

Dinosaur nests at the sea shore

SIR — Beach deposits of the Arenisca de Arén Formation¹ (southern Pyrenees, Maastrichtian, Upper Cretaceous) are rich in dinosaur eggs and bones, distributed over an area of about 15 km² along the northern flank of the Tremp syncline (Fig. 1). At one locality (Bastús, Lleida) we have estimated the presence of up to 300,000 eggs in a rock volume \approx 12,000 m³, suggesting some kind of 'site fidelity'.

Dinosaur remains have frequently been reported from marine sediments. Efremov²

ing well-preserved dinosaur nests (Fig. 1). Farther down, the red layer passes into shore face sandstones with well-preserved, wave-induced sedimentary structures, forming part of a near-shore environment passing landward into mudflat deposits, occasionally dissected by small channels⁵. As the beach-ridge plain was built up to seaward (progradation), the dinosaurs nested in the exposed sandy sediment.

A huge number of egg shell fragments are scattered along the outcrop (Fig. 2).

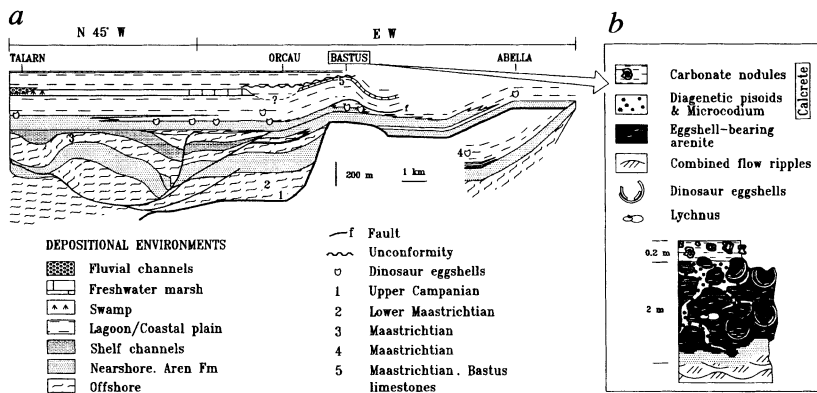


FIG. 1 a, Cross-section through the NW-prograding depositional sequence, Late Campanian and Maastrichtian in age, along the northern flank of the Tremp syncline (South Central Pyrenees, Spain). The succession formed under high terrigenous influx and relative sea-level rise. The sequence passes from coastal plain (Tremp Fm. p.p.) through strand-plain/barrier island (Arenisca de Arén Fm.) to offshore sediments. It overlies near-shore deposits of a similar sequence, which are affected by growth faults⁵. The dinosaur nests have been preserved mainly at the top of the Arenisca de Arén Formation. b, Log of the Bastús section. The matrix of the eggshell-bearing rock is a calcite-cemented, medium-to-coarse-grained arenite of mixed siliciclastic and carbonate grain composition. Carbonate grains comprise different types of worn skeletal fragments dominated by shallow-marine elements, intraclasts as well as dinosaur eggshell debris, and are generally affected by microbial micritization. This, along with the local preservation of relict dripstone and meniscus cement fabrics, suggests that lithification was initiated at the strand line (beach rock). Shoreline progradation then caused the incipiently cemented sand to become exposed to meteoric waters. Diagenesis in the freshwater phreatic environment involved selective leaching of initially aragonitic components and concomitant precipitation of new diagenetic calcite. Further diagenetic alteration before final burial of the sediment occurred in the meteoric vadose zone, as documented by the presence of a variety of petrographic features diagnostic of pedogenic calcare development. c, Aspect of the outcrop.



suggested a marine habitat for some dinosaurs, although these were later interpreted as the remains of individuals that had been washed to the sea. Chanda³ proposed a shallow-water marine environment for the dinosaur egg-bearing Lameta Formation of India, however, Shani *et al.*⁴ conclusively argued for the continental (palustrine) origin of these sediments. Thus, the Arenisca de Arén egg sites represent the first unambiguous evidence of dinosaurs nesting at a sea shore.

The top of the Arén Formation at Bastús is 2-m-thick red sandstone contain-

Other fossils include large bone fragments; tiny smashed bones, probably belonging to young dinosaurs; a fairly complete skeleton of a small lizard and land snails (*Lychnus giganteus* Repelin). As the original disposition of some nests and bones are well preserved, post-depositional transport can be excluded as a primary cause of fragmentation. Rather, the eggs were destroyed by the animals' nesting and trampling activities and later by paedogenesis (Fig. 1). The nesting probably occurred in the unconsolidated sands of the beach ridge, in a short time interval between beach abandonment

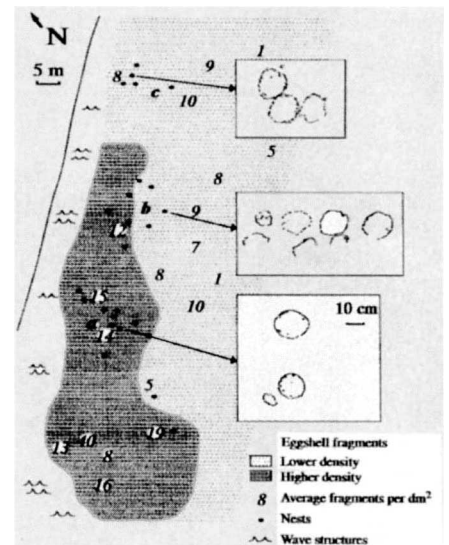


FIG. 2 Map illustrating nest distribution and eggshell density. The preserved nests, probably the last clutches, are in three main clusters. The distance between nests is 1–5 m (a), 4–6 m (b) and 3–8 m (c). The distance between some nests (1 m) is too short with respect to the presumed body size of the adult dinosaurs, suggesting that the nesting strategy is different from the colonial nesting behaviour described elsewhere⁶.

and early diagenesis (meteoric leaching, incipient lithification and paedogenesis).

The eggshell structure is tubospherulitic, and most of the preserved egg sections show subcircular contours. Some complete eggs from this locality, housed in the Freie Universität, Berlin, have a subspherical shape, with a diameter \approx 20 cm. The outcrop has yielded the remains of 24 nests arranged in three clusters on a surface \approx 6,000 m² (Fig. 2). Each nest contains 1–7 eggs, most of them having only two or three preserved eggs, representing portions of nests deposited in a hole dug in the sediment.

The Bastús nests are closely spaced but do not overlap (modal distance 2.5 m; Fig. 2). As the preservation state of the nests is generally good, some factor has precluded the destruction of previous clutches by newcomers. Some territorial behaviour may account for this feature. A high population density and/or scarcity of favourable substratum could explain the observed short distances between nests.

Using a 1-dm² grid in 20 randomly chosen areas (Fig. 2), we calculate that the eggshell material amounts to \approx 0.5% of the total rock volume. Given the volume of the sandstone body (12,000 m³) and assuming an average egg size of 20 cm diameter and 1.45 mm shell thickness, we estimate

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6. Horner, J. R. *Nature* **297**, 675–676 (1982).

that the lithic body contains the remains of some 300,000 eggs. This suggests that the area was a nesting ground, and that the dinosaurs may have returned to this same area during several reproductive seasons.

The Bastús egg site is the result of the nesting behaviour of a dinosaur population living very near the sea shore and may represent a local example of a widespread phenomenon in the Upper Cretaceous of the south-central Pyrenees.

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Codon bias targets mutation

SIR—Serine is encoded by two sets of triplets (AGC and AGT (grouped as AGY) and TCA, TCC, TCG and TCT (grouped as TCN)). We have analysed the usage of the two types of codon in human immunoglobulin variable region (V) gene segments and have found a preference for AGY codons in the complementarity-determining regions (CDRs; particularly CDR1) and for TCNs in the frameworks (Fig. 1). This bias is not a peculiarity of human immunoglobulin V genes, but also occurs in mice (not shown) and *Xenopus*¹.

Immunoglobulins undergo functional

diversification by somatic mutation, with nucleotide substitutions being introduced throughout the V-gene segment. Diversification is greatest in CDRs, the portions most strongly implicated in contacting antigen. We propose that the biased serine codon usage in immunoglobulins has evolved to help the somatic hypermutation machinery target these parts of the antibody molecule. Thus, mutations are targeted to residues that could yield increased affinity for antigen and away from places where they are more likely to destroy the structural scaffold. Consistent with this, the V genes of the T-cell receptor (TCR), where there is no evidence for functional diversification by somatic mutation, do not show a bias in their CDRs (Fig. 2).

During maturation of the antibody response, the V regions in those B lymphocytes selected by the initial antigen challenge contain many nucleotide substitutions. Cells expressing antibodies with improved binding characteristics are then selected from the population of somatically mutated daughter cells. The nucleotide substitutions are not targeted randomly. In mouse variable-region κ -chains (V_{κ} s), for example, there is intrinsic favouring² of CDR1, and hotspots of mutation have been identified that are intrinsic to the mutational process^{2,3}. The consensus [A/G G C/T A/T] has been proposed⁴ as a preferred target for mutation and, indeed, the most striking of the hotspots are often associated with AGY serine codons^{2,3}. Thus, the preponderance of AGY over TCN serines in the CDRs of germline V genes (and the converse in frameworks) suggests that the DNA sequence of germline V genes has evolved in response to selection for appropriately targeted mutability.

There are some significant exceptions to this biased codon usage. In germline V_{κ} s, a

FIG. 1 Distribution of TCN and AGY serine in immunoglobulin V segments. *a, b*, Sequences of functional human V_H genes (kindly provided by G. Cook and I. Tomlinson⁷) and V_{κ} genes⁸ have been grouped into families and, at each serine position, the number of family members containing a TCN codon is depicted above the line and an AGY codon below. The name of each family and the number of its members are given. CDRs are shaded. *c*, The ratio of serines encoded by AGY triplets to those encoded by TCN triplets is given for CDR1, CDR2 and the framework portions (in FR1, FR2 and FR3 are summed together) of human immunoglobulin (Ig) V_H and V_{κ} , and TCR V_{α} and V_{β} segments. This compilation has been made by combining all Vgene sequences for each locus. Using a large sample test for a binomial distribution, the AGY/TCN ratios in the V_H CDR1 and CDR2 and in the V_{κ} CDR1 are significantly skewed ($p < 0.03\%$) from the ratio (0.64) expected from the GenBank protein database⁹. There is slight inverted skewing of the AGY/TCN ratio in the case of the TCR CDRs, although this is of much less statistical significance.

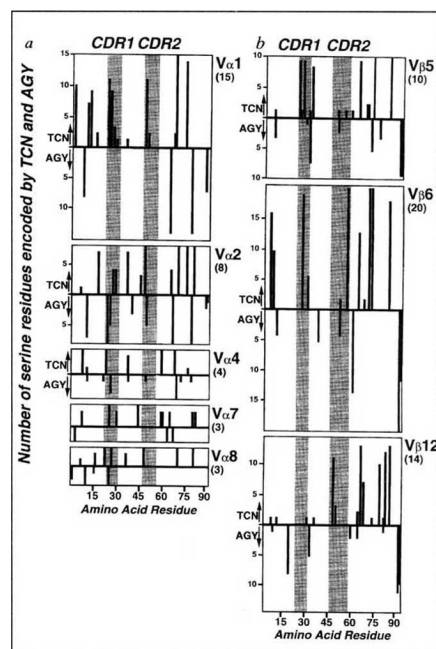
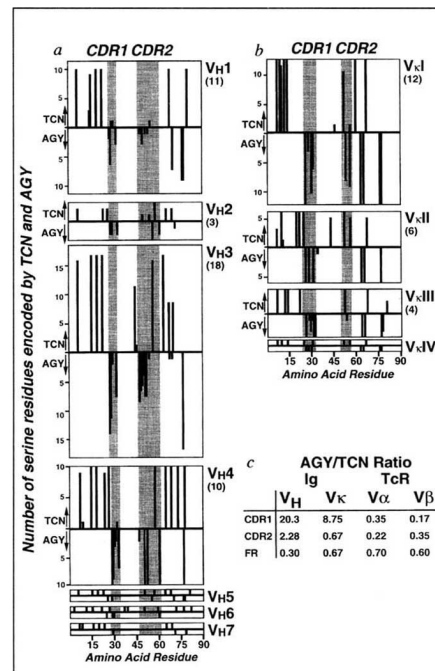


FIG. 2 Distribution of TCN and AGY serine in TCR V segments. Sequences of human TCR V_{α} and V_{β} genes (classified as in ref. 10) have been grouped into families and the serine codon usage displayed as in Fig. 1. Only the largest TCR Vgene families were used for the analysis.

TCN serine (position 52) is conserved in CDR2 presumably because it contributes to the structural integrity of the CDR loop⁵. On the other hand, V_{κ} framework position 77, a conserved AGY in human and mouse germline V_{κ} s, is one of the intrinsic mutational hotspots located outside a CDR². There is also a preference for serine AGY codons in positions at the 3' end of TCR V_{β} genes flanking the heptamer/nonamer recombination signals which may reflect a role in the gene rearrangement process.

It has been noted that serine AGY codons are more mutable than serine TCNs in protein coding sequences of humans and mice. This observation parallels the mutability of V-gene serine codons during somatic hypermutation and is consistent with them being common features of the molecular mechanism of evolutionary and somatic mutation.

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