Rapid Genetic Diversity with Variability between Replicated Digital Organism Simulations and its Implications on Cambrian Explosion

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Abstract
Cambrian Explosion resulted in substantial increase in biodiversity, which may be attributed to both environmental and biological factors. Although increased genetic evolution rate had been shown during this period, the role of genetic evolution in increased biodiversity is unclear. Re-creating Cambrian Explosion experimentally is not feasible. In this study, we used digital organisms (DOs) at high rate of random point mutations in the absence of selective pressure to examine the extent genetic evolution possible during Cambrian Explosion. Our simulation results suggest rapid and significant genetic divergence in the absence of selective pressure can occur at a species level and at local population level with significant differences between each local population (F ≥ 15.97, p-value ≤ 1.4E-79). Hence, the emergence of biodiversity in Cambrian Explosion may be due to the release of accumulated adaptive potential.

Keywords: Cambrian Explosion; Biodiversity; Digital Organisms (DOs)

Introduction
Cambrian Explosion or Cambrian Radiation around 540 to 515 million years ago [1] saw sudden and substantial increase in biodiversity [2], from about 30 classes of organisms pre-Cambrian to about 130 post-Cambrian period [3]. This coincides with rise in temperature, seawater and oxygen levels and the transition from aragonitic sea to calcite sea [4]. Increased oxygen and the appearance of zooplanktons may drive metabolism and support more complex organisms and food webs [5,6] while increased calcium level may result in widespread biomineralization as an adaptive response to calcium toxicity [7]. Carnivory predation may also increase the pace of evolution [6]. Molecular estimates suggest the divergence of the phylogenetic tree occurred between late Ediacaran and early Cambrian; implying that Cambrian is a real evolutionary event, not just an increase in fossil records [8]. These factors may jointly result in increased biodiversity [3]. However, the role of genetic evolution in increased biodiversity is unclear despite evidence [9] of increased genetic evolution rate.

As it is not feasible to re-create Cambrian Explosion experimentally; digital organisms (DOs), which are computer-simulated organisms [10,11] and had been used to explore various evolutionary scenarios [12-18], can be a tool to explore the impact of genetic evolution. Here, we use DOs to examine the extent of genetic divergence due to high rate of random point mutations in the absence of selective pressure [14,17] as possible evolutionary backdrop during Cambrian Explosion. Our results suggest high sequence diversity may evolve within a short period of time with possibility of significant differences between each isolated population. This suggests the possibility of a build-up of adaptive potential prior to Cambrian Explosion.

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Methods

30 replicates of 100 DOs, each DO with 1200-base chromosome as genome comprising of 300 repeats of "ATGC", were constructed and simulated using Digital Organism Simulation Environment (DOSE) [19,20] for 2500 generations with 10% background point mutation rate [21,22] similar to that used in previous studies [23-25]. The genomes of 100 DOs from five generations (500, 1000, 1500, 2000, and 2500) of each replicate were pairwise aligned using Smith-Waterman algorithm [26] in SeqProperties [27] for sequence divergence. The pairwise alignment score for minimum sequence divergence can be calculated using two identical 1200-base DNA sequences. The pairwise alignment score of the maximum sequence divergence can be estimated as the third standard deviation below the mean (-3 sigma) of pairwise alignment scores using 100 pairs of 1200-base randomly generated sequences.

Results and Discussion

The minimum sequence divergence gives a pairwise alignment score of 1200 by Smith-Waterman algorithm [26] while the average pairwise alignment scores from 100 pairs of 1200-base randomly generated sequences is 777.29 with a standard deviation of 4.7996. The pairwise alignment scores of randomly generated sequences are normally distributed (Kolmogorov-Smirnov D = 0.084, p-value = 0.453); hence, the pairwise alignment score of 762.89 can be used estimate the score of maximum sequence diversity.

30 simulations were performed, yielding 4,950 pairwise alignment scores for each of the five generations (generations 500, 1000, 1500, 2000 and 2500). The pairwise alignment scores were combined by generation and analyzed (148,500 pairwise alignment scores per generation). The maximum and minimum pairwise alignment score across the five evaluated generations are 766 and 789, respectively. Our results show that the average population reached 96.6% maximum diversity within the first 500 generations (Figure 1). The maximum pairwise alignment score observed in any generation (n = 30 simulations x 4,950 scores x 5 generations = 742,500) is 806, which is 90.1% maximum diversity; while the minimum pairwise alignment score observed in any generation is 751, which is 102.9% maximum diversity or 5.48 standard deviations below the average pairwise alignment score of two randomly generated sequences.

![Figure 1: Average pairwise alignment score by combining all 30 simulations. Error bars denote standard deviations.](image-url)
While it is surprising to observe near maximum sequence diversity within 500 generations, it may demonstrate the extent of possibilities in random mutations. As genetic variation, which translates to sequence diversity, is precondition to selection [28]; it may be beneficial for the species in the long run to maintain high diversity as materials for future adaptation needs [29]. This has been shown to be the case in Taiwan Parrotbill [30].

Analyzing our alignment results using two-way ANOVA on simulations and generations, our results suggest that both simulations ($F = 23.19, \ p-value = 6.6E-123$) and generations ($F = 18.04, \ p-value = 8.0E-15$) are significant. Further analysis into the 30 simulations from 500th and 2500th generations (Figure 2) show that the average sequence diversity is significantly different between simulations ($F \geq 15.97, \ p-value \leq 1.4E-79$).

![Figure 2: Average pairwise alignment score between simulations. Panel A and B are data for 500 generation and 2500 generation, respectively. Error bars denote standard errors.](image)

This suggests that identical initial population evolve differently even in the same external condition. This has been observed in Richard Lenski’s long-term experimental evolution using 12 flasks of *Escherichia coli* cultures founded from the same ancestor where citrate utilization evolved in one of the 12 identically maintained cultures [31]. Further work demonstrates that adaptive potential may have its origins substantially prior to the emergence of the citrate utilization phenotype [32]. Hence, this may suggest the possibility of genetic
divergence and the accumulation of adaptive potential prior to environmental conditions conducive for phenotypic divergence, which leads to emergence of biodiversity in Cambrian Explosion.

**Conclusion**

Using DOs, our results suggest rapid and significant genetic divergence in the absence of selective pressure can occur at a species level and at local population level with significant differences between each local population. This may suggest that emergence of biodiversity in Cambrian Explosion may be a result of accumulated adaptive potential waiting for opportune environment.

**Conflict of Interest**

The authors declare no conflict of interest.

**Bibliography**


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