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Report

Rates of Phenotypic and Genomic Evolution during the Cambrian Explosion

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Summary

The near-simultaneous appearance of most modern animal body plans (phyla) ~530 million years ago during the Cambrian explosion is strong evidence for a brief interval of rapid phenotypic and genetic innovation, yet the exact speed and nature of this grand adaptive radiation remain debated [1–12]. Crucially, rates of morphological evolution in the past (i.e., in ancestral lineages) can be inferred from phenotypic differences among living organisms—just as molecular evolutionary rates in ancestral lineages can be inferred from genetic divergences [13]. We here employed Bayesian [14] and maximum likelihood [15] phylogenetic clock methods on an extensive anatomical [16] and genomic [17] data set for arthropods, the most diverse phylum in the Cambrian and today. Assuming an Ediacaran origin for arthropods, phenotypic evolution was ~4 times faster, and molecular evolution ~5.5 times faster, during the Cambrian explosion compared to all subsequent parts of the Phanerozoic. These rapid evolutionary rates are robust to assumptions about the precise age of arthropods. Surprisingly, these fast early rates do not change substantially even if the radiation of arthropods is compressed entirely into the Cambrian (~542 mega-annum [Ma]) or telescoped into the Cryogenian (~650 Ma). The fastest inferred rates are still consistent with evolution by natural selection and with data from living organisms, potentially resolving "Darwin's dilemma." However, evolution during the Cambrian explosion was unusual (compared to the subsequent Phanerozoic) in that fast rates were present across many lineages.

Results

The abrupt appearance of most modern animal body plans (often ranked as phyla and classes) over half a billion years ago is one of the most important evolutionary events after the origin of life [1–5]. This initial diversification of major metazoan groups—termed the Cambrian explosion—is suggested by the fossil record to have occurred largely in the Terreneuvian (~542–521 mega-annum [Ma]) [1–3], although molecular analyses suggest a cryptic Precambrian interlude of up to several hundred million years [6–9]. An emerging consensus favors an intermediate interpretation closer to the first scenario: trace fossil, microfossil, and biomolecular evidence has slightly extended the stratigraphic record of some metazoan groups [3, 4, 10], while recent studies employing molecular dating have typically greatly reduced the postulated cryptic Precambrian branches between phyla [4, 11, 12]. Many other fundamental aspects of this event remain uncertain, notably the relative importance of environmental, genetic, developmental, and ecological triggers [1–5]. Morphological evolution is also widely postulated to be very rapid during the Cambrian explosion [1–3]. A relatively short Precambrian prelude dictates this pattern, while even a lengthy "phylogenetic fuse" can be interpreted to be consistent with it: phyla could have diverged deep in the Precambrian but still acquired many novelties simultaneously across the early Cambrian [8, 18] (though it is hard to interpret innovations shared by multiple phyla as evolving in the Cambrian under the second scenario). Darwin suggested that the sudden appearance of a range of advanced animals in the Cambrian was difficult to reconcile with gradual evolution by natural selection [2, 4], as they would have required a lengthy period of Precambrian evolution. This view has been echoed by subsequent workers arguing for a lengthy but poorly preserved prelude [6–8]. A much shorter cryptic period would be required if rates of evolution in the late Precambrian and early Cambrian could be demonstrated to be substantially elevated. However, precise estimates of rates of evolution during the Cambrian explosion remain elusive, as the patchy stratigraphic record during this pivotal interval precludes direct paleontological estimates of evolutionary rates.

Arthropods are the exemplar group for investigating questions about such macroevolutionary rates and patterns. They are the most abundant and diverse phylum in the early Paleozoic, have very complex preserved morphologies, and occupied extensive morphospace by the middle Cambrian [1, 19, 20]. However, rapid early expansion of morphospace does not demonstrate faster early evolution. Steady evolutionary rates coupled with strong constraints would also produce this pattern: morphospace occupation would increase initially before plateauing once constraints are reached, but lineages could maintain their evolutionary rates even after morphospace is fully occupied (by moving around within this permitted morphospace). Thus, although occupation of morphospace and lineage-specific evolutionary rates are often correlated, they represent independent concepts. Although studies have quantified morphospace occupation in the Cambrian [19, 20], no analyses have directly quantified the rate of anatomical change during the Cambrian explosion and compared it with subsequent evolution. Tracking changes in individual fossil lineages across stratigraphic sequences is problematic, as most continuous sequences spanning substantial durations typically preserve only hard external parts, and the small number of absolute dates for the Cambrian means that temporal resolution can be obtained to only within a few million years [21]. Burgess Shale-type Lagerstätten preserving soft tissue are patchily distributed across time and space and still retain only a fraction of the anatomy observable from living animals; in arthropods, the most complete fossils preserve less than one-third of the anatomical characters used to infer evolutionary relationships among living forms [22]. Similarly, there have been few attempts to quantify rates of genomic evolution in the Cambrian: pioneering efforts based on short sequences then available suggested elevated

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rates [23, 24] but employed problematic methods that could have generated such patterns even on rate-constant data [25, 26].

We here simultaneously infer rates of phenotypic and genomic change in arthropods during the Cambrian explosion and subsequent Phanerozoic, using a novel approach that exploits (1) the extensive phenotypic and genomic data available for living arthropods, (2) the calibration information available from the rich arthropod fossil record, and (3) adapting molecular clock methods for use on both genetic and morphological data (see the Supplemental Experimental Procedures online for detailed methods, and see Data Set S1 online for data sets). Given DNA sequences from living taxa, and (ideally multiple) calibration points, there are numerous phylogenetic dating methods that can estimate evolutionary relationships and divergence dates. For each branch, such methods simultaneously infer both duration (length in time units) and change (length in molecular substitutions), thus directly computing evolutionary rates. Notably, "relaxed clock" methods [13] can reveal how rates have changed with time by permitting rates of molecular evolution to vary across branches, both internal (between two nodes, always extinct) and external (leading to a tip, which is typically extant). Such approaches have been recently applied to morphological data [27, 28] but have not been used to explicitly address the issue of past rates of evolutionary change. We here analyze an extensive data set of arthropods, consisting of 395 phenotypic characters [16] and 62 protein-coding genes [17, 29], with 20 calibration points taken from the fossil record (Table S1). The combined data were analyzed using Bayesian relaxed-clock methods in BEASTMC3 [14] to simultaneously estimate tree topology, divergence dates, and morphological and molecular evolutionary rates across branches (and thus across time). Phenotypic data were allowed to influence inferred tree topology and divergence dates, as such data, even in combination with genomic data, can be crucial for reconstructing topology and thus relative and absolute divergence times [30]. Unlike other recent studies [4, 9, 11, 12], which estimated divergence dates, our study explicitly aimed to estimate how rates of evolution varied through time. We also tested the sensitivity of these results to analytical methods (by using maximum likelihood to infer tree topology [31] as well as divergence dates and evolutionary rates [15]), to data (by analyzing the molecular data alone), and to topology (by enforcing novel arthropod clades recently proposed [32]). We also test robustness to calibration assumptions by randomly deleting internal calibrations, as well as employing a range of root age constraints for panarthropods (hard bounds of <542 to <700 Ma, as well as soft bounds) that encompass the majority of recent age estimates [4, 9, 11, 12].

The topology of the dated arthropod trees (Figures 1 and 2) is broadly consistent with the trees obtained from the same nuclear genes alone across a broader sample of exemplars [9, 12, 17, 29, 32] as well as from other extensive molecular and combined data sets [11, 16, 33]. The relative amounts of molecular and phenotypic evolutionary change across the tree (Figures 1B and 1C) are also consistent with other analyses (e.g., [16, 17, 29]): most basal branches of arthropods exhibit substantial molecular and phenotypic change (many synapomorphies). However, a striking pattern involves the time frame for these basal arthropod divergences, and attendant rates of molecular and phenotypic evolution, under all root age constraints broadly consistent with the fossil record [1–5, 10] and the majority of recent molecular studies [4, 11, 12] (i.e., 542–650 Ma). The first occurrence of many advanced arthropod clades in the Cambrian, as underscored by recent discoveries of several crustacean lineages [34] (Table S1), implies that the major arthropod clades all diverged and evolved within a brief time window (~40 million years [Myr]; Figure 1A). This compresses into a very narrow interval the extensive molecular (Figure 1B) and phenotypic (Figure 1C) changes that occurred on the branches leading to euchelicerates, myriapods, mandibulates, pancrustaceans, oligostracans, vericrustaceans, and miracrustaceans (crown ages all >500 Ma). Accordingly, rates of evolution in most early arthropod lineages are substantially faster than rates for the subsequent Phanerozoic (Figures 2A, 2D, 3A, and 3D). The fastest molecular rates occur in the branches leading to Arthropoda and Pancrustacea (>10× average subsequent rates); the fastest phenotypic rate occurs in Mandibulata (>16×). The average rate of phenotypic evolution in early Cambrian lineages (0.561% per million years [pMyr]) is ~4× the average rate in subsequent lineages (0.136% pMyr; Tables S2 and S3; Figure S3). Similarly, the average Cambrian rate of molecular evolution (0.117% pMyr) is ~5.5× the average subsequent rate (0.022% pMyr); the latter rate is highly consistent with rates inferred for conservative nuclear genetic data in modern invertebrates [23, 24, 35].

These rate estimates are robust to a wide range of root age assumptions (Table S3). Surprisingly, early rates increase only slightly under an extreme Cambrian explosion scenario, where maximum root age is tightened to 542 Ma and basal arthropod divergences are compressed into <10 Myr within the lowermost Cambrian. Early rates also remain highly elevated if root age is relaxed to 650 Ma, resulting in a "long fuse," where basal arthropod divergences are telescoped across >100 Myr. Panarthropods are unlikely to be younger than 542 Ma (when there are already putative arthropod trace fossils; Table S1) or older than 650 Ma (which exceeds many recent molecular estimates for the age of panarthropods [4, 11, 12] and the age of the oldest fossil evidence not only for arthropods but for any animals [3, 4, 10]). Placing a soft bound on root age reveals that, in order to reduce early Cambrian evolutionary rates to subsequent levels, one has to assume that panarthropods originated ~940 Ma (Table S3).

These rate patterns also persist but are slightly dampened (Table S3) if the phylogeny is constrained to conform to an alternative recent phylogeny of arthropods in which pancrustaceans form two primary clades [32]. The pattern of elevated early rates also holds in analyses in which tree topology and branch lengths are based solely on molecular data (Figure 4), if the internal calibrations are greatly relaxed (e.g., by deleting 50% of the calibrations) and if rates are compared across a consistent interval (50 Myr sliding window; Table S3B).

Maximum likelihood analysis (Supplemental Experimental Procedures SI_12), using solely molecular data to first infer only topology (using RAxML) and then dating this topology (using r8s), retrieves a very similar chronogram (Figure S2C) and very similar patterns and rates of evolution (Figures 2B, 2E, 3B, and 3E; Table S3); average rates of phenotypic and molecular evolution after the early Cambrian are within 5% of Bayesian estimates. Phenotypic traits diverge at 1.17% pMyr in the early Cambrian, ~8× the average rate for the remainder of the Phanerozoic (0.139% pMyr); molecular divergence is 0.204% pMyr, ~9× the subsequent rate (0.021%).

All of these sensitivity analyses are discussed in Supplemental Experimental Procedures SI_1–SI_12, along with corrections for other potential biases: oversampling of basally

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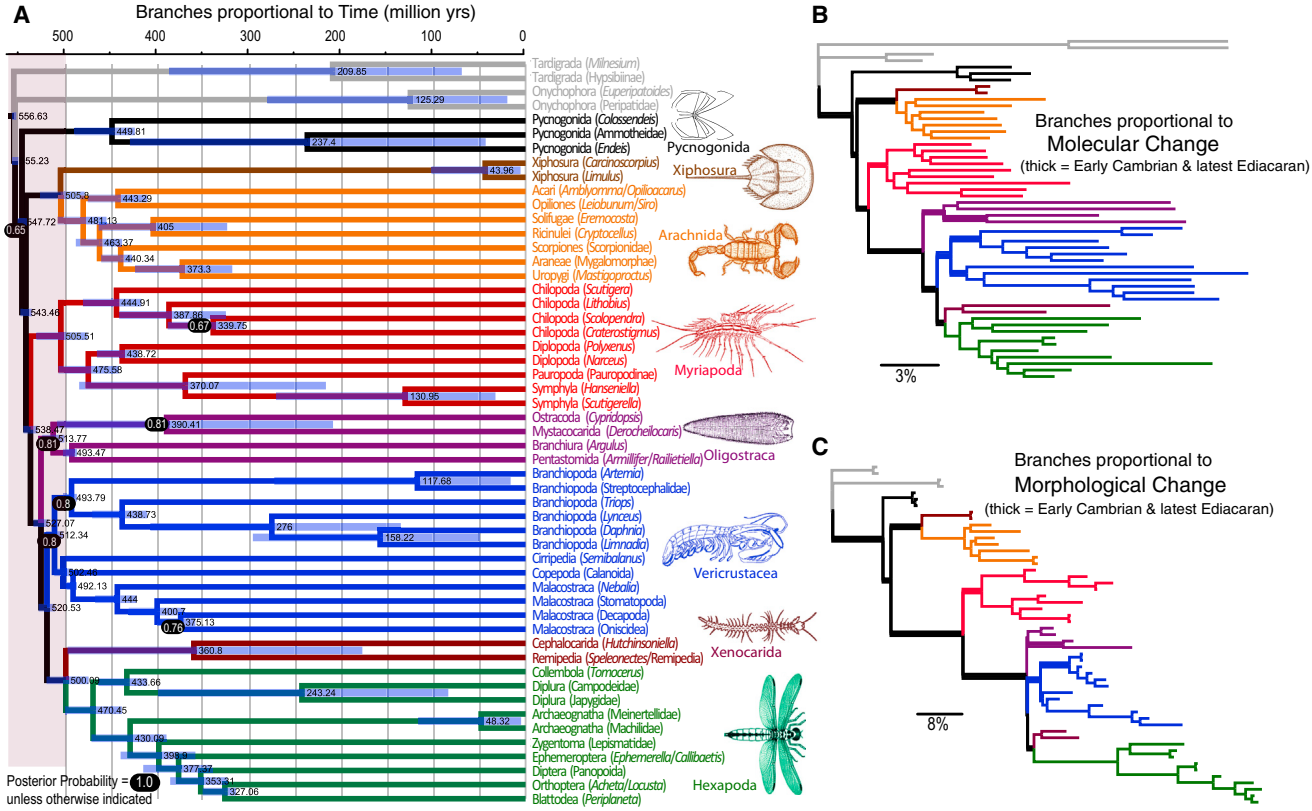

Figure 1. Arthropod Lineages during the Cambrian Explosion Are Short in Duration yet Undergo Large Amounts of Phenotypic and Molecular Change

Bayesian (BEASTMC3) analysis of 395 phenotypic characters (Supplemental Experimental Procedures SI_2) and 62 nuclear genes (Supplemental Experimental Procedures SI_3). Branches and taxa in these three trees are in identical order and have the same color coding. Note the large amount of change indicated by the long bold branches in (B) and (C), and the corresponding short durations of these branches in (A). For details of specimen illustrations, see Supplemental Experimental Procedures SI_14.

(A) Tree with branch lengths and divergence dates in terms of time; pink shading highlights the period before the late Cambrian (>500 Ma), during which most high-level ("phylum" and "class") diversity appears in the animal fossil record. Blue bars denote 95% highest posterior densities (HPDs) for divergences, which are often large due to extreme rate heterogeneity.

(B) Tree with branch lengths proportional to molecular change; bold denotes branches with ages > 500 Ma (branch age = midpoint of upper and lower node).

(C) Tree with branch lengths proportional to phenotypic change; bold denotes branches with ages > 500 Ma.

changing traits (and undersampling of unique derived traits) in the morphological data, saturation and/or underparameterization of molecular sequence data, and parameter uncertainty associated with chronologically short Cambrian branches.

Discussion

This study provides the first explicit estimates of rates of both phenotypic and molecular evolution during the Cambrian explosion, permitting illuminating comparisons with later Phanerozoic rates. The 4- and 5.5-fold increases in phenotypic and molecular evolutionary rates respectively provide quantitative support for the widespread view that evolutionary rates were elevated during the Cambrian explosion. Notably, both the patterns and magnitude of the rate elevations are strikingly similar for two very different suites of characters (one set dominated by anatomical traits, the other consisting of protein-coding nuclear genes). This is most consistent with proposed drivers that could directly affect these two disparate sets of features (and by implication most other systems), such as ecological opportunism coupled with a more complex fitness landscape, which might have unleashed latent evolutionary capabilities [4, 8], or smaller body size and shorter generation time in basal metazoan (including arthropod) lineages [35]. However, the results offer less support for scenarios that do not necessarily entail elevated rates across both morphology and housekeeping genes: for example, that the explosion was driven largely by changes in gene regulation (e.g., [18, 36]) or by evolutionarily labile developmental systems that congealed after the Cambrian (e.g., [37]). Such scenarios do not predict that genes involved in basic metabolic and cellular processes common to all panarthropods should have faster evolutionary rates in earlier panarthropod lineages.

The elevated Cambrian rates of phenotypic evolution are unlikely to be an artifact of oversampling of characters changing on early arthropod branches, for two reasons. First, the data set explicitly surveyed phenotypic characters changing at all levels in the phylogeny, not only those occurring on early, basal branches (see Supplemental Experimental Procedures SI_1). Second, the molecular data exhibit an almost identical Cambrian rate burst yet cannot suffer such ascertainment bias (because gene sequencing samples every single nucleotide in a gene region, not just sites changing on particular branches). The congruence of the morphological pattern with the molecular pattern suggests that the morphological pattern is biologically meaningful rather than artifactual.

These findings also directly answer a powerful argument for a lengthy Precambrian phylogenetic fuse. One of the few

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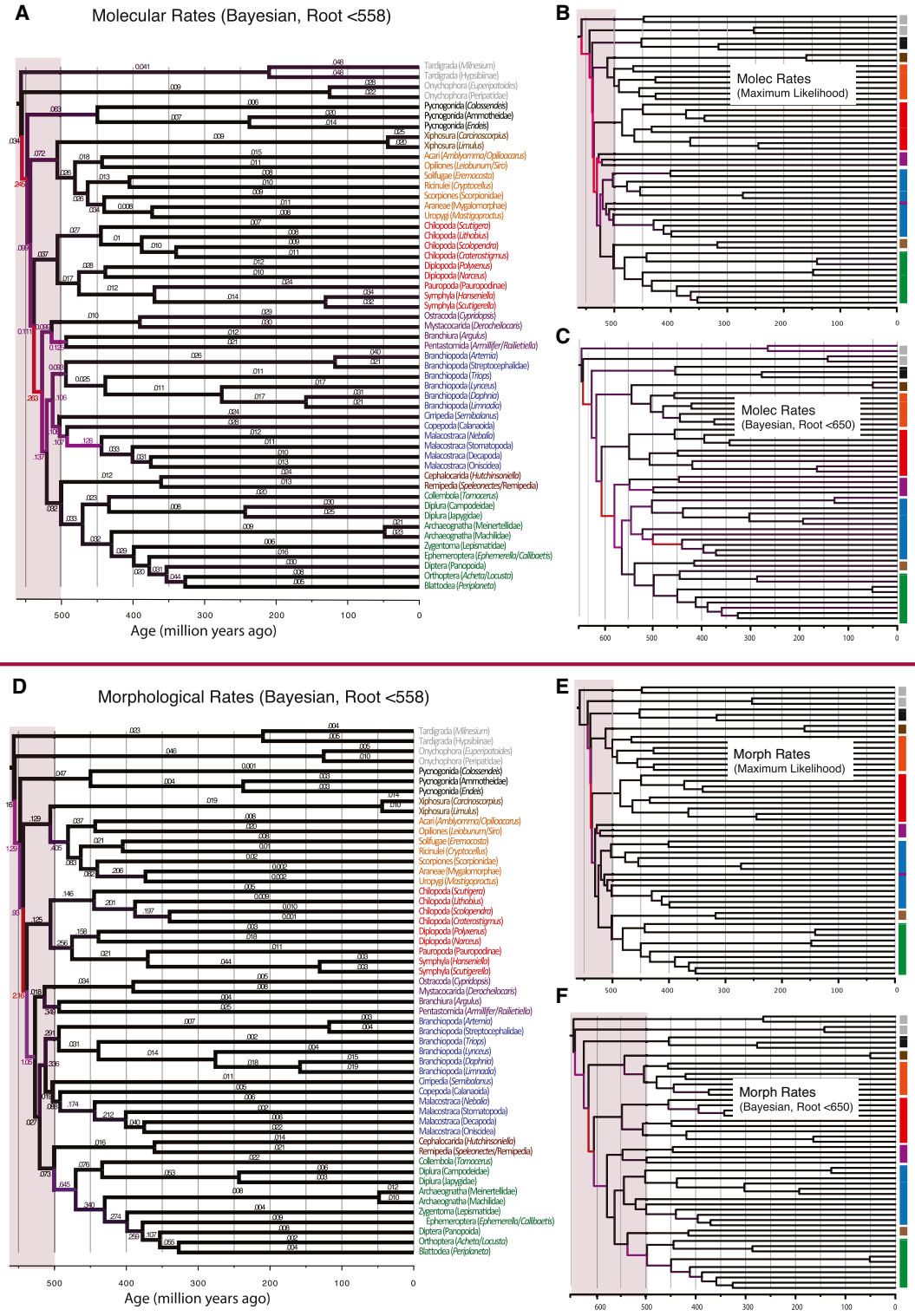

Figure 2. Fastest Rates of Phenotypic and Molecular Change in Arthropods Are Concentrated in Lineages Spanning the Cambrian Explosion

Warmest colors (red) denote fastest-evolving branches; numbers beside branches in (A) and (D) denote evolutionary rates in % change per million years (pMyr) (95% HPDs in Table S2). These results are robust to dating methods and calibration assumptions (Table S3). Taxa are color coded according to major clade as in Figure 1. Branch rates for (B), (C), (E), and (F) are detailed in Figure S2.

(A–C) Molecular evolutionary rates inferred using BEASTMC3 assuming panarthropods are no older than 558 Ma (A), maximum likelihood and penalized likelihood rate smoothing (B), and BEASTMC3 assuming panarthropods are no older than 650 Ma (C).

(D–F) Phenotypic (morphological) evolutionary rates inferred in the same three analyses as (A)–(C).

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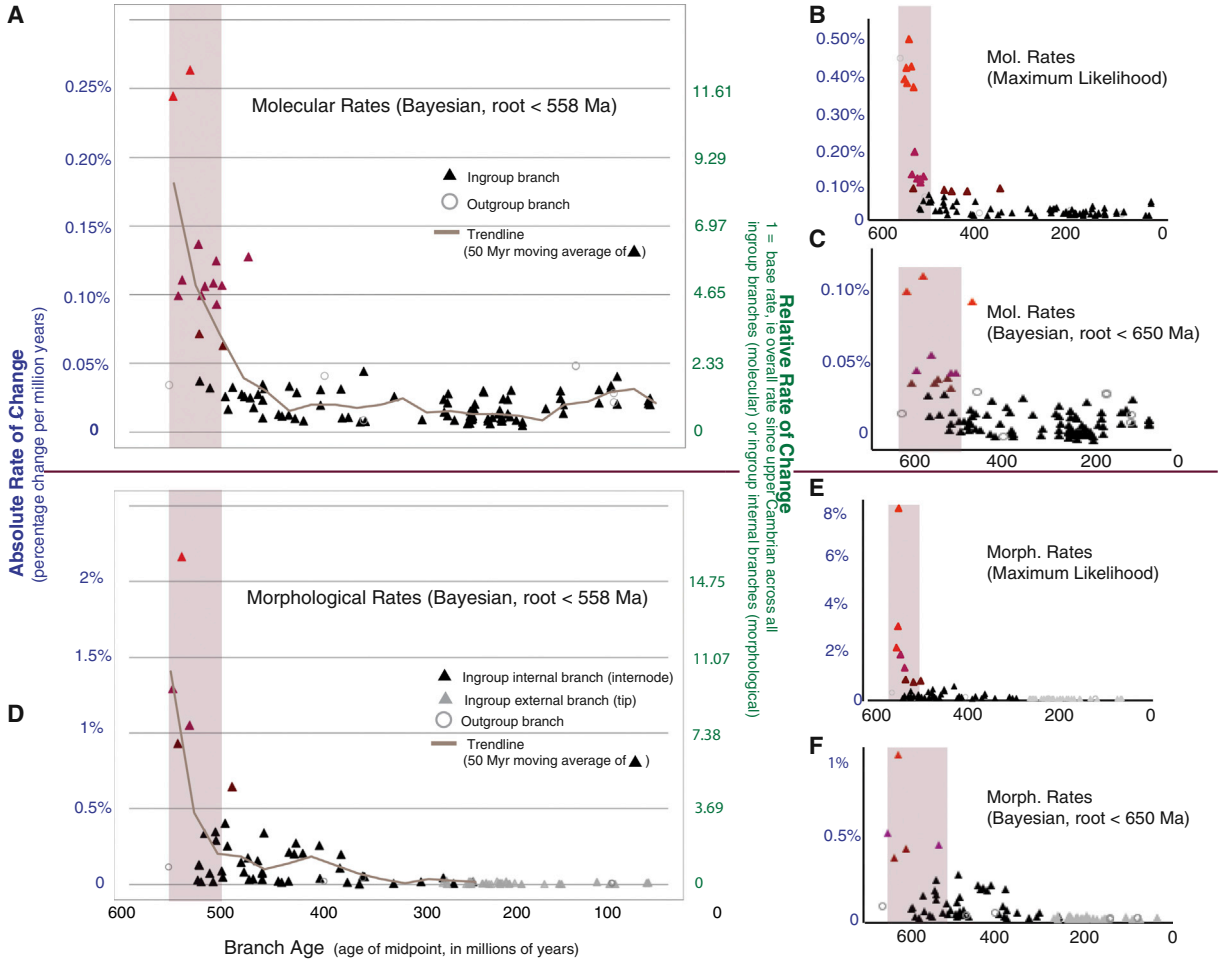

Figure 3. Rates of Phenotypic and Molecular Evolution in Arthropods Increased ~4 and ~5.5-Fold during the Cambrian Explosion

Rates (in % change pMyr) plotted against branch age (= midpoint of branch). Fastest branches (warm colors) are concentrated in an interval from 558 to 500 Ma (pink shading). Brown line represents the moving average inferred using a sliding window of 50 Myr. Because of potential bias, outgroup branches (for molecular change) and outgroup and terminal/tip branches (for morphological change) are plotted using different symbols and excluded from sliding-window calculations. See Supplemental Experimental Procedures SI_1–SI_12 for full methods and Figures 1 and S2 for rates on all individual branches.

(A–C) Molecular evolutionary rates inferred using BEASTMC3 assuming panarthropods are no older than 558 Ma (A), maximum likelihood and penalized likelihood rate smoothing (B), and BEASTMC3 assuming panarthropods are no older than 650 Ma (C).

(D–F) Phenotypic (morphological) evolutionary rates inferred in the same three analyses as (A)–(C).

studies comparing Cambrian and subsequent evolutionary rates investigated phylogenetic signal in simulated DNA data [7]. That study suggested that reconstructing relationships between Cambrian lineages would be essentially impossible assuming constant evolutionary rates, unless they diverged gradually over a time period of >100 Myr. However, the same study [7] showed that phylogenetic relationships in a "Cambrian bush" are resolvable if elevated evolutionary rates during the critical time window generated large suites of changes on chronologically short branches. Our results support this possibility, and the numbers are highly consistent. The simulations found that the optimal DNA substitution rate for recovering Cambrian evolutionary relationships was 0.01% pMyr; the average substitution rate inferred here is 0.02% pMyr, confirming the appropriateness of this filtered genomic data for inferring deep arthropod divergences [29] (Figure S1). Simulations suggested that ability to recover a series of divergences compressed into ~35 Myr improved significantly if evolutionary rates in this interval were increased 5-fold; in the focal analysis (root < 558 Ma), most basal arthropod divergences are compressed into ~40 Myr, with a 5.4-fold increase in the early Cambrian (Figures 1 and 3).

The present study reveals that in order to equalize morphological and molecular evolutionary rates before and after the Cambrian explosion, the root of panarthropods needs to be ~940 Myr old (Table S3: soft-bounded root). Thus, the assumption of rate constancy through time means that extensive morphological and molecular divergences between the primary arthropod groups must be accommodated on chronologically long branches, leading to very early basal divergences inconsistent with the fossil record. Notably, other recent molecular studies, without hard bounds on root ages, obtained similar dates for panarthropods [9, 38]; such approaches allow the analysis to freely "telescope" the age of the root to reduce rate heterogeneity across time. However, those studies did not investigate how rates vary if the maximum root age is successively reduced to be compatible with the fossil record (and the majority of recent molecular studies). The present study reveals that constraining root age to any figure even loosely consistent with the fossil record

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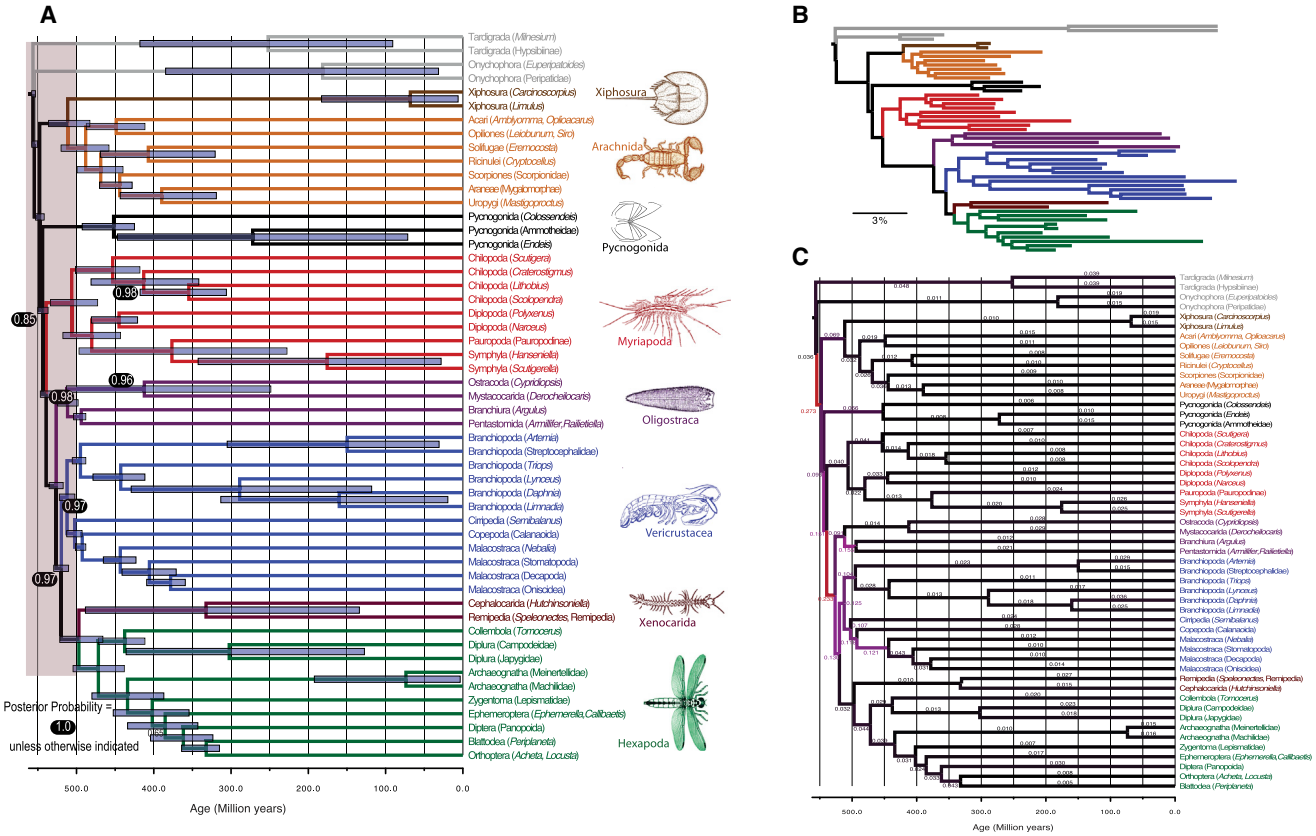

Figure 4. Evolutionary Rates in Arthropods Based on Molecular Data Only

Arthropod phylogeny, divergence dates, and evolutionary rates based on a BEASTMC3 analysis of 62 protein-coding genes (Supplemental Experimental Procedures SI_9); the patterns retrieved here are very similar to those found in the context of a combined morphological and molecular analysis (Figures 1 and 2).

(A) Consensus tree with branch lengths and divergence dates in terms of time; all posterior probabilities = 1.0 unless indicated. Shaded bars denote 95% HPDs for divergences, which are often large due to extreme rate heterogeneity.

(B) Tree with branch lengths proportional to amount of molecular change; taxon order is identical to (A). Large amounts of change occur on chronologically short basal branches.

(C) Tree with rates of evolutionary change on each branch (% pMyr); warmest colors (red) denote fastest-evolving branches.

(e.g., <650 Ma) implies elevated rates of morphological and molecular evolution in the late Precambrian.

Darwin famously considered that the sudden appearance of complex morphologies in the lower Cambrian was at odds with normal evolutionary processes [2, 4]. Many subsequent workers have reasonably contended that this pulse of diverse fossils is not explicable without either positing a lengthy cryptic Precambrian prelude or invoking "unknown evolutionary mechanisms" [6–8]. Similarly, initial molecular analyses concluded that the extensive molecular divergences between arthropods, echinoderms, and chordates could not be fully reconciled with the abrupt Cambrian fossil record, assuming even the fastest modern rates of molecular evolution [35]. These legitimate reservations have predictably been exploited by opponents of evolution. However, "Darwin's dilemma" [2, 4] might be resolvable. The initial molecular analyses [35] demonstrated that observed rates of molecular evolution could be reconciled with divergences between metazoan phyla as recent as ~586 Ma, which (although still pre-dating the Cambrian) is now broadly congruent with recent discoveries of the earliest metazoans (Table S1). Our results, using updated methods on morphological and genomic-scale data, show potentially even greater congruence. Inexplicably fast rates are not required to explain the Cambrian explosion of arthropods, even under an extreme scenario in which all divergences are compressed into the Cambrian. Rather, the pattern is consistent with many Cambrian lineages exhibiting accelerated—yet plausible—rates of morphological and molecular evolution. Typical directional selection can increase phenotypic evolutionary rates by orders of magnitude over short timescales [39], and even conserved genomic regions can exhibit 10-fold differences in evolutionary rates in living sister lineages [40]. More specifically, in arthropods, data sets of first- and second-position codons alone often exhibit 2-fold, and occasionally 5-fold, differences between closely related taxa (Figure S2D).

While this study examined only arthropods, the patterns found here may be general across other metazoan groups, though broader taxonomic studies are required. Arthropods are by far the most abundant and well-known Cambrian phylum, typically representing nearly 40% of species and more than half of the specimens in Burgess Shale-type biotas [41], a dominance that has persisted until the present [33]. The patterns found in arthropods have accordingly been routinely extrapolated as representative for all Cambrian taxa (e.g., [6]). Clades characterized by major phenotypic innovations often exhibit higher rates of evolution initially (e.g.,

[42]); if this pattern characterized most metazoan phyla, rates in the Cambrian would be uniformly elevated [1–4].

Supplemental Information

Supplemental Information includes three figures, three tables, Supplemental Experimental Procedures SI_1–SI_14, and two supplemental data files and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.07.055.

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