that do not come directly from the epicenter. The generation process of such a surface wave is reproduced here from the real records, and its physical mechanism is interpreted as refraction to compensate for a wavefront discontinuity.

To confirm the interpretation of the ground motion pattern, we performed ray tracing for Love waves in models of the structure in the Kanto basin (*9*, *10*). The S-wave velocities in the basement and sedimentary layers are estimated from P-wave data because they are not well constrained from the previous studies. The model for ray tracing uses the local-mode approximation (*11*), employing a 100 × 100 grid with a spacing of 2.00 km (E/W) and 1.75 km (N/S). A horizontally layered structure is retrieved from the three-dimensional (3D) structural model at each point, and the phase velocity of the fundamental mode of the Love wave is then calculated for each grid point at a period of 8 s. We carried out ray tracing in this phase velocity distribution with the shooting method (*12*). The calculated rays were traced to 40 s after the origin time of the earthquake (Fig. 2C). Because rays are defined as normal to a wavefront, the tips of the rays indicate the theoretical wavefronts at 40 s, which agree well with the observed wavefronts.

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Evolutionary Exploitation of Design Options by the First Animals with Hard Skeletons

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The set of viable design elements available for animals to use in building skeletons has been fully exploited. Analysis of animal skeletons in relation to the multivariate, theoretical "Skeleton Space" has shown that a large proportion of these options are used in each phylum. Here, we show that structural elements deployed in the skeletons of Burgess Shale animals (Middle Cambrian) incorporate 146 of 182 character pairs defined in this morphospace. Within 15 million years of the appearance of crown groups of phyla with substantial hard parts, at least 80 percent of skeletal design elements recognized among living and extinct marine metazoans were exploited.

Fundamentally different strategies for constructing hard skeletons emerged in the evolution of each animal subkingdom (*1*) now recognized on the basis of ribosomal RNA sequences (*2*, *3*) and patterns of early embryonic development (*4*). The Ecdysozoa (*2*) consist largely of animals that molt their skeletons periodically, as the name implies. The Lophotrochozoa (*3*, *5*) include protostomes with external skeletons that typically grow by accretion. Internal skeletons that can be remodeled, especially in more derived taxa, are characteristic of the Deuterostomia. The Skeleton Space (*6*, *7*) is a theoretical morphospace that provides a framework, independent of time and the characters of any given group of organisms (*8*), in which to assess rates and patterns of exploitation of morphospace by these animals. We use it here to analyze the initial, early Cambrian emergence of hard parts that complemented, and to varying degrees replaced, hydrostatic skeletons of metazoans that evolved to larger sizes.

A span of 40 million years (*9*) embraces the appearance of the first small, simple shells that may have been secreted by metazoans and the subsequent exuberant diversity of Chengjiang (*10*) and the Burgess Shale (*11*–*14*). This is not so short a time for an evolutionary "explosion." However, the proliferation of animals with the well-differentiated hard parts characteristic of specific metazoan phyla was largely restricted to the last 15 million years of this interval (*15*). We address three issues: How rapidly were the opportunities of skeletal morphospace taken up in this evolutionary radiation? What patterns of change over time are expressed in the skeletal designs of these early metazoans? To what extent were changes in the genetic basis of pattern formation, the expansion of taxonomic diversity, and the exploitation of design options concurrent?

Our theoretical morphospace is based on seven general properties of animal skeletons or their components. Each has two, three, or four broadly defined states, illustrated here schematically and by examples drawn from animals of the Burgess Shale, of early Middle Cambrian age, in British Columbia (Fig. 1):

1) A skeleton may be internal, as in the elephant, or external, as in the lobster.

2) The skeleton may be composed of rigid material like vertebrate bone, or it may be flexible like the notochord of the lancet.

3) A skeleton may consist of one element, as in most snails; two, like the bivalved shells of animals evolved independently in several different classes and phyla; or multiple elements, as in crinoids and crabs.

4) In shape, the parts of skeletons are essentially rods, plates, or solids. Rods define and support spatial frameworks, like the scaffolding formed by sponge spicules, or they are used as levers, like vertebrate limb bones. Interlocking plates, like those of tortoise shells and sand dollars, enclose space. A cone is a folded plate; we set these apart because they are so widely used as external skeletons. Three-dimensional solids are typically machine parts, like ankle bones, vertebrae, and teeth.

5) Growth of a skeleton that must function continuously as it develops can be accomplished by accretion, as in molluscan shells; by molting and replacement; by the addition of units to a modular structure, as in colonial organisms; or by the sort of remodeling that makes ball-and-socket joints possible in mammals.

6) Most skeletal parts grow in place, where they function, but some are prefabricated and then moved into working position, such as shark's teeth.

7) Multiple components are integrated in

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four basic ways. They may stiffen soft material, without direct contact with one another. They may be linked by joints, which provide flexibility and mechanical advantage. They may be sutured or fused together, like the bones of the human skull. They may overlap, providing a sliding articulation like that between tergites, which allows a woodlouse or trilobite to roll up.

The Skeleton Space constitutes a system of theoretical designs against which the skeletal parts of living and extinct organisms can be compared. Each structure is characterized by a seven-letter formula that defines its form. The skeleton of the human finger has the formula ACWGNXQ. It consists of multiple, internal, rigid, jointed rods that are remodeled as they grow in place. Given 2 × 2 × 3 × 4 × 4 × 2 × 4 combinations of characters, the Skeleton Space embraces 1536 possible designs. To simplify the analysis of this large number of cases, we compare the skeletons used by different groups of organisms in a matrix of all possible pairs of character states.

Thomas and Reif (*7*) have shown that more than half of these character pairings are abundantly used, generally by animals in several phyla; two-thirds of these pairings are common. Only logically or functionally implausible combinations are unrecognized among living and extinct animals. To determine how rapidly this exploitation of available morphospace took place, we sought to establish a benchmark early in metazoan evolution. We elected to assess the occurrence of design options among animals found in the Burgess Shale. The fossils are exceptionally well preserved, detailed descriptions of most taxa are available, and this marine fauna has long been regarded as the epitome of early metazoan life (*12, 14*).

We determined the design formula for each kind of skeletal element present in 104 well-documented Burgess Shale genera with hard

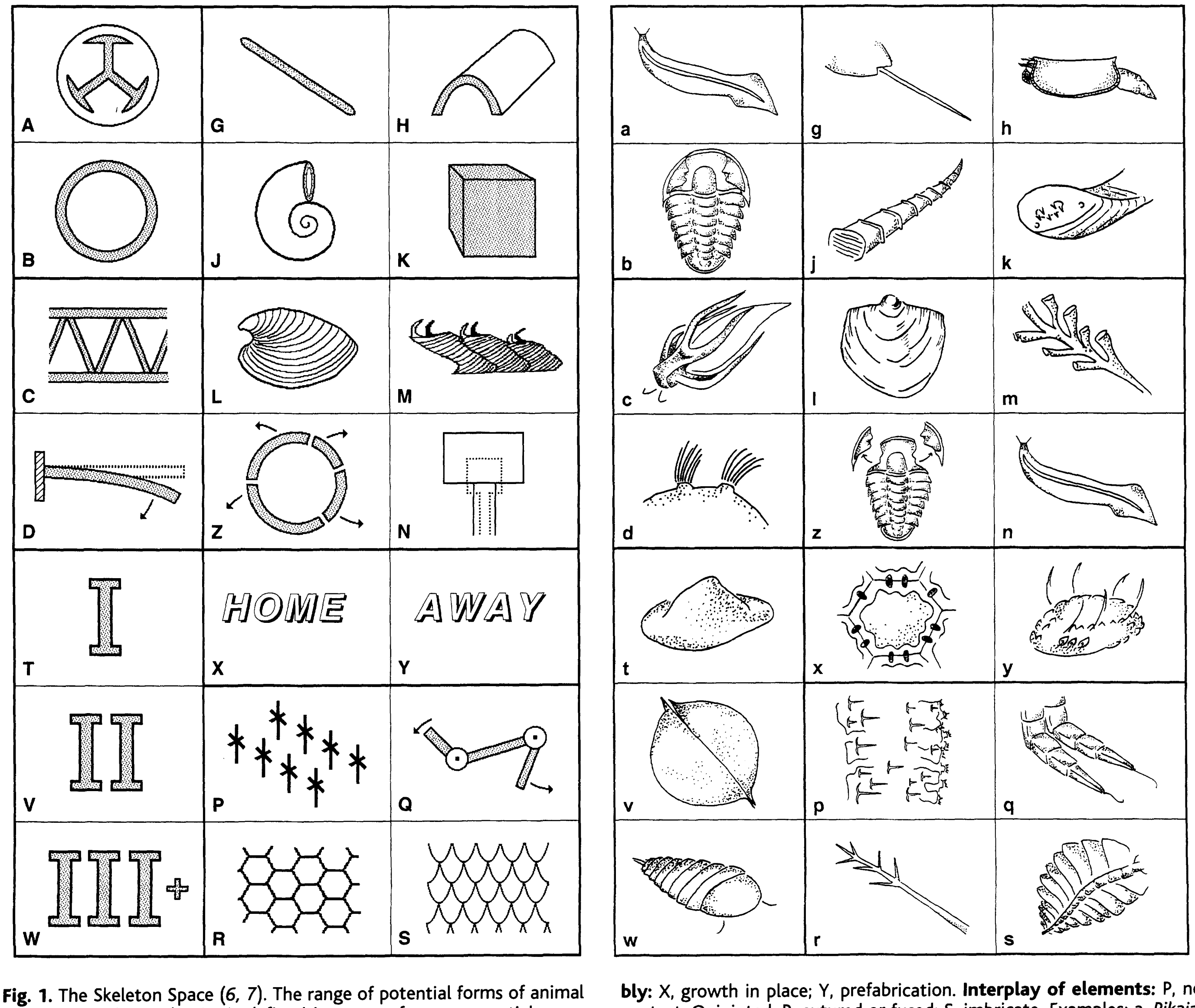

Fig. 1. The Skeleton Space (*6, 7*). The range of potential forms of animal skeletons or their subunits is defined in terms of seven essential properties, each with two to four possible states, yielding a total of 21 variables. Graphics characterizing parameters of the skeletal morphospace are shown on the left. Examples of skeletal elements from the Burgess Shale fauna representing each descriptor are illustrated on the right. **Situation:** A, internal; B, external. **Material:** C, rigid; D, flexible. **Number:** T, one element; V, two elements; W, more than two elements. **Shape:** G, rods; H, plates; J, cones; K, solids. **Growth:** L, accretionary; M, serial units + branching; Z, replacement/molting; N, remodeling. **Assembly:** X, growth in place; Y, prefabrication. **Interplay of elements:** P, no contact; Q, jointed; R, sutured or fused; S, imbricate. Examples: a, *Pikaia*, notochord; b, *Elrathia*, exoskeleton; c, *Marrella*, paired spines; d, *Burgessochaeta*, setae; t, *Scenella*, shell or velum; v, *Isoxys*, bivalved carapace; w, *Emeraldella*, thoracic tergites; g, *Burgessia*, telson; h, *Branchiocaris*, carapace; j, *Haplophrentis*, shell; k, *Odontogriphus*, teeth; l, *Paterina*, shell; m, *Chaunograptus*, thecae; z, *Elrathia*, exoskeleton; n, *Pikaia*, notochord; x, *Gogia*, plates; y, *Wiwaxia*, sclerites or paleae; p, *Diagoniella*, spicules; q, *Leanchoilia*, great appendages; r, *Saratrocercus*, fused spines; s, *Anomalocaris*, lateral lobes.

parts. These formulas are listed in a database that includes comments and quotations from the primary literature, documenting our assessment of each skeletal structure and any uncertainties that may be associated with our interpretation of its characteristics (*16*). Frequencies of the pairs of character states represented among these design formulas were tabulated (Fig. 2A). Some frequencies greatly exceed the number of taxa because different parts of an organism may have characters in common. To compare these data with a compilation for all living and extinct marine organisms (*7*), we arbitrarily consider a character pairing to be abundant if it is represented in ≥50 and common if it occurs in ≥10 skeletal elements of the Burgess Shale animals. Differences between the two data sets are represented graphically (Fig. 2B).

A

	Internal	External	Rigid	Flexible	One element	Two elements	> two elements	Rods	Plates	Cones	Solids	Accretionary	Unit/serial	Replace/molt	Remodeling	In place	Prefabrication
Rigid	37	301															
Flexible	9	27															
One element	2	95	88	8													
Two elements	0	74	67	6													
> two elements	44	161	183	22													
Rods	35	122	141	15	16	31	110										
Plates	9	181	169	20	76	37	77										
Cones	0	12	11	1	5	6	1										
Solids	2	14	16	0	0	0	16										
Accretionary	11	37	44	4	14	11	23	16	17	12	3						
Unit/serial	0	4	4	0	F	0	4	2	2	0	0						
Replace/molt	F	264	245	17	78	58	128	100	151	0	12						
Remodeling	1	0	0	1	1	0	0	1	0	0	0						
In place	46	328	336	36	97	74	203	157	188	12	16	48	3	264	1		
Prefabrication	0	1	1	0	0	0	1	0	1	0	0	0	1	0	F		
No contact	21	26	32	15	7	2	38	34	9	3	1	16	1	7	1	47	0
Imbricate	1	52	53	0	9	7	37	0	49	4	F	6	2	43	0	52	1
Jointed	3	129	128	4	49	29	54	52	74	2	3	6	0	122	0	132	0
Fused, sutured	21	119	124	15	29	36	75	71	54	3	12	20	1	91	0	140	0

B

	Internal	External	Rigid	Flexible	One element	Two elements	> two elements	Rods	Plates	Cones	Solids	Accretionary	Unit/serial	Replace/molt	Remodeling	In place	Prefabrication
Rigid	o																
Flexible	O	o															
One element				o													
Two elements																	
> two elements	o																
Rods	o	•	•	•	•	●											
Plates	O			o	●												
Cones	o	o	o		O	O											
Solids	o		o		o		o										
Accretionary	o	o	o	o	o	o	o		o	o	o						
Unit/serial	**O**	O	O	O			O	O	O	**O**	o						
Replace/molt				o	●	•				O	o						
Remodeling	O	O	O	o		o	**O**		**O**	O	o						
In place	o			o						o		o	O		O		
Prefabrication	o	o	o	o	o		O	o	•		O	o	o	O			
No contact		•		•	O	•	o	•		O		o	o	•	•	o	o
Imbricate							o	o	o			o		•			•
Jointed	O			O		o				O		O	**O**		O		o
Fused, sutured	o	●		•		•					•	o	O	●	o		O

Fig. 2. Exploitation of the Skeleton Space by animals of the Burgess Shale. (**A**) Numbers of skeletal elements in 104 genera with hard parts that exhibit each pair of characteristics. (**B**) Differences in frequencies of character pairs between the Burgess Shale and later marine faunas. Among skeletons of all living and extinct marine animals, the frequency of each character pair was scored as absent (0), present (1), common (2), or abundant (3) by Thomas and Reif (7). Here, a character pair is considered to be abundant if its frequency is ≥50, and common if it is represented in ≥10 skeletal elements of the Burgess Shale animals. Differences in scores between the two data sets are shown graphically: bold open circles, –3; open circles, –2; small open circles, –1; small solid circles, +1; large solid circles, +2.

A large proportion of the design options available for making animal skeletons is represented in the Burgess Shale fauna. Within 15 million years of the appearance of metazoans sufficiently large and well-differentiated to appear in the fossil record (*17*), at least 146 of 182 of the options presented by the Skeleton Space had been exploited (Table 1). Put another way, >80% of character pairs represented among the design elements observed in skeletons of all living and extinct animals were used within the first 6% of the time span of metazoan evolution.

The most striking features of this initial radiation into skeletal morphospace are the high frequencies of single-element rods and multi-element, broadly metameric exoskeletons. These reflect the abundance of sponges, arthropods, and arthropod-like taxa of uncertain affinity. The skeletal structures of these taxa are linked to the simplest possible strategies for increasing body size. They support sessile structures with limited cellular integration in sponges, and they house modular animals with varying degrees of anterior-posterior differentiation in the arthropod-like forms. In contrast, three-dimensional solids and structures whose growth involves continuous remodeling are underrepresented, reflecting the limited diversification at this time of animals with internal skeletons.

The small number of cones in this fauna is an artifact of facies and preservation. The Burgess Shale accumulated on a muddy sea floor at the foot of a steep carbonate ramp (*18*). Contemporaneous and earlier small shelly faunas favored shallow platform environments, as did archaeocyathid reefs of the Lower Cambrian. Preliminary data for the Tommotian stage (~530 million years ago), based on faunas of the Lena River region in Siberia (*19*), show that at least 89 character pairs were already used by this time. The low frequency of structures that grow by serial addition of elements or individuals reflects the absence of colonial cnidarians with hard parts from faunas of this age. This may reflect a diversity of zooplankton, on which cnidarians feed, that was still limited at this time [(*20*), but see (*21*)].

These results lead to the following conclusions:

1) A large proportion of the available morphospace was exploited very rapidly,

Table 1. Comparative exploitation of the Skeleton Space.

Group	Designs used
Tommotian metazoans	89
Burgess Shale metazoans	146
Cnidaria (living and extinct)	89
Mollusca (living and extinct)	122
Arthropoda (living and extinct)	90
Vertebrata (living and extinct)	133
All marine metazoans	178
All living and extinct animals	180

once animals with skeletons appeared on the evolutionary stage. This is consistent with the later rapid exploitation of more narrowly defined morphospaces, documented for crinoids and other taxa, in the Ordovician (*22*).

2) Predominance of serial segmentation and design elements broadly comparable with those of arthropods indicates strong, early selection for rapid duplication and subsequent specialization of structural subunits (*7, 23*).

3) The disparate types of skeletal elements that occur together in some organisms (*14*) suggest a low level of morphological integration that would later be streamlined by more precise gene regulation (*24*). However, even the most seemingly bizarre taxa, such as *Anomalocaris* (*25, 26*), do not have more distinct types of skeletal elements than contemporary arthropods assigned to the Crustacea. Some Burgess Shale crustaceans already had skeletons as structurally specialized as those of their close living relatives (*27*).

4) Skeletons of terminal Proterozoic and earliest Cambrian animals (*28*) consisted of scales and spicules, weakly mineralized shells, and structures built largely with radiating aggregates of crystals. The rapid, late Early Cambrian exploitation of opportunities presented by the Skeleton Space was facilitated by the parallel evolution of complex, biologically tailored multilayer composites constructed from a variety of organic and inorganic materials. Recurring features of these structural materials suggest that their development is controlled by a common regulatory network of genes that was already established in ancestral stem-group bilaterians (*29*).

5) Internal skeletons and growth by remodeling are uncommon among the Burgess Shale animals. These options have since been extensively exploited by vertebrates, after two duplications of the *Hox* gene cluster (*30*). No causal relation between regulatory functions of *Hox* genes and the emergence of internal skeletons has yet been established. However, the duplication of high-level regulatory genes would have made it possible to bypass constraints set by established lower-level linkages, opening the way for the establishment of a novel *Bauplan* with more independent controls over the development of local structural units. Basal agnathan chordates are now known from the Lower Cambrian of Chenjiang (*31*), so the fossil record is consistent with two phases of vertebrate *Hox* duplication, one preceding the early Cambrian radiation and the other that could come much later, in the Ordovician, if it was associated with the development of bony endoskeletons.

After the divergence of protostomes and deuterostomes, a major clade within each group went in for active locomotion, evolving strong anterior-posterior differentiation and jointed-lever skeletons. In arthropods, these emerged as exoskeletons; in vertebrates, they are predominantly internal. These distinctive types of skeletal development reflect prescriptive patterns of embryogenesis that appear to have evolved long before hard skeletons emerged (*29*). Convergent patterns of evolutionary diversification in the two metazoan clades with the most varied modes of life reflect common geometric constraints of growth process and mechanical function on skeletons with radically different origins.

Viable design options are fixed point attractors that actual skeletons must approach, leading to the evolutionary convergence emphasized by Conway Morris (*14*). Real animals evolve as strange attractors, far-from-equilibrium systems with combinations of properties that are unpredictable in detail. Rapid exploitation of the Skeleton Space by early Cambrian animals confirms that evolution follows rational and consequently predictable patterns, as Niklas (*32*) and McGhee (*8*) have shown for land plants and a variety of animals in the context of analogous theoretical morphospaces.

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Distinct Classes of Yeast Promoters Revealed by Differential TAF Recruitment

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The transcription factor TFIID contains the TATA box binding protein (TBP) and multiple TBP-associated factors (TAFs). Here, the association of TFIID components with promoters that either are dependent on multiple TAFs ($\mathrm{TAF_{dep}}$) or have no apparent TAF requirement ($\mathrm{TAF_{ind}}$) is analyzed in yeast. At $\mathrm{TAF_{dep}}$ promoters, TAFs are present at levels comparable to that of TBP, whereas at $\mathrm{TAF_{ind}}$ promoters, TAFs are present at levels that approximate background. After inactivation of several general transcription factors, including TBP, TAFs are still recruited by activators to $\mathrm{TAF_{dep}}$ promoters. The results reveal two classes of promoters: at $\mathrm{TAF_{ind}}$ promoters, TBP is recruited in the apparent absence of TAFs, whereas at $\mathrm{TAF_{dep}}$ promoters, TAFs are co-recruited with TBP in a manner consistent with direct activator-TAF interactions.

TFIID is a general Pol II transcription factor (GTF) that initiates transcription complex assembly by binding to the TATA box through its TBP subunit. TFIID has also been implicated in the mechanism of action of certain promoter-specific activator proteins (activators) (*1*). Whereas TBP is a general factor, TAFs are highly promoter selective, which raises the