic feature of much palaeontological data for such methods is that the data are often ordered by (geological) time. The original bootstrap method was designed for data that are identical and independently distributed; time series do not satisfy this criterion. A method applicable to palaeontological (time series) data sampled at intervals that may or may not be constant is now available. In particular, the method recognizes the necessity of coupling the magnitude of evolutionary change with the magnitude of the time interval over which that change is measured (Kitchell *et al*. 1987) (Section 2.11). The method also works with two types of time series: those in which a change in the time series is recognized on the basis of independent criteria, and those in which a segment of the time series is identified as exceptional simply on the basis of that change (*post hoc* recognition). Such computer-intensive methods of statistical inference will undoubtedly play an increasing role in fields such as palaeontology that rely little on laws, axioms, and deductions to gain understanding.

Sensitivity of initial conditions

Palaeontologists have used computer simulation methods to generate samplers of patterns produced by a variety of random processes, because much of the evidence in palaeontology since the nineteen-seventies is pattern data. Mathematicians and statisticians had already shown that random processes are capable of producing orderly pattern. Many of the properties of random processes were known by analytical solution. However, the ability to display these randomly-produced patterns *graphically* and by simulation did most to convince palaeontologists of the fallacy of the expectation that orderly patterns required deterministic explanations. It was shown that palaeontologically significant patterns, such as some trends and the topology of branching patterns, could be produced by random models (see review by Raup 1977). The purpose of this work was both to enlarge the intuitive understanding of palaeontologists so that they would not incorrectly equate pattern with non-randomness, and to better identify non-randomly produced patterns.

The opposite side of this coin, namely that completely deterministic processes lacking randomness can nevertheless produce random patterns, required the computer for its development. Until recently

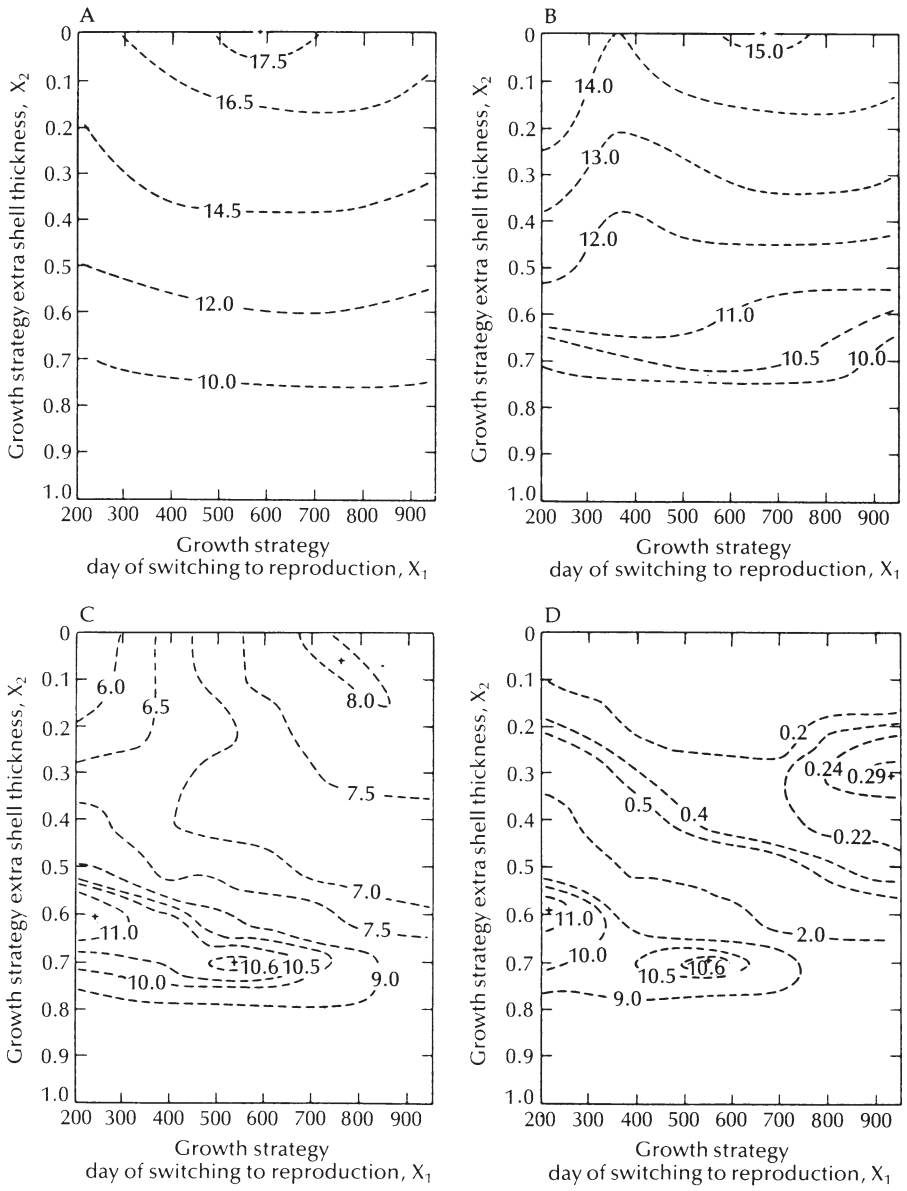


Fig. 2 This example of computer-intensive analysis involves simulation modelling of a set of simultaneous algorithms that represent the opposing interests of a species interaction. The axes represent trade-off energetic options of a prey species (vertical axis = morphological allocation option; horizontal axis = reproductive strategy option). The contours depict the fitness consequences (in terms of total expected reproduction of the prey over its lifetime) for all possible combinations. Both the magnitude and positions of the contours change dramatically as predation intensity is increased, from zero in panel A to increasingly high values towards panel D, despite there being no change in the predatory strategy of prey selection. (From DeAngelis *et al.* 1985.)

within all the sciences, complex patterns were considered to be the consequence of complex causes. It has now been shown, however, that apparently random behaviour can derive from even simple deterministic processes. A small difference in initial conditions, for example, can lead to unexpectedly divergent behaviours. The term 'chaos' has been applied to such patterns and processes, to distinguish them from randomness. In chaos, the disorder is ordered. Such ordering is apparent in the detail of the patterns, a detail made increasingly evident by computer techniques and images.

In palaeontology, it was shown that the most simple model of diversification, and the one being applied to empirical analyses of taxonomic diversity, had chaotic behaviour. Using computer simulation runs to map the surprising array of behaviours and their abrupt and ordered thresholds, Carr & Kitchell (1980) showed that the 'coupled logistic' model of Sepkoski (1979) could produce not only logistic patterns of diversity change with time but also extremely complex and chaotic patterns of diversity change. In this latter case, the oscillations are driven internally, without external perturbation. Whereas earlier work, by warning that a high degree of order can be generated by purely random processes, had tried to dispel the palaeontologist's bias that randomness implies a random pattern, Kitchell & Carr (1985) warned against the bias that determinism implies an ordered pattern. They showed

that even a completely deterministic and remarkably simple process can produce patterns of bewildering complexity. The understanding of chaotic behaviours is now being pursued in a number of cognate fields within biology, physics, and chemistry, promising to revolutionize our collective understanding of a class of complex phenomena, until recently unknown.

Phylogenetic inference

The methodology of inferring phylogenetic (evolutionary) relationships among organisms has become both increasingly explicit and empirical (Section 5.2). Phylogenies are constructed from data on the distribution of characters (the empirical component, such as that resulting from morphometric studies), according to some criterion made operational by a computing algorithm (the explicit component). These criteria and associated algorithms used to form phylogenetic hypotheses rely either on parsimony methods, maximum likelihood methods, or compatability methods; reviews that examine the fundamental assumptions of each method were given by Felsenstein (1983).

These methods are derived from a class of problems in mathematics and statistics that focus on maximizing or minimizing some aspect of the data. In such optimality methods, the assertion is not that the historical process of evolution is optimal. Rather, optimization methods are used to choose among all tree topologies generated by an algorithm for a given set of data. Parsimony methods, for example, evaluate phylogenetic hypotheses on the basis of number of homoplasies (convergences and parallelisms); the 'best' genealogy is the one of minimum homoplasy. Because the criteria for evaluating phylogenies are unique to the method, comparing methods in terms of finding the 'true' genealogy is not possible. Instead, types of parsimony, maximum likelihood, and compatability algorithms can be compared with one another in terms of a practical goal (efficiency in computer time) and a methodological goal (minimizing tree 'length' or the required independent origins of each character).

Although small data sets may be analysed by hand (using the 'brute force' method of generating all possible cladograms; there are 15 possible for four taxa), large data sets *require* computer-assisted analyses (there are more than two million cladograms for only nine taxa, and more than 10^{20} cladograms for 20 taxa). Even the latter is too much for computer analysis. This raises an interesting situation; there is no exact solution to the problem of finding *the* minimum tree for even moderate-sized data sets. This problem may not be soluble: among mathematicians, there is agreement that NP- (not polynomial)-complete optimization problems (such as these) cannot be solved given current approaches and algorithms. Within palaeontology, phylogenetic approaches principally make use of morphological character data. An interesting discussion was provided by Gauthier *et al.* (1988) who showed, using both palaeontological and neontological character data, the importance of palaeontological data. Stratocladistic methodology may also prove useful as a means of integrating both character data and stratigraphic data in an analysis of phylogeny, where a total parsimony debt (summed from morphology and stratigraphy) serves as the minimization criterion.

A problem in need of redressing is that most palaeontological analyses of taxonomic data sets (e.g. patterns of diversity change, extinction, rates of evolution) have made use of data currently available. Much of these data do not reflect the methodology discussed above. As a recognized consequence, monophyletic and non-monophyletic groups are not distinguished from one another. This presents a problem of interpretation since 'monophyletic groups have a unique history that exists and is to be discovered, whereas paraphyletic groups may start off with a unique history, but their boundaries are adjusted *a posteriori* and they are in part a human invention' (Benton 1988).

Computer-aided vision systems

The most severe restriction on palaeontology today is the lack of adequate databases to test hypotheses of interest. It is likely that major advances in the future will be made in the rapid acquisition of morphological and character-state data from automatic vision systems. Although the systems described below have not yet been widely used in palaeontology and are still in stages of development, the future of advanced computer techniques in palaeontology will undoubtedly move in these directions.

With laser disc technology, it is now possible (and currently in use in some research laboratories) to store all known species' images (e.g. holotypes) and their descriptions, and to make use of them with a dichotomously driven, interactive algorithm to resolve the identification of an unknown species. This technology permits exact comparisons on the

screen. Access is also virtually instantaneous, with more than 50 000 analog images currently capable of being stored per disc and with the ability to access more than one disc at a time. The system utilizes answers provided by the user to a computer-driven key to select the most likely known species. It then automatically compares the unknown image with these selected known species, making comparative diagnostic measurements. Because of the interactive nature of the algorithm, the user maintains control of the final decision.

Programs designed for palaeontological applications that make use of artificial intelligence programming have also begun to be developed (e.g. Riedel *in press*). These programs explicitly attempt to deal with objects that are naturally variable (organisms), and may be made even more variable by preservational processes, yet are members of a single category (the species). These systems use character-state descriptions entered by the user and work within a hierarchy of character-states necessary for discrimination between possible species. As above, the final result is a 'narrow as possible' reporting of species that have these characters.

Algorithms associated with image analysis systems are also now available (and being developed) for converting data from serial sections of any fossil (whether actually sectioned or not) to three-dimensional models of that fossil, thereby allowing the user in many instances to bypass the building of physical models. The reconstructed three-dimensional form can also be viewed from all perspectives by rotation and movement simulation algorithms.

Acquiring morphometric data by image analysis

Palaeontologists, of necessity, rely on morphological data to make evolutionary inference. A recurring problem in the sciences is that the theories of a field may occasionally far exceed the capacity of that field to acquire and analyse data necessary for evaluating those theories. Such a situation occurred in palaeontology, e.g. with the proposal of punctuated equilibrium and its associated prediction of morphological stasis. The imperative quantitative data on morphological change within and between species, and over time and geography, were not copiously available. Much of the problem stemmed from the difficulties of acquiring quantitative data on morphology in a rapid and accurate manner.

Widespread interest within numerous fields in the study of biological shape and its transformation has resulted in a series of important advances. In terms of technique, advances in computer technology have made possible increasingly powerful image analysis systems that combine image acquisition and image processing capabilities with pattern recognition analyses. Such image analysis or optical pattern recognition systems have made the acquisition of quantitative data on morphology rapid, accurate, and affordable.

The field of *morphometrics* has been redefined recently as 'the analysis of biological homology as well as geometric change' (Bookstein *et al*. 1985). Morphometrics is relevant to questions of phylogenetics, ontogenetic trajectories and their evolutionary potential for heterochrony, patterns of anagenesis and cladogenesis, ecophenotypy, and morphological integration. Such analyses are particularly informative when they combine hypotheses of phylogenetic descent with hypotheses of morphological (character) transformation.

Reviews of methodology and examples of the application of outline methods and landmark methods were given by Lohmann (1983) and Reyment (1985), respectively. The approach recommended by Bookstein *et al*. (1985) focuses more on the dynamics of change in shape. Analyses begin with a study of the major dimensions of morphological variation in time and space that characterize each species. Analytical procedures determine which parameters contribute most to intraspecific characterization and to interspecific discrimination within respective geographical and temporal contexts. A recent application of outline and landmark methods was given by Stanley & Yang (1987) who assessed the rates of morphological evolution in separate lineages of Neogene bivalves. Schweitzer *et al*. (1986) used the same basic techniques to evaluate the relative contribution of development (heterochrony) and structural regulation in two closely related species.

Prospects

Palaeontology today is actively engaged in computer-aided research programs. The evolution of the interaction between palaeontology and computer technology is following much the same path as that of the evolution of the human brain, as we currently understand it. The computer has not simply resulted in an increase in the speed, efficiency, and size of the problems we analyse. It has introduced novelty or true innovation. It is well recognized that the biological and evolutionary sciences deal with a much greater degree of complexity in their systems

of study than do the physical sciences. Computer techniques are beginning to open up the field of study of complex systems and, through vision systems, to relieve the human investigator of some of the effort in amassing empirical data.

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6.2 Practical Techniques

6.2.1 Preparation of Macrofossils

P. J. WHYBROW & W. LINDSAY

Mechanical methods

A rock is invariably physically weakened by the presence of fossils, usually because the chemical constituents of fossils differ from those of the enclosing matrix. For at least three centuries, palaeontologists have exploited this difference by using percussion methods, normally a hammer and a chisel, to expose and to collect fossil material. Following the introduction of electricity into museums and universities in the nineteenth century, power tools were developed that 'automated' the basic manual techniques. Today, three mechanical techniques are widely used in palaeontology laboratories: *percussive*, *grinding*, and *abrasive* (Rixon 1976).

Percussive and grinding techniques. Percussive electric or pneumatic engraving pens (Fig. 1) are hand-held and equipped with a tungsten carbide tip. Invariably the tip supplied by the manufacturer is too coarse for most preparations and has to be substituted by tungsten carbide rod welded onto the oscillating shank of the pen. The fitting of the rod also enables a choice of either chisel or pointed tips to be fashioned. Before commencing preparation not only should the concealed morphology of the fossil be imagined (by reference to published information concerning similar fossils) but also the petrology of the matrix must be investigated (in case acid techniques can be better utilized). If

Fig. 1 A hand-held, pneumatic engraving pen used to remove rock matrix. In the foreground is a heavy duty pneumatic chisel.

the rock cover is excessive, it can be removed by grinding. Diamond or carborundum wheels and burrs used in dentistry are ideal; for larger blocks, parallel grooves are cut using a pneumatic diamond saw and the thin rock wedges then removed by percussive methods. All preparations should be carried out at high magnifications using a binocular microscope so that the fossil–rock interface can be easily seen; a cold-light, fibre optic light source is invaluable for this (especially a system with contrasting colour filters). The position of the percussion point should ideally be at right angles to the plane of the fossil surface being exposed. The degree of force required to chip or flake away the rock and leave an unmarked specimen comes about by trial and (infrequently) error. Extreme care must be taken when microbedding planes pass through and around a fossil as flakes may contain part of it. Extensive preparation gradually weakens the structural integrity of a fossil but the percussive force used normally remains constant. Therefore, the specimen must be supported firstly by a shock absorbing cushion (such as a sandbag) and secondly by embedding in a water soluble polyethylene glycol wax of high molecular weight. For supporting delicate areas of a vertebrate skull, this wax is essential and can itself be strengthened while in its fluid state by the addition of surgical gauze (Whybrow 1982).

Abrasive techniques. 'Airbrasive' or 'sand-blast' machines are quick and effective aids for removing rock that is softer than the fossil. An inert gas (compressed air, nitrogen, or carbon dioxide) propels an abrasive powder, which is kept in a fluid state in a vibrating pressure vessel, through a nozzle of small diameter. Various hardnesses of powder can be used, ranging from sodium bicarbonate to the cast iron shot used in large industrial machines. Similarly, various diameters of nozzle can be selected. The abrasive action depends on particle size and the amount of gas pressure used. Exposed parts of a fossil can be protected by a coating of rubber latex from any polishing effect of the powder, and a box with a dust extraction system protects the operator from possibly hazardous particulates. A binocular microscope is essential for this work to see the degree or variability of abrasion of the rock.

Chemical methods

Rocks and the fossils they enclose do not always respond well to mechanical techniques. The hardness of an ironstone or some limestone matrices may prohibit mechanical preparation, while the complexity or abundance of fossil remains may defy methods reliant on manual dexterity. As with mechanical methods, chemical methods aim to remove the matrix without damaging the specimen. However, in both cases, there are occasions when the information required can only be obtained by destroying the fossil and retaining the natural impression left in the rock.

Chemicals used in fossil preparation are chosen for their ability to disrupt or dissolve the rock matrix, but they must achieve this without causing the same effect on the fossil. Such differentiation is determined by the chemistry of both rock and fossil. Furthermore, the long-term conservation of the fossil in a collection, with all the hazards associated with handling, must be considered.

Chemical disruption. Water, sometimes in conjunction with a detergent, readily breaks down some soft shales and muds. The clay minerals swell as the strongly polar water impregnates their structure. Detergents and other surfactants assist the process by reducing surface tension at the clay–water interface. A similarly disruptive effect occurs in the presence of hydrogen peroxide (H_2O_2). Solutions of H_2O_2 are unstable and deteriorate giving off oxygen. In the presence of alkalis, rough surfaces, and metals, the process is accelerated. In rock matrices the oxygen bubbles released within the pores disrupt the sediment and weaken the matrix (see also Section 6.2.2).

Sequestrants and chelating agents. Polyphosphates, such as sodium hexametaphosphate $(NaPO_3)_6$, act

as water softeners, sequestering the calcium, magnesium, and iron salts present. Clayey and muddy sediments are broken down in solutions of polyphosphates. In a manner similar to that of water softeners, chelating agents form stable complexes of metalic ions (such as calcium and magnesium) in rock forming minerals. Ethylene diaminetetracetic acid and its sodium salts in solution can corrode rock matrices, but it will also attack fossil material and careful control is therefore required.

Acids. Acids are extensively used in chemical methods of preparation (Lindsay *in* Crowther & Collins 1987). Hydrochloric acid was used in the late nineteenth century to dissolve limestone containing carbonized graptolites. Subsequently hydrofluoric, nitric, formic, acetic, and thioglycollic acids have been used in both vertebrate and invertebrate palaeontology. Hydrofluoric and nitric acids are employed for the maceration of sediment samples containing fossil pollen (Section 6.2.2) and pose particular problems of safety.

The development of vertebrate material using aqueous solutions of acetic acid was first carried out in the nineteen-forties and followed from earlier techniques devised at the British Museum (Natural History) (Rixon 1976). Acetic acid is the most commonly used acid for this work and is readily controlled and reasonably safe at low concentrations. Used in solutions of 1−10%, the reaction between the acid and calcium carbonate in the matrix occurs more readily than that between the acid and phosphates in fossilized bone (Fig. 2). The differential rate of dissolution is controlled by varying the length of immersion time and the acid concentration. The time of exposure to acid at each step of the process may vary from a few hours to several days, and the development of a specimen may take years to complete. Bone that undergoes prolonged exposure to acid will be significantly affected; for this reason the dissolution of the matrix is interrupted regularly to wash, dry, and lacquer any newly exposed bone.

Consolidants and adhesives

Consolidation (hardening) of a specimen must be carried out during preparation in order to conserve it for subsequent study. A number of adhesives and consolidants are used; they should be reversible in the long term as further work on a specimen may be required. In mechanical preparation the surface of the fossil is coated with a consolidant as the rock is removed in order to prevent fractures caused by any excessive vibration (Fig. 3). Polyvinyl butyral resin, dissolved in a variety of solvents, has now replaced polyvinyl acetyl resins and serves as an adhesive when dissolved in ethyl acetate. Polymethyl-methacrylate, also dissolved in ethyl acetate, is a useful adhesive but shrinks markedly on drying and should never be used as a consolidant. Supplied as a powder monomer with a liquid polymer catalyst, polymethyl-methacrylate effectively seals wide cracks. Cynoacrylate adhesives are effective for the fast repair of small pieces of fossil, but their long-term stability is at present poorly understood and they are practically insoluble when set. Chemical methods of preparation require adhesives and consolidants that protect the fossil from chemical attack as well as supporting and strengthening it.

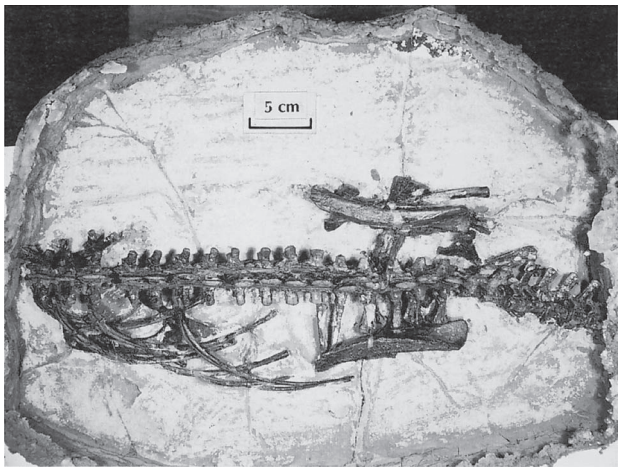

Fig. 2 The partially exposed, post cranial skeleton of the Jurassic dinosaur *Scelidosaurus harrisoni* during preparation with acetic acid.

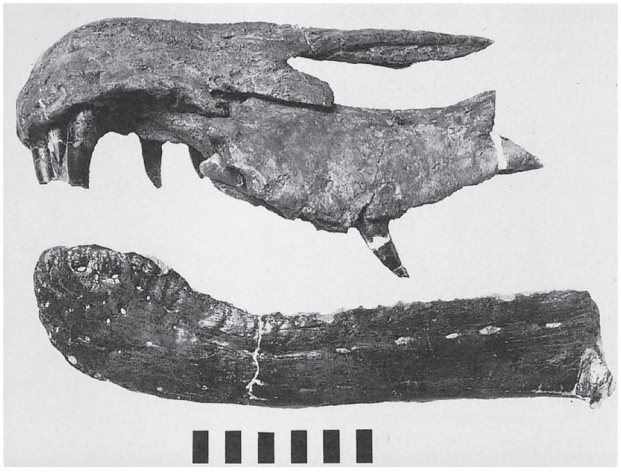

Fig. 3 Anterior skull and jaw elements of the Lower Cretaceous dinosaur *Baryonyx walkeri* after mechanical and chemical preparation. Scale in cm.

Polybutyl-methacrylate is used as an acid resistant consolidant and can withstand long periods of immersion in acids. Polymethyl-methacrylate as an adhesive is similarly resistant to attack by organic acids; the cynoacrylates also seem to be unaffected.

In *all* methods of preparation, which by necessity expose the fossil to risk, good records must be kept (Rixon 1976). Photographs, drawings, and written descriptions are essential and must be prepared as the specimen passes through various stages of treatment. Their value can only be appreciated when a dismembered fossil needs to be reassembled.

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6.2.2 Extraction of Microfossils

R. J. ALDRIDGE

Extraction techniques have been developed principally to recover microscopic fossils from rock samples, but may also be adopted for larger specimens. A variety of chemical and mechanical procedures for rock disaggregation are employed, dependent upon the composition of the rock and of the fossils sought. Residues from these processes are often large, and some concentration of the microfossil specimens may be required. Many of the chemicals used in dissolving samples and in concentrating residues are highly hazardous or toxic and the safety aspects of all techniques should be fully investigated before they are applied. Full attention must be given to hazard warnings given by the suppliers of chemicals.

Releasing microfossils from rocks

Calcareous rocks. Limestones, dolomites, and calcareous clastic rocks can be broken down with dilute organic acids (e.g. acetic acid, CH_3COOH; formic acid, (HCOOH) to release microfossils composed of calcium phosphate (conodont elements, fish remains) or with resistant organic walls (scolecodonts, chitinozoans, palynomorphs) (Fig. 1). Some workers crush the samples into 1–3 cm chips, but this is only necessary for very impure limestones. Standard procedure is to place the sample in a polythene bucket or beaker which is then filled with warm, 10–15% acetic acid; formic acid acts more rapidly and may be used at higher concentrations, but is more corrosive and hazardous. Phosphatic material may be attacked by acetic acid in the absence of calcium acetate to buffer the solution, so powdered calcium carbonate should be added to samples with low lime content. Alternatively, samples may be buffered by using a solution comprising 7% concentrated acetic acid, 63% water, and 30% of filtered liquid remaining after digestion of previous samples.

Hydrochloric acid (HCl) dissolves phosphate, but may be used at a concentration of about 10% to recover organic-walled microfossils and siliceous (e.g. radiolarians) or silicified material. When buffered by calcium acetate, HCl can be used to extract phosphatic, siliceous, and organic specimens from a single sample, but there is always a risk of damage to the phosphate, especially when all the limestone is allowed to dissolve.

When effervescence fades or ceases, the sample is sieved; the mesh sizes of the sieves employed are dictated by the sizes of the microfossils sought. For conodont elements, an upper sieve of 1 mm mesh and a lower of 75 μm are adequate, but chitinozoans and palynomorphs require much finer bottom sieves, down to 5 μm. Undissolved rock remaining on the upper sieve is placed in new acid solution, while the sieved residue is dried and retained for concentration and picking.

There is no easy technique for recovering calcareous microfossils from calcareous rocks. Soft limestones and marls may be treated in a similar way to soft shales, but for hard limestones and chalks only crude mechanical methods are available. Normally, these involve pounding the moistened sample with a pestle in a mortar, followed by washing and concentration. An intermediate step is sometimes inserted in which the pulverized sample is washed into a container and placed in an ultrasonic cleaner for a period of two minutes to two hours. Delicate microfossils will not survive these techniques and are best studied in thin section. The procedure may be successful, though, for calcareous nannofossils such as coccoliths.

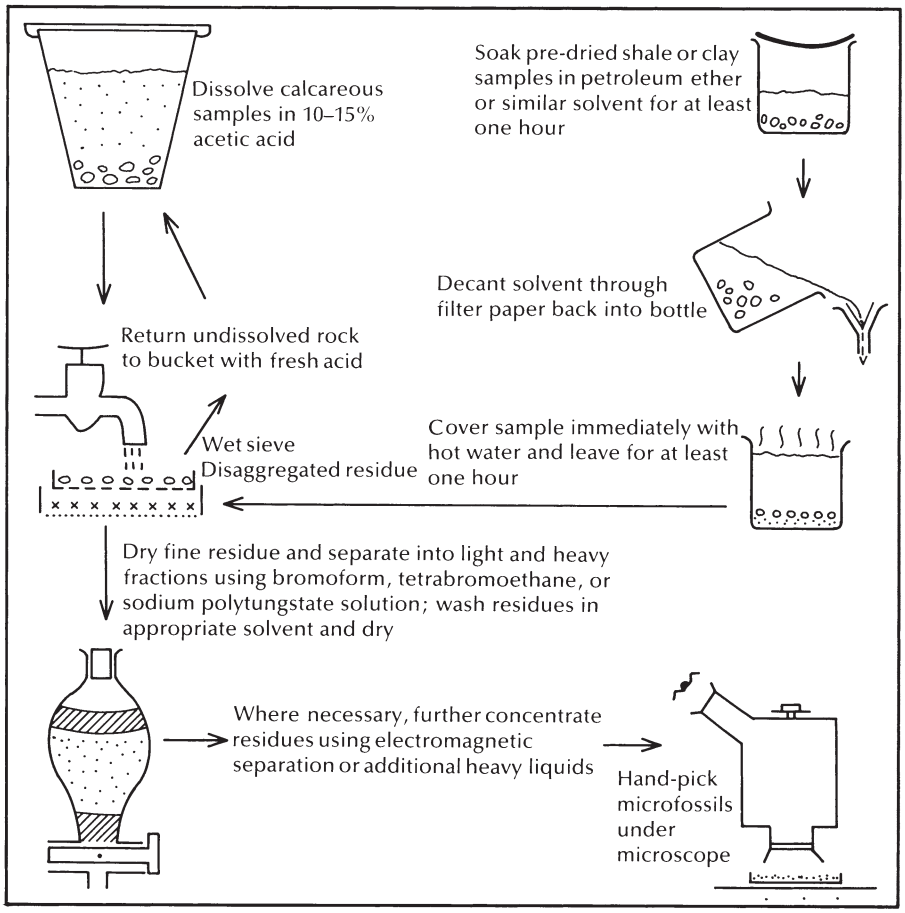

Fig. 1 Techniques for extracting and concentrating microfossils from limestones, calcareous clastic rocks, and soft or partly indurated mudrocks.

Argillaceous rocks. Soft or partly indurated clays and shales may be disaggregated by a number of techniques. A relatively gentle procedure involves the use of petroleum ether, paraffin, or similar solvent on thoroughly pre-dried samples (Fig. 1). All of these solvents are highly flammable, and due regard must be given to fire risks. The rock is soaked in solvent for at least one hour; the solvent is then poured off and the rock immediately inundated with hot (not boiling) water. The clay is reduced to an uncohesive, muddy slurry, which then can be wet-sieved as appropriate. Black shales and other mudrocks that do not respond to this treatment may disaggregate on immersion in a 10–15% solution of hydrogen peroxide (H_2O_2) in water (see also Section 6.2.1). The reaction involves the oxidation of organic matter, which may also be accomplished by other oxidizing agents, such as sodium hypochlorite (NaClO).

Hard clays may also disintegrate when boiled in water with a dispersing agent. Those commonly used include a few grams of sodium carbonate (Na_2CO_3) or 20% sodium hydroxide (NaOH). Some samples respond to boiling in the detergent Quaternary 'O', with a 20% solution added to boiling water containing the sample. A combination of techniques may be applied, perhaps involving treatment with buffered acetic or formic acids for samples containing some calcium carbonate. Mechanical disaggregation may sometimes be achieved by alternate freezing and thawing of samples soaked in water, or by boiling the rock in sodium thiosulphate ($Na_2S_2O_3.5H_2O$), which will crack the shale apart as it crystallizes when allowed to dry.

Sandstones. For most microfossils there is no technique for extraction from sandstones or siltstones, unless the rock is poorly-cemented, when mechanical methods may be successful, or calcareous, when acids may be employed. For organic-walled microfossils, palynological techniques (below) may be tried, but palynomorphs are not normally well preserved in coarse clastic rocks.

Cherts. Phosphatic microfossils, such as conodont elements, can be recovered from cherts and other

siliceous rocks using dilute hydrofluoric acid (HF). The sample is crushed into 1–5 cm fragments, any carbonate removed with acetic acid, and the fragments placed in 5–10% HF in an acid-resistant plastic container in a fume cupboard. After 24 hours the HF is decanted off and neutralized with calcium hydroxide; the residue is first washed with dilute HCl, then several times with water before being sieved and reprocessed as necessary. The technique works through fluoridization of the apatite of the conodont elements and is accompanied by some fracturing and distortion of specimens. *Hydrofluoric acid is extremely dangerous* and must be used in properly designed fume cupboards with the handler wearing *full protective clothing*.

Concentration techniques

Residues from disaggregation procedures can be concentrated into light and heavy fractions by using various heavy liquids. Bromoform ($CHBr_3$, specific gravity 2.89) and tetrabromoethane ($C_2H_2Br_4$, specific gravity 2.96) are commonly used to produce a heavy concentrate containing phosphatic microfossils, but these chemicals are severely toxic. A safer alternative involves the use of water-soluble sodium polytungstate ($3Na_2WO_4.9WO_3.H_2O$), which can be made up at any required specific gravity, but is best at 2.75 or slightly higher to avoid problems of high viscosity and crystal precipitation. Light or buoyant microfossils, such as hollow foraminiferans, radiolarians, and chitinozoans, may be removed in a light concentrate by adjusting the specific gravity of the sodium polytungstate accordingly. Electromagnetic separation is useful in dealing with large residues containing iron oxides or iron-rich dolomite grains.

Palynological techniques

Procedures for the recovery and concentration of palynological microfossils are complex, with the steps tailored to the nature of the sample being processed. A full account was given by Phipps & Playford (1984), who emphasized the dangers of HF, zinc bromide ($ZnBr_2$), and other chemicals used. *Palynological processing should only be undertaken in a purpose-built laboratory with efficient fume-cupboards, full protective clothing, and neutralization and disposal facilities available.* All equipment must be kept absolutely clean to avoid contamination.

Rock samples should be thoroughly cleaned by scrubbing and, if necessary, etching in HCl or HNO_3 (nitric acid) prior to crushing to 1–2 mm fragments. Any carbonate in the rock must be completely removed using warm 10% HCl, followed by thorough washing in distilled water. Silica and silicates are dissolved using HF. Cold, concentrated HF is poured onto the sample in a polypropylene beaker and stirred daily with a teflon rod until all the rock has disaggregated. The reaction may be speeded up by warming the containers in a water bath. After digestion the sample is washed with warm water and fluoride precipitates are removed by treatment with warm 40–50% HCl, followed by at least four washes in warm water. Ten per cent HCl is added to the last washing to discourage flocculation. Mineral particles may be separated from the organic residue by centrifuging in zinc bromide solution (specific gravity 2.0); if examination reveals the presence of pyrite, 10% HNO_3 may be added to the organic fraction for ten minutes to remove it. Unwanted, undecomposed, or partially decomposed organic material can be removed by careful oxidation (although experience is needed to avoid destruction of microfossils during this process). Concentrated HNO_3 is a commonly used oxidant. Fine organic debris may be removed by alkali treatment with 5% potassium hydroxide (KOH).

After processing, the remaining organic-rich residue is sieved, using appropriate mesh sizes for the palynomorphs present. Generally a 53 μm sieve is employed to retain chitinozoans and large palynomorphs, while a fine sieve of 5–7 μm is necessary for the smallest specimens. The fossils may be further concentrated prior to sieving by swirling in a large watch glass. The palynological concentrate, or a representative fraction of it, is finally strew-mounted onto slides, using glycerine jelly for temporary mounts and Canada balsam or a plastic mounting medium for permanent mounts.

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6.2.3 Photography

D. J. SIVETER

Introduction

The photography of fossils involves a wide range of techniques, materials, and object sizes. Large fossils, in excess of about 15 cm in length, fall within the range of normal cameras with standard lenses; specimens up to about 2–3 mm long are best photographed using the scanning electron microscope (SEM). The middle ground between normal and SEM photography (Section 6.2.4) is generally known as *macrophotography*, and covers a magnification range on the negative from about × 0.2 to × 20 or more. Macrophotography in incident light, for which there are numerous systems available, is the type of photography used for most macroinvertebrates. The Leitz 'Aristophot' system (Whittington *in* Kummel & Raup 1965) was first used for the macrophotography of fossils in the nineteen-fifties, and has since been widely adopted (Fig. 1H). It was modified in various ways before production was discontinued in the early nineteen-eighties. In its image range the quality of photographs produced by this apparatus is excellent. The comparable Nikon 'Multiphot' system gives similar results and is still (1991) marketed. In the last decade Wild-Leitz (now Leica) have introduced a quite different system for the macrophotographic range, the photomacroscop. The most useful source on the photography of fossils is Kummel & Raup (1965); many of the techniques described therein have not been superseded.

Preparation: cleaning and coating

Prior to photography any extraneous sediment should be removed from the surface of the fossil (Section 6.2.1). If the specimen is embedded in matrix, particular effort should be concentrated on cleaning its margins. This obviates the need for any retouching of or cutting round the fossil outline to delete non-organic material on the final print. The handling of testaceous specimens should be minimized, and they should be cleaned with an organic solvent (such as acetone) to remove any surface grease marks.

When photographing most fossils, particularly those that are of variable or light shade, better results are obtained if the specimen is first coated. A matt, uniformly dark surface is applied to the fossil which is then lightly dusted with a whitening agent for contrast. Fountain pen ink and particularly black photographic opaque have been used as darkening agents; these should ideally be applied to impart a dark grey (not black) colour. The former can be removed in large part with a mixture of ammonia and hydrogen peroxide solutions, and the latter with warm soapy water. The cleaning of darkening agents from natural mould specimens (especially in medium to coarse clastics) is very difficult or impossible, as they are fully absorbed into porous sediments; this is particularly so where Indian ink has been used. The excellent opaque produced by Phillips and Jacobs (Philadelphia) is now discontinued. Practitioners should experiment with alternatives; poster paint has, for example, been successfully used. Various inks and carbon powder (soot) have been used to darken latex and silicone rubbers.

A whitening agent sympathetically applied on the darkened surface considerably enhances the contours and surface sculpture of the fossil, as it falls more densely on those areas of greater relief, which are thus highlighted (Fig. 1). It also provides an even, glare-free reflecting surface for photography and results in prints of a similar tone — which are desirable when making plates for publication. Ammonium chloride, magnesium oxide, and antimony oxide have all been used for whitening. Ammonium chloride and antimony oxide are heated in a glass bulb and the resulting sublimate cloud directed onto the fossil (Teichert 1948; Marsh & Marsh 1975). Magnesium oxide is produced by burning magnesium ribbon and the fossil is held over the smoke. Ammonium chloride should be washed off immediately after use as it combines with water vapour in the air to form hydrochloric acid capable of etching the fossil; its deliquescence also renders it impracticable for use in areas or on days of high humidity, as the sublimate quickly becomes coarse-grained after coating. Nonetheless, many authors favour the use of ammonium chloride as control on the application of magnesium oxide is not very precise. All coating should be done in a fume cupboard, but the draught should not be so strong as to affect the flow direction of the whitening agent. After coating and prior to photography a check should be made under a binocular microscope for hairs or other artifacts. The implications for future conservation of the specimen should be considered before employing these techniques.

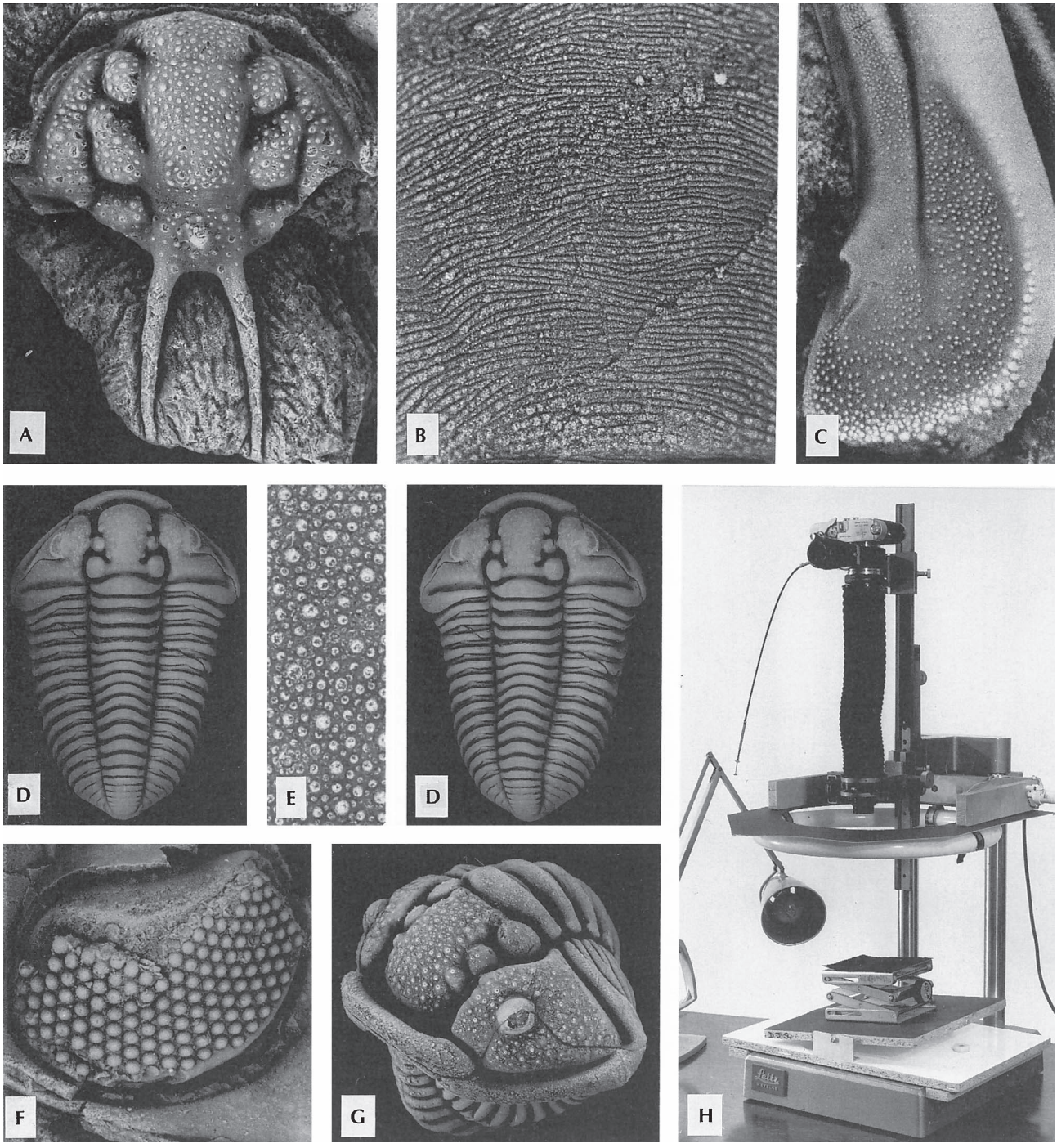

Fig. 1 A and B taken using Nikon 'Multiphot', C–G using Leitz 'Aristophot'. A taken with the Nikon 'Makro-Nikkor' 12 cm lens, B with Nikon 'Makro-Nikkor' 6.5 cm lens, C–G with the Leitz 'Summar' 12 cm lens. A–C, E, G photographed on Kodak 'Panatomic X' film, D and F on Ilford 'Pan-F' film. All specimens are coated with ammonium chloride on top of matt black opaque. A–G, Silurian trilobites. A, Cranidium, odontopleurid, Ireland; dorsal view, × 4. B, Glabellar sculpture, proetid, Ireland; dorsal view, × 22. C, Thoracic pleural facet, calymenid, Gotland; lateral view, × 10. D, Stereo-pair, complete specimen, calymenid, West Midlands, U.K.; dorsal view, x 2. E, Glabellar sculpture, calymenid, Welsh Borderland; dorsal view, × 10. F, Eye, phacopid, Ireland; oblique view, × 7. G, Complete specimen, calymenid, Gotland; oblique view × 2.5. H, Leitz 'Aristophot' with anglepoise and ring light illumination, laboratory jack, and tilt-table for taking stereo-pairs.

Macrophotographic equipment and methods

The photographic film should be fine grained (50 ASA or less) and have good resolving properties so that when enlarged it suffers minimal loss of definition; Ilford 'Pan-F' and Kodak 'Panatomic X' are both suitable. Fossil size on the final print depends on the negative magnification multiplied by that selected on the enlarger. Macrophotography of fossils is for most purposes adequately and economically performed with the use of 35 mm format, with final prints of up to × 30 to × 40 being satisfactorily obtained. Recourse to larger format apparatus and film (e.g. 9 × 12 cm) is preferable only where excessive enlargement is demanded, or where a wider field of view is required at a given magnification. The photographic stand should be sturdy and capable of absorbing vibrations.

The camera body is not one of the more critical pieces of equipment but the action of the shutter should be smooth if this is to be used to control exposure, and those with a reflex mirror lock-up facility that negates the vibrations from this source are most useful. Leica 'M' cameras have been used on the 'Aristophot' in combination with a separate reflex mirror unit that also incorporates a focusing magnifier and focusing screen. Nikon 'F' cameras for use with the 'Multiphot' house the reflex mirror and focusing system within the camera body. At high magnifications requiring long bellows extensions, where the slightest vibration is ruinous, it is best to control the exposure by means of the lens shutter rather than the camera shutter. When using the 'Aristophot' in the 35 mm format, the correct exposure time is best assessed empirically with the use of test films and records of film speed, lens type, aperture setting, lighting, and magnification. Through the lens metering (TTL) is available in this format with the 'Multiphot', utilizing in particular the Nikon F3 camera. However, with over-long exposures (in excess of about 1 second the readings from any type of metering system will be inadequate due to reciprocity failure, and extra time must be allowed, depending on the film type. Much macrophotography of fossils falls within the 1–15 second exposure time.

The focusing screen on the camera should be of the finely ground glass or clear glass type and focusing done at full aperture. The specimen–lens and lens–film (bellows length) distances combine to determine magnification on the negative, and at any given magnification these distances will vary according to the focal length of the lens employed. Manufacturers' handbooks normally contain graphs plotting magnification against distance for each lens. Sometimes it is desirable to produce negatives at set, whole number magnifications; this requires retention of the camera and lens in the appropriate positions and focusing by moving the specimen vertically, either by means of a heavy duty laboratory jack or a rack and pinion operated 'lift'. The specimen can be mounted by plasticine onto the jack or 'lift', the surface of which should be painted matt black to provide a contrasting background to the whitened fossil. Photographs other than those of surface sculpture should not be focused on the upper surface of the fossil but more towards the median plane of the specimen to take into account depth of field.

Lighting comprises two basic components. A directional light source, by convention shining from the northwest, is beamed at the fossil at an angle (normally low) suitable for emphasizing its relief. The shadows thus produced are partially filled in and the specimen lit overall by means of soft, diffuse, even illumination. One of the several ways of achieving the desired effect is to use an anglepoise lamp with a frosted bulb, the light strength of which is controlled by a dimmer switch, together with a fluorescent ring light (about 30 cm diameter and 60 watts) capped by a reflector (Fig. 1H). Any extraneous light should be prevented from entering the lens.

It is important to ensure that any lens used for enlarging small objects gives, in addition to sharp resolution at the plane of focus, good imaging throughout the depth of the specimen. Increased depth of field is achieved by reducing the size of the lens aperture, but beyond a certain limit (which can be empirically determined for each lens) the effect of diffraction gives progressively poorer resolution and makes it pointless to stop down further. Lenses optically corrected for the macrophotographic range, for use with the 'Aristophot' or 'Multiphot', come in several focal lengths from about 12 mm to 120 mm. Lens selection depends on the desired scale of reproduction, those with shorter focal lengths being used for greater magnifications. The original 'Aristophot' lenses, the Leitz 'Milar' and particularly the 'Summar' range, and also the later, compatible first generation 'Photar' range, give excellent results; Nikon have consistently produced four macro lenses with high resolving power for use with the 'Multiphot'. The latest, more restricted generation of 'Photar' lenses reproduce over the × 1 to × 16 range and are combined with the Leica 'R' system of