Evolution of Diversity and Complexity by Cryptic Variations in Gene Regulatory Networks

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学位記番号	生博第２６３号
学位授与年月日	平成２５年９月２５日
学位授与の要件	学位規則第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）生態システム生命科学専攻
論文題目	Evolution of Diversity and Complexity by Cryptic Variations in Gene Regulatory Networks （遺伝子制御ネットワークの隠蔽変異による多様性と複雑性の進化）
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論文内容の要旨

Organisms have various forms and life-styles, which surprise me with their diversity and complexity. The traits, without exception, have been developed in the history of evolution with their roots in a simple common ancestor. This fact makes me conscious of some mechanistic principles behind the diversification and complexification of life. My major goal as an theoretical biologist is to formulate this stochastic process. This attempt will be a critical step toward a comprehensive understanding of "Tree of Life". I have been working on the problem focusing on the two properties combined in gene regulatory networks (GRNs)—robustness and evolvability.

Driving force of evolution is genetic variation, the quantity of which in a population determines the speed and direction of phenotypic evolution (Hansen and Houle 2008; Lande 1976; Lande and Arnold 1983). While adaptive phenotypic evolution depends on heritable variation in phenotypes, selection on phenotypes exhausts genetic variance, resulting in a limit to the selection response (Blows and Hoffmann 2005; Blows 2007). The maintenance of genetic variation has therefore been a major concern in evolutionary biology. Furthermore, concerted action of multiple genetic modifications are often necessary for organisms to produce a new complex traits (Monteiro and Nogueira 2010; Muller and Newman 2005). It is a long-disputed question how organisms could go through useless or deleterious intermediate stages, fitness valleys (Masel 2006; Stern 2011).

A group of organisms exhibit larger phenotypic variance when they encounter a novel environment than they usually do (Schlichting 2008; Takahashi 2013). It indicates the existence of invisible variations, i.e., cryptic genetic variations (CGVs), which would emerge as diverse phenotypes in response to the changes in environmental or genetic background. Such mechanisms that enable accumulation and release of CGVs are called evolutionary capacitor and considered to contribute to macro-evolutionary patterns such as saltatory evolution and adaptation to novel environments (Gibson and Dworkin 2004; McGuigan and Sgro 2009; Rutherford and Lindquist 1998; Schlichting 2008; Wagner 2005).

However, it is not always possible for organisms to produce any desired traits with recurrent mutations; although mutations occur at random positions in a genome, their effects are not random nor additive, but are constrained and biased by their developmental pathways (Pigliucci and Preston 2004; Smith et al. 1985; Wagner and Altenberg 1996; Wilkins 2007). Therefore, assuming the simple allelic effects on phenotypes is insufficient to understand the

evolution of phenotypic novelty; instead, considering how genetic variations are translated into phenotypic variations is necessary. A novel phenotype is not necessarily the product of a novel gene, but rather often emerges when a novel expression pattern is created with existing genes (Prud'homme et al. 2007; Shubin et al. 1997, 2009). Gene regulatory network (GRN) is in this sense the key stone of evolutionary novelty. GRNs control the spatial and temporal patterns of gene expression and are ubiquitously involved in biological processes such as cell differentiation, environmental responses, pattern formation and circadian rhythm (Davidson 2006; Evans and Marcus 2006; Farkas et al. 2006). Modularity of GRN enables co-option of a existing functional unit for another context and provide the useful material for phenotypic novelty (Carroll et al. 2004; Fraser et al. 2009; Masel and Trotter 2010; Monteiro 2012; Wilkins 2007).

Also GRNs are considered to be a candidate of evolutionary capacitor because of their epistatic behavior and mutational robustness (Siegal and Bergman 2002; Wagner 1996); thus GRNs can facilitate macro-evolution not only by modularity, but also through cryptic variations. However, the nature of cryptic variations in GRNs is poorly understood because most studies on the evolvability of GRNs hardly paid careful attention to population dynamics (Aldana et al. 2007; Ciliberti et al. 2007; Draghi and Whitlock 2012; Espinosa-Soto et al. 2011; von Dassow et al. 2000). CGVs should be accumulated through population genetic processes, such as mutations, genetic drift, and natural selection. It is therefore essential to understand how GRNs are modified in evolutionary processes under various conditions and how they can contribute to the phenotypic evolution through cryptic variations.

Here I constructed an individual-based model of GRNs that controlled gene expression in response to environmental stimuli. The model enabled the analysis of network properties in the context of population genetics. It demonstrated that populations of GRNs accumulate and release cryptic variations, the number of which varies depending on the properties of the GRNs and the environments to which they have been subjected across the generations. Large and complex GRNs are preferentially evolved under heterogeneous and fluctuating environment; such GRNs tend to exhibit higher potential for accumulation and release of CGVs and thus for new adaptation. These findings indicate that the expansion of GRNs and adaptation to novel environments are mutually facilitating, resulting in a sustainable sources of evolvability. This study thus provides important insight into the origins of biological diversity and complexity. The

progress in genome decoding techniques will soon enable the analyses of GRN structure within and among populations. For the future, this study provides the theoretical framework to understand how GRN structure and cryptic variations in a population will behave on an evolutionary timescale.

An important factor I ignored in this thesis is stochastic noise in gene expression. The expression of duplicated genes was more diverse than that of singletons (Dong et al. 2011; Ha et al. 2009; Kliebenstein 2008); individuals with larger GRNs genes may have advantages in diverse environments because they produce more genetically variable offspring. Therefore, considering stochastic effects of gene duplication may expand the parameter range in which environmental fluctuations facilitate the GRN evolvability.

Stochastic noise has importance aside of that aspect; it may facilitate GRN evolution and phenotypic novelty especially in unicellular organisms through the intermediate state called phenotypic accommodation (West-Eberhard 2003), partial penetrance (Eldar et al. 2009) or persistence (Wakamoto et al. 2013). Whereas deterministic dynamics is dominant when the cellular activity is high, stochastic fluctuation overwhelms deterministic component of the dynamics when the cellular activity is low under stressful environments (Kashiwagi et al. 2006). Then cells can find the new optimal phenotypes in stressful environments without guided by programmed pathway to express them. Genetic basis that more stably express such novel phenotypes that originally produced with stochasticity or plasticity can evolve and be fixed afterward (phenotypic assimilation; West-Eberhard 2003). I think it will be a major route for a horizontally transferred free gene to be integrated as a terminal node of GRNs, and that is why genes derived from horizontal transfer are abundant in terminal genes, not transcription factors (Lagomarsino et al. 2007).

This scenario can be extended to multicellular organisms, which have capacity to produce functional outcomes despite physiological, developmental, environmental change. A striking example is the evolution of tetrapod forelimb to a bird or bat wing. It needs concerted changes in bones, muscles, nerves, and vessels, but co-evolution of all these tissues with many regulatory changes in parallel is not necessary. Each component are developed through interactions with each other called exploratory processes (Kirschner et al. 2005) or self-organization (Kauffman 1993). Complex phenotypic changes can be produced with a small

number of genetic modification in this way. Such "facilitated variations" will be a key player that literally facilitate the evolution of complex traits (Gerhart and Kirschner 2007).

A theory of macro-evolutionary dynamics should fulfill two requirements. First, the potential of "facilitated variations" has to be quantified. A possible solution today is to measure the degree of phenotypic integration (Pigliucci and Preston 2004) by morphometrics, or some statistics on modularity of a GRN may be good proxies for that. The effect of facilitation can be examined by phylogenetic analysis. Second, the model must be designed from the viewpoint that individual GRNs constitute their own environment and thus ecosystem; niche construction should be included in phenotypes, and phenotypes should affect the evolutionary trajectories of other genotypes. It can be considered as a kind of game theory, but is different in that a new theory aims not at reaching an optimum nor equilibrium, but at divergence toward diversification and complexification. Modeling the interplay between ecology and development in this manner will lead us to a comprehensive understanding of macro-evolutionary pattern.

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論文審査結果の要旨

進化の原動力は遺伝的変異であるが、生物は、通常の環境では発現していない変異が、新しい環境のもとで出現することがあり、それを隠蔽変異という。このような隠蔽変異は、生物が新しい環境に遭遇したとき、より大きな変異を生じるため、新しい環境に適応できる変異が出現する可能性が高くなる。これまで、このような隠蔽変異がどのような機構で生じているかについては、いくつか集団遺伝学的なモデルが構築されていたが、表現型を創り出す遺伝子制御ネットワークがどのように隠蔽変異の蓄積に関与しているのかについては明らかになっていなかった。岩嵜航氏は、環境要因と遺伝子制御ネットワークがどのように隠蔽変異の蓄積に影響するかと、個体ベースモデルをつかって調べた。

モデルでは、各個体は環境からのシグナルにより活性化する遺伝子制御ネットワークを持ち、一倍体で無性生殖する単細胞生物を仮定した。シミュレーションはクローンの個体群からスタートし、ある一定期間特定の環境刺激と最適な遺伝子発現量で規定される安定化選択の下で進化させた後、環境刺激や最適値が変動する環境でさらにある期間進化させる。その間、さまざまな環境刺激を与えてみたときに生じる表現型多様性の変化を観察し、隠蔽変異を評価する。本研究では、遺伝子制御ネットワークに関与するパラメータ（遺伝子数やシス制御領域の長さ）と、環境条件に関するパラメータ（選択圧、環境変動の強さ、個体が生涯で経験する環境の数）を変化させてその影響を調べた。

その結果、集団内の隠蔽変異の蓄積量は遺伝子数、突然変異率と正の相関があったが、各個体の相互作用密度、クラスタ係数、自己制御数、直径などほかのネットワーク特性とは相関がなかった。また、選択圧が強いほど遺伝的変異は減少し、通常時の表現型変異は減少したが、新規環境で出現する表現型変異（隠蔽変異）の量は影響を受けなかった。さらに、個体が生涯で経験する環境の数が多い場合と確率的な環境変動が強い場合には全体の遺伝的変異とともに隠蔽変異も減少した。その一方で遺伝子制御ネットワークはより大きなものが進化した。

これらのことから、環境の異質性や変動性の高さは変異の蓄積を妨げるという点で短期的な進化可能性は低下させるが、より複雑な遺伝子制御ネットワークの進化を促進するという点では長期的な多様化には正の効果をもたらすことが示唆された。これは、遺伝子制御ネットワークの複雑化と、多様な環境への進出や新たなニッチ構築が相互に促進し合うことで、生命システムの進化が駆動されているという新しい仮説を提唱する研究であり、非常に重要な結果となった。よって、岩嵜航氏提出の論文は，博士（生命科学）の博士論文として合格と認める。