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Cooperation between phenotypic plasticity and genetic mutations can account for the cumulative selection in evolution

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We propose the cooperative model of phenotype-driven evolution, in which natural selection operates on a phenotype caused by both genetic and epigenetic factors. The conventional theory of evolutionary synthesis assumes that a phenotypic value (*P*) is the sum of genotypic value (*G*) and environmental deviation (*E*), *P*=*G*+*E*, where *E* is the fluctuations of the phenotype among individuals in the absence of environmental changes. In contrast, the cooperative model assumes that an evolution is triggered by an environmental change and individuals respond to the change by phenotypic plasticity (epigenetic changes). The phenotypic plasticity, while essentially qualitative, is denoted by a quantitative value *F* which is modeled as a normal random variable like *E*, but with a much larger variance. Thus, the fundamental equation of the cooperative model is given as *P*=*G*+*F* where *F* includes the effect of *E*. Computer simulations using a genetic algorithm demonstrated that the cooperative model realized much faster evolution than the evolutionary synthesis. This accelerated evolution was found to be due to the cumulative evolution made possible by a ratchet mechanism due to the epigenetic contribution to the phenotypic value. The cooperative model can well account for the phenomenon of genetic assimilation, which, in turn, suggests the mechanism of cumulative selection. The cooperative model may also serve as a theoretical basis to understand various ideas and phenomena of the phenotype-driven evolution such as genetic assimilation, the theory of facilitated phenotypic variation, and epigenetic inheritance over generations.

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In his *Blind Watchmaker*, Dawkins argues that evolution can be accelerated by the "cumulative selection" (Ref. 1, Chap. 3). For example, suppose that 10 genetic mutations (advantageous allele pairs) are required for the evolution of a certain trait. Even if mutations can be exchanged by genetic recombination, it is very difficult for the 10 mutations to accumulate in an individual. If, however, one mutation is not lost in subsequent recombinations and each recombination will only accumulate mutations, then the evolution is completed as soon as all the mutations have accumulated in an individual. According to Dawkins, this mechanism of the cumulative evolution drastically accelerates evolution so that most complicated organs such as the eye is possible within a certain geologic time scale. However, there seem to be no textbooks mentioning the concept of the cumulative evolution (e.g., Ref. 2), and the authors have also failed to find original papers mentioning it. It appears that this concept is completely ignored by experts. This may be an inevitable consequence of the fact that Dawkins himself does not explain by what mechanism and/or under what condition the cumulative evolution is possible. In the present study, we show that the cumulative evolution is impossible according to the conventional theory of evolutionary synthesis, which implies that the conventional theory cannot explain the evolution of complex phenotypes in a plausible time scale. We demonstrate, however, that the cumulative evolution can be made possible by a slight modification of the conventional theory.

In general, one may say that "natural selection operates on the phenotype of individuals." Differences of phenotypes determine the survival probability of individuals, affect the ability to reproduce, and lead to the evolution of the population. In the evolutionary synthesis, it is assumed that only the (change of) phenotypes due to genetic mutations are subject to natural selection. This is justified by the argument that acquired traits are not inherited to the next generations so that they can be safely ignored when considering evolution. However, according to the "general argument" above, acquired traits, although not inherited, certainly comprise the phenotype, and hence they should be also subject to natural selection. Acquired traits are therefore one of the factors affecting evolution and cannot be ignored. The "slight modification" mentioned above refers to this inclusion of acquired traits.

Acquired (non-heritable) traits are the changes of phenotypes induced by the phenotypic plasticity. In modern terms, they are epigenetic changes or alteration. Even if the genome itself is unchanged, environmental factors, food, lifestyle (such as smoking and alcohol intake), physical and mental stresses, famine (starvation) may cause epigenetic changes, which, in turn, cause phenotypic changes[3,4]. Such epigenetic changes do affect the reproduction rate of individuals, and therefore, should be subject to natural selection.

Studies of epigenetics have been elucidating molecular mechanisms of epigenetic regulations such as DNA methylation and histone modification[5,6]. The whole set of epigenetic regulatory pattern is called epigenome, and individual chromatin modifications are called epigenomic marks[7]. Although a change in epigenome is expected to induce a macroscopic phenotypic change through the regulation of genes, there are not so many studies available that report such complicated relationships.

A famous example is the tragedy known as the "Dutch Hunger Winter" at the end of the World War II. Severe prenatal undernutrition was found to have epigenetic effects on health in adulthood[8,9]. Many of these effects are illnesses such as obesity, metabolic diseases, cardiovascular diseases, and breast cancer. Some examples show clear connections between epigenetic marks and phenotypes of animals. For example, an infant rat exhibits reduced anxiety response to stresses if it is sufficiently taken care of by its mother after the birth, this anti-stress response (a phenotypic change) is induced by the DNA methylation of a glucocorticoid receptor gene[10]. The difference between fertile queen bees and sterile worker bees, sharing the same genotype, is another example of epigenetic changes that cause a phenotypic change. It has been shown recently that being fed on royal jelly changes the DNA methylation pattern which induces the phenotype of the queen bee[11]. These examples all show that environmental effects in general cause changes in epigenomes which in turn cause life-long phenotypic changes in individuals.

As we have seen above, epigenetic changes are closely related to diseases. Diseases in general, especially lifestyle-related diseases such as diabetes, cardiovascular diseases, cancers, are conspicuous examples showing that the phenotypic changes of individuals are caused by the combination of genetic and epigenetic factors. These lifestyle-related diseases are known to be multifactorial, that is, they involve many genes[12,13]. At the same time, they are also known to depend on epigenetic changes caused by environment factors such as food, smoking and stress[14,15].

Generalizing the examples of diseases, we may state that "phenotypic changes are composed of genetic mutations and epigenetic changes." Based on this principle, we propose the "cooperative model" of evolution in the following. We have found that the simulations based on this model give results that are completely different from those based on the evolutionary synthesis. In particular, the "cumulative selection" and accelerated evolution were observed only with the cooperative model.

Let us make an additional remark to avoid possible confusion. When epigenetic changes are concerned in the context of evolution, what is often assumed is the inheritance of epigenomic marks, that is, transgenerational epigenetic inheritance. However, this phenomenon is not widely observed in organisms. In particular, only a handful of examples are known in which the transgenerational epigenetic inheritance in its strict sense (i.e., through the germline) is observed[16]. Our model of evolution presented in the following aims to be applicable to a wide range of organisms (eukaryotes), and hence does not assume the trangenerational epigenetic inheritance. In other words, the epigenetic changes referred to in our model are not transmitted to the next generation although they persist for individuals' life span and induce phenotypic changes.

Methods

Phenotype caused by epigenetic factors

According to the theory of modern evolutionary synthesis, the phenotypic value, P, of an individual in a population may be expressed as the sum of its genotypic value, G, and environmental deviation, E[17]:

$$P = G + E. \quad (1)$$

The environmental deviation E expresses the fluctuation among individuals in a genetically uniform population, and this fluctuation is supposed to be induced by different growth environments as well as ontogenetic noises among individuals. In other words, the term E is regarded as a noise or fluctuation of the phenotypic value in the absence of conspicuous environmental changes, and hence its variance, $\sigma^2(E)$, is not very large (compared to that of the genotypic value).

In contrast, the cooperative model that we propose foremost assumes that evolution is triggered by an environmental change. A species in a certain ecological as well as natural

environment tends to maintain the state that is most adapted to the environment so that evolution will not occur. In order for the species to evolve, its environment should change first, and the change should persist for a sufficient period of time. While organisms attempt to adapt to the changed environment, the degree of adaptation is not uniform among individuals so that those that are better adapted to the change tend to be better selected, those that are not well adapted tend to be eliminated. We assume that an individual organism responds to the environmental change by its phenotypic plasticity. Let the contribution of the phenotypic plasticity to the phenotypic value be F, then we have

$$P = G + F. \tag{2}$$

The change due to the phenotypic plasticity (F) may be regarded as an epigenetic change caused by the environmental change. The value of F varies depending on individuals. We assume that F follows the normal distribution with mean 0 and standard deviation $\sigma(F)$.

The noise term E of Eq. (1) is usually assumed to follow the normal distribution as well. The first terms G in these equations are identical as we shall show in the following. Therefore Eqs. (1) and (2) are nearly identical. The only difference between the two models is the variance of the distributions:

$$\sigma(E) < \sigma(F), \tag{3}$$

that is, the standard deviation of F is greater than that of E. In other words, the acquired phenotypic change in response to an environmental change is clearly greater than the phenotypic fluctuation in the absence of an environmental change. Furthermore, the difference between F and E is not limited to the quantitative difference of the variance, but F incorporates the effect of a qualitative phenotypic change (with the use of the truncation selection scheme, see below). It is noted that, technically, we should have $P=G+E+F$, but both E and F are considered to be normal random variables and the sum of two such variables is also a normal random variable, so that Eq. (2) may be employed without losing generality.

The first term G on the right-hand side of Eqs. (1) and (2) is the genetic factors contributing to the phenotype. As in quantitative genetics, we assume that a macroscopic trait depends on multiple genetic factors in general. For example, a recent study indicates that the genetic factors affecting human height are scattered on 180 loci in the human genome[18]. Another study shows that there are more than 30 loci associated with the type-2 diabetes in addition to regulatory genetic mutations (such as those in promoter regions)[19]. The more mutations an individual accumulates, the higher the probability of diseases. From this observation, we assume that the effect of multiple genetic factors is additive (see Discussion below).

In the following, we regard Eqs. (1) and (2) as the fundamental equations for the conventional model (i.e., modern synthesis) and the cooperative model, respectively.

Formulation of the cooperative model

We formulate the cooperative model more explicitly as follows. Let there be a population of N individuals and each individual has L genes related to a particular trait. A genetic mutation of the gene $j(=1,...,L)$ in the individual $i(=1,...,N)$ is represented as $g(i,j)$ which takes the value 0 for the wild type and 1 for the mutant. Let $w(j)$ be the contribution of the gene j to the phenotype after the environmental change. This is independent of individuals, and takes the value 1 (advantageous) or −1 (disadvantageous). Furthermore, the phenotype of each individual is affected by epigenetic changes. The epigenetic effect $F(i)$ is assumed to be a random variable following the Gaussian distribution with mean 0 and standard deviation $\sigma(F)$. $F(i)>0$ corresponds to an adaptive epigenetic effect, and $F(i)<0$ to a disadvantageous one. It should be stressed that $F(i)$ is specific to the individual i and is not inherited. The phenotypic value $P(i)$ of the individual i is given as

$$P(i) = \sum_{j=1}^{L} w(j)g(i,j) + F(i). \tag{4}$$

By comparison with Eq. (2), we have

$$G(i) = \sum_{j=1}^{L} w(j)g(i,j). \tag{5}$$

By replacing $F(i)$ in Eq. (4) with $E(i)$ (a normal variable with a smaller variance), we obtain the conventional model.

Simulation

We simulated the evolution of a population after an environmental change using a genetic algorithm[20]. Two kinds of simulations were performed. One kind of simulations is based on the cooperative model in which the phenotypic value of individuals consisted of both genetic and epigenetic factors as in Eq. (2). The other is based on the evolutionary synthesis in which the phenotypic value consisted of the genetic factor and the environmental deviation (Eq. 1).

In both the cases, an individual is represented as a linear array ("chromosome") of L genes. We set the number of genes L=20, and $w(j)$=1 for j=1,...,10 and $w(j)$=−1 for j=11,...,20. The initial population size was set to N_0=100,000 and the maximum population size to N_m=100,000. The initial mutation rate was set to p=0.01, and the rate of random selection to q=0.15. We set the threshold for selection T=5 (see Discussion for more explanation about these parameters). The standard deviation of the non-genotypic factors was set to $\sigma(F)$=3 and $\sigma(E)$=0.5 in accordance with Eq. (3). The value $\sigma(E)$=0.5 was chosen to be comparable to the standard deviation of the genotypic value G in the initial population[21] (<0.45, c.f., Supplementary Tables S1 and S2).

It should be noted that we have employed a truncation selection model by using the threshold T in order to express a "qualitative" phenotypic change of an individual in re-

sponse to the environmental change. That is, the individuals with their phenotypic value P greater than the threshold T are considered to have qualitative phenotypic changes. Since we assume the normal distribution with mean 0 and $\sigma(F)=3$, the probability that an individual with $G=0$ may surpass the threshold ($T=5$) is approximately 4.8%. In case of the conventional model ($\sigma(E)=0.5$), the corresponding probability is negligibly small ($\approx 10^{-23}$). Therefore, in the latter case, no qualitative change is expected so that the E term may be regarded as a quantitative fluctuation.

We have applied the following simulation protocol to both the conventional model and the cooperative model in the same manner.

1. Generate N_0 individuals having mutations at randomly chosen genes with the probability p. Set $N=N_0$.
2. Select $2N$ pairs out of N individuals. For each pair, crossover the chromosomes at a randomly chosen site to produce 2 new individuals ("offsprings"). There are $4N$ offsprings in total. The parent individuals are discarded in the following. Assign a normal random number $F(i)$ to each individual.
3. A new individual is selected or discarded with probability 1 if the phenotypic value is greater than or equal to the threshold, $P(i)\geq T$, or is less than 0, $P(i)<0$, respectively. In the case when $0\leq P(i)<T$, the individual is selected with probability q.
4. If the population size is greater than N_m, randomly select N_m individuals. Set N to the number of remaining individuals.
5. Iterate the steps 2 to 4.

   (Replace $F(i)$ with $E(i)$ for the conventional model.)

Note that in the present implementation of the genetic algorithm, no new genetic mutations are introduced during evolution. Instead, minor genetic mutations are assumed to preexist before the environmental change. We performed 5 simulations with different random numbers for each model (i.e., the cooperative model and evolutionary synthesis).

Results

The results of the simulations are shown in Figure 1. After the environmental change, the wild type species becomes unstable, and individuals that have succeeded in adapting to the new environment, or those that have been simply lucky, are selected whereas those that have failed are eliminated.

Figure 1A shows that the population of the conventional model (red lines) monotonically decreases and become extinct by the 20-th generation. The population of the cooperative model shows a totally different trend: the initial population of 100,000 individuals rapidly decreases down to around 1,000 for the first several generations, but then starts to increase up to 100,000 ($=N_m$) by the 20-th generation.

Figure 1B indicates that this change of population size is due to the initial leap and the subsequent gradual increase of the average phenotypic value of the population, which in turn leads to an increased number of individuals whose phenotypic values surpass the threshold ($T=5$). At the same time, the average effect of genetic mutations per individual $\langle G\rangle$, corresponding to $G(i)$ in Eq. (5), is persistently increasing through generations (Fig. 1C, blue lines). This is an important point implying that, although the population decreases for the first several generations, adaptive genetic mutations continuously increase on average by genetic recombination. On the contrary, such accumulation of minor genetic mutations does not occur in the conventional model, and the average effect of genetic mutations remains less than 2 (Fig. 1C, red lines). Figure 1D clearly indicates that such a difference between the conventional model and cooperative model is caused by the large difference between the environmental deviation $E(i)$ (red lines) and the epigenetic factor $F(i)$ (blue lines). In particular, the initial leap in $\langle F\rangle$ (in the first generation) indicates that the epigenetic factor (the second term of Eq. 2) contributes most significantly to the initial phase of the evolution. That is, individuals whose phenotypic values surpass the threshold are limited to those having large values of F in the initial phase. As the average genotypic value $\langle G\rangle$ increases as generations proceed (Fig. 1C), more individuals can surpass the threshold, and as a result, $\langle F\rangle$ decreases. The initial leap in $\langle P\rangle$ (Fig. 1B) is due to that in $\langle F\rangle$.

The details of the evolution of $\langle G\rangle$ are provided in Supplementary Tables S1 and S2 (The results of 5 simulations based on the cooperative models in Tables S1 a–e; those of the conventional model are in Tables S2 a–e). Table S1 shows that, in the initial population (the 0-th generation), there are individuals with $G(i)=0$, $G(i)>0$ as well as $G(i)<0$, but as generations proceed, those with $G(i)<0$ rapidly decreases and virtually diminishes at around the 7-th generation (details vary depending on the random seeds in the simulations). On the other hand, the individuals with $G(i)>0$ constantly increase through generations. This increase is astonishing, considering the fact that the total population is greatly decreasing during the first several generations. It is interesting to note that individuals with $G=5$ (those that can surpass the threshold without a positive F) start to appear when the decreasing population starts to increase. Table S1 shows that the mode of the distribution of $G(i)$ shifts from $G=0$ (the 0-th to 5-th generations) to $G=1$ (the 6-th to 8-th generations), $G=2$ (the 9-th to 11-th generations), $G=3$ (the 12-th to 14-th generations), $G=4$ (the 15-th to 17-th generations), $G=5$ (the 18-th to 20-th generations), and finally to $G=10$ (after 64-th generation), constantly increasing to higher values. Individuals with $G(i)\geq 5$ have the phenotypic value greater than or equal to the threshold on average, and they are unconditionally selected. This phenomenon, the increase or accumulation of advantageous genetic mutations through generations, is the cumulative selection proposed by Dawkins.

In the case of the conventional model (Table S2), even

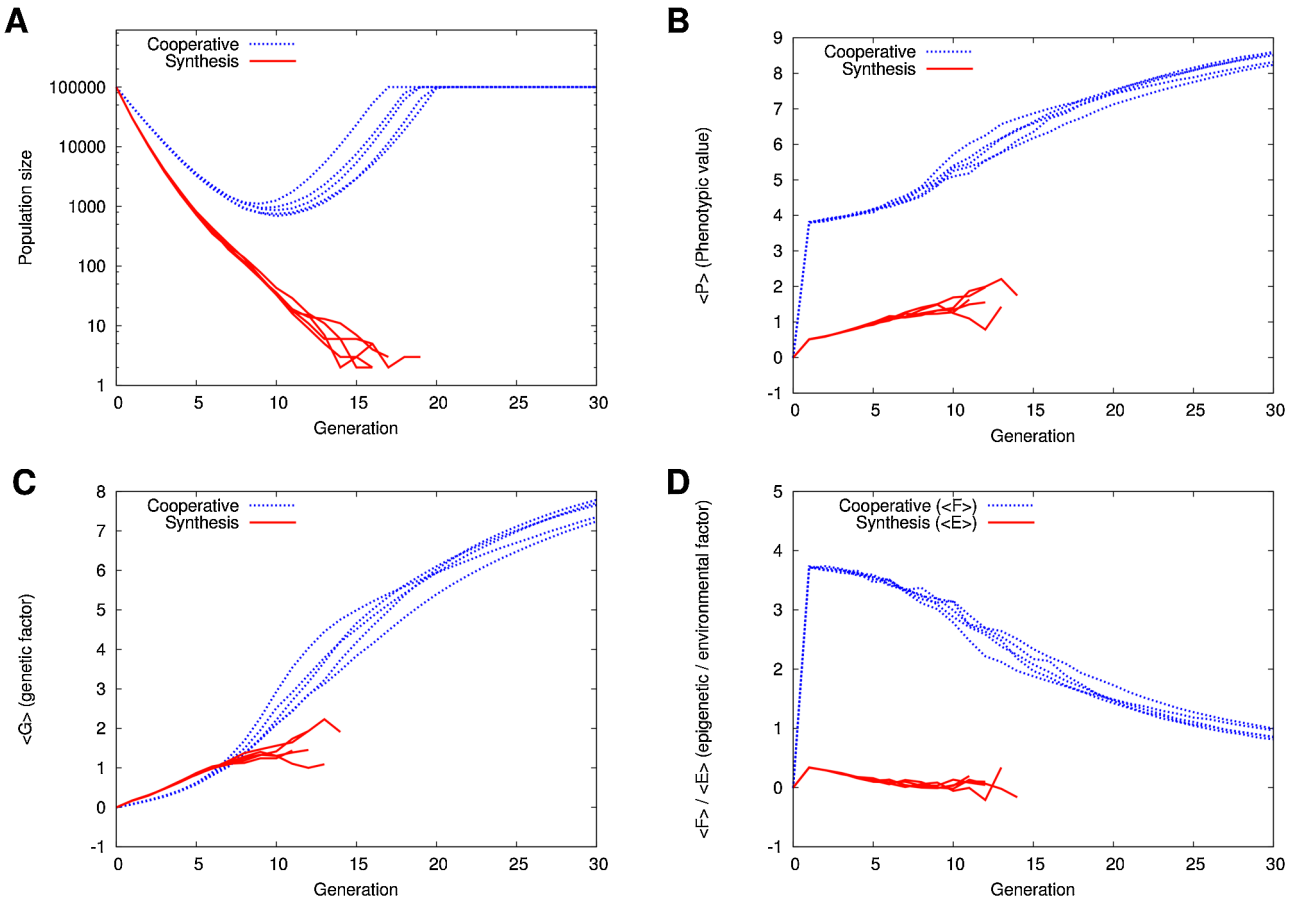

Figure 1 Simulation results. Dashed blue lines and solid red lines indicate simulations based on the cooperative model (Eq. 2) and the evolutionary synthesis (i.e., the conventional model, Eq. 1), respectively. A: The evolution of population size. B: The evolution of the average phenotypic fitness, $P(i)$ in Eqs. (1) and (2). C: The evolution of the average genotypic value $G(i)$ in Eq. (5). D: The evolution of the average epigenetic value (blue), $F(i)$ in Eq. (2) and environmental deviation (red), $E(i)$, in Eq. (1). Data points for the population size less than 10 were not plotted in B, C and D due to the (deceivingly) large variances.

though the simulations start with the same initial conditions (Table S1a and S2a have the same initial population, as for S1b and S2b, and so forth), the distributions of the population after the first generation are very different from those in Table S1. In particular, individuals with G=0, G=+1, G=+2, etc. are constantly eliminated as the total population decreases, and the mode of the distribution always stays at G=0–2 and does not shift to higher values. The latter observation clearly indicates that the conventional model does not realize the cumulative selection. According to Dawkins, such an evolutionary mechanism is called the "single-step selection"[1]. In such case, the average genetic contribution $\langle G \rangle$ remains nearly constant irrespective of generations. Figure 1C clearly shows the difference between the cumulative selection and the single-step selection.

The above comparison between the simulations based on the cooperative model and those based on the conventional model leads to the conclusion that the cumulative selection is caused by the additive effect of the genetic and epigenetic factors. It is essential in the cumulative selection that (incompletely) accumulated mutations are maintained and irreversibly fixed by some "ratchet" mechanism. In the case of the cooperative model, the ratchet mechanism is realized in the way that an individual is naturally selected when the sum of its genetic and epigenetic contributions to the phenotype surpasses the threshold. The simulations also show that the evolution of a new phenotype is accelerated once the ratchet mechanism triggers the cumulative selection, as Dawkins has suggested.

Discussion

On cumulative selection

We have shown that the cumulative selection is realized by the addition of the epigenetic factor (F) to the genetic factor (G). It is noted that the epigenetic value F is not inherited to the next generation, in accordance with the general principle that "acquired traits are not inherited." In addition, the epigenetic value F may be not only positive, but also negative, following the normal distribution with zero mean.

Table 1 Comparison of disease, evolutionary synthesis, and cooperative model

	genetic factor	epigenetic factor	natural selection	cumulative selection
Disease	○	○	×	×
ES[a]	○	×	○	×
CM[b]	○	○	○	○

○: applicable; ×: not applicable. [a]Evolutionary synthesis; [b]Cooperative model.

In spite of these properties of the epigenetic effect, it is notable that the addition of F to the genetic factor G caused the significant deviation (i.e., the cumulative selection) from the conventional model in which Eq. (1) holds instead of Eq. (2). The cooperative model may be regarded as an extension of the conventional model by replacing the E term in the latter with the F term in the former. In fact, when the effect (variance) of F is sufficiently small, the cooperative model reduces to the conventional model of evolutionary synthesis.

We assumed that the number of beneficial mutations was 10 while the threshold value was $T=5$. Accordingly, the mode of the distribution of the genotypic value G continued to increase after passing $G=5$ ($=T$), and finally reached $G=10$ (Table S1). The reason for G not staying at 5, but reaching 10 is that the average survival rate of individuals of a given genotypic value is greater for greater G. Figure 1A shows that, while the population size is recovered by the 20-th generation, the genotypic value continues to change. According to Table S1, an order of 100 generations are required for the new genotype to be completely fixed in the population.

Let us summarize the similarities and differences among diseases, the theory of evolutionary synthesis and the cooperative model (Table 1). Diseases may be regarded as phenotypes that have negative fitness, and therefore, should be eliminated from the (human) population from an evolutionary point of view. But they never disappear for various reasons. For example, diseases that are cured naturally are not disadvantages in evolution. Genetic diseases that are recessive tend to remain in the population; lifestyle-related diseases including cancers that develop after individuals' reproductive period do not affect the next generation, and hence, are not subject to natural selection. In summary, although diseases are genetic phenomena, they are not subject to natural selection, and hence, are not evolutionary phenomena. In Table 1, only the cooperative model assumes genetic factors, epigenetic factors and natural selection at the same time. Only when these 3 conditions are met, the cumulative selection is possible.

Comparison with experiments

The cooperative model may be validated if we could find examples of evolutionary events which could be well explained by the model, but there have been no such examples reported to date. We therefore compare our model with the phenomenon of "genetic assimilation" as they share many features in common.

Genetic assimilation is a classical phenomenon discovered by Waddington, and exemplified by the famous experiments using fruit flies[17,22]. In one experiment, exposed to a heat shock (40°C) after puparium formation, approximately 40% of the adult flies in the population exhibited the *cross-veinless* phenotype. In another experiment, the *bithorax* mutant of flies were obtained by submitting eggs to a treatment with ether vapor. In either experiment, the mutant flies were collected (artificial selection), mated, and reproduced, and the offsprings were exposed to the same stresses (heat shock or ether vapor). By repeating this process for 10 to 20 generations, the mutant phenotypes started to appear without the stress. That is, the mutant phenotypes due to epigenetic changes were genetically assimilated[22].

These experiments share common features with the cooperative model except that the former utilizes artificial selection and stresses. It is important to note that external stresses induced significant phenotypic mutations in the experiments of the genetic assimilation. The phenotypic mutants were observed in the initial population so that they did not depend on the genotypes. In other words, these mutations were acquired changes. This point is consistent with the assumption of the cooperative model. In addition, it is a typical characteristics of acquired mutations (by phenotypic plasticity) that a certain fraction of the population exhibit the same phenotype, and such a trend is different from the case of genetic mutations[23]. In fact, the heat shock experiment induced the cross-veinless mutants in the 40% of the population. In the case of the cooperative model, approximately 4.8% of the population in the first generation can surpass the threshold value ($T=5$). (One of the distinguishing features of phenotype-driven theories of evolution is the assumption that an environmental change induces phenotypic mutations in a certain fraction of the population[24].) After being exposed to the same external stimulus (environmental change) for many generations, phenotypic mutations become genetically fixed, and that "many" generations can be as few as 10 to 20 generations. This observation is common to the experiments and our simulations. According to the cooperative model, we can understand that such rather rapid evolution is made possible by the mechanism of cumulative selection. In the simulations, we have assumed truncation selection which was also used to interpret the experiments (Ref. 17, Fig. 14.20; Ref. 22).

Finally, we note that there are several other examples of genetic assimilation in natural environments (Ref. 25, Chap. 19).

Assessing the generality of the cooperative model

The cooperative model assumes several conditions and hypotheses. We examine the generality of these assumptions in the following.

Assumption: An environmental change induces epigenetic changes of phenotype

This is the most fundamental assumption of the cooperative model. As we have reviewed in first section, various examples are already known (the Dutch Hunger Winter, stress resistance of rats, honeybees' caste, etc.) so that this assumption can be validated. Waddington's experiments also demonstrate that external stimuli such as heat shock can induce acquired phenotypic changes.

The theory of Lande[26] is similar to ours in that organisms respond to a sudden and large-scale environmental change by phenotypic plasticity. His theory also predicts that a species adapts to the environmental change by phenotypic plasticity which is gradually genetically assimilated. However, Lande assumes classical "phenotypic plasticity" which is to be compared to our "epigenetic change." In addition, Lande does not treat qualitative phenotypic changes, and does not assume truncation selection. His model also does not exhibit the cumulative selection, which may be the reason for the rather long time-scale (10^3–10^4 generations) for the genetic assimilation to take place. However, as the experiments of Waddington as well as our simulations suggest, such a slow rate of genetic assimilation is questionable.

Assumption: There exist adaptive epigenetic changes of phenotypes

Even if an environmental change causes a phenotypic change due to epigenetic changes, evolution will not take place unless the phenotypic change is adaptive. Consider the case of the Dutch Hunger Winter. The fetuses during that time had epigenetic changes to endure poor nutrition. But the famine ended in several months, and the children were now exposed to overnutrition, which would lead to obesity and other lifestyle-related diseases in their later life. However, what if the state of undernutrition had persisted for a longer time, say, years? In that case, the people would have been able to take nutrition efficiently from a small amount of food. That is, the epigenetic change would have been adaptive. This hypothetical situation suggests that phenotypic changes that are both adaptive and epigenetic are plausible in actual evolution of animals[27].

Assumption: Genetic factor, G.

The genotypic value, G, in Eqs. (1) and (2) is identical to the formulation of the additive polygenic model in quantitative genetics[28]. In our definition, we assumed that the genotypic value may be either positive or negative so that the coefficients $w(j)$ are included. This definition expresses that a macroscopic phenotypic change is induced by multiple, possibly many, genetic mutations. Strictly speaking, this definition holds only for quantitative traits. We nevertheless assume that possibly qualitative macroscopic phenotypes may be modeled as quantitative traits combined with truncation selection, and hence, Eq. (5) should be regarded as an approximation.

We have assumed that a half ($L/2=10$) of the genetic mutations are positive, which is greater than the threshold value ($T=5$). This means that the combination for the genetic mutations to have $G \geq T$ is not unique. This corresponds to the observation that multifactorial diseases are caused by different genetic (and epigenetic) factors for different individuals.

In an evolutionary time scale, the F term represents a fast response to the environment. This means that even the individuals of the 0-th generation can exhibit phenotypic changes due to the F factor. This contrasts with the G factor, which represents a much slower response. Thus, the cooperative model is characterized by the combination of the fast and slow responses represented by the F and G terms, respectively.

Assumption: Accumulation of genetic mutations

In the absence of any environmental changes, evolution does not occur, but genetic mutations (including single-nucleotide polymorphisms) do accumulate in the population. For example, it is suggested that a significant amount of genetic mutations have been accumulated in the present human population[29–31]. Most of these genetic mutations are neutral. When the environment changes, those genetic mutations that contribute to advantageous phenotypic changes will be naturally selected and accumulate in the population. The cooperative model suggests that in order for genetic mutations to accumulate, the cumulative selection must operate, which in turn requires non-genetic factors (phenotypic plasticity or epigenetic changes) in addition to genetic factors. Thus, the cooperative model requires that a sufficient variety of genetic mutations already exist in the population before the environmental change. It is interesting to note that these genetic mutations are neutral before the environmental change, but they become advantageous or disadvantageous, if they are related to relevant phenotypes, after the environmental change[32]. This indicates that the neutrality of genetic mutations is relative to the environment.

Assumption: Sexual reproduction

This is a prerequisite, rather than an assumption, on which the cooperative model is based. What is important here is that parental genomes are shuffled by genetic recombination when being transmitted to offsprings. This process makes it possible for an individual to accumulate mutations that existed at different loci of its parents. Thus, sexual reproduction is a necessary condition for the cooperative model and cumulative selection. This, in turn, implies that the cumulative selection will not be observed in prokaryotes, because asexual reproduction is usually not accompanied by genome shuffling. The evolutionary synthesis is perhaps more appro-

priate for the evolution of prokaryotes than the cooperative model.

To be more precise, genome shuffling accompanied by sexual reproduction is affected by the initial gene arrangement in the genome, recombination rate, linkage disequilibrium, and so on. However, the details of genetic recombination were not taken into account in this model, as they are not essential at the present level of abstraction.

Assumption: Evolution begins with an environmental change

The cooperative model assumes that evolution is triggered by an environmental change. Although some investigators[26,33–35] have recently studied the effect of abrupt environmental changes on evolution, the evolutionary synthesis traditionally assumes that evolution starts from the (genetic) variation of individuals in the population. This assumption of the evolutionary synthesis is based on the idea of Darwin who regarded selective breeding of domestic animals as the model of evolution. That is, selective breeding (evolution) proceeds by artificially (naturally) selecting advantageous mutants. Although Darwin did not deny the possibility of evolution by environmental changes, he rather stressed that (gradual) evolution may occur even in the absence of environmental changes. However, whether the gradual evolution is possible in the absence of environmental changes requires a closer examination. In terms of the adaptive (fitness) landscape, evolution proceeds towards a local maximum so that the evolution will halt when the maximum is reached. When the environment changes, the whole landscape will change so that the previous maximum point is no longer a maximum and new maxima will appear at different points[36]. This change makes previously stable species unstable so that they need to change their positions in the landscape toward a new maximum. This argument based on the adaptive landscape suggests that evolution will eventually stop in the absence of environmental changes (except for genetic drift) and that evolution will occur after an environment change.

Assumption: Selection by threshold (truncation selection)

In the conventional theory of evolutionary synthesis, individuals with relatively greater fitness in the population are naturally selected whereas in the cooperative model, individuals with a phenotypic value greater than a given threshold are naturally selected. This difference is related to the treatment of environmental change. The cooperative model assumes that evolution is triggered by an environmental change which exerts an external selective pressure on individuals. Such an external pressure may be well modeled by the truncation selection or threshold model. The evolutionary synthesis usually does not consider environmental changes and the selective pressure exerted thereby so that threshold models are hardly employed (Ref. 17, Chap. 17.2).

In addition, the onset of diseases is explained by threshold models in population genetics (Ref. 28, Chap. 5). Our assumption that a qualitative phenotypic change occurs due to epigenetic changes is similar to the case of diseases. Therefore, the application of the threshold model in the present study is reasonable.

In summary, the assumptions in the cooperative model are reasonable, and are expected to hold for a wide range of species. For example, sexual reproduction is observed in virtually all eukaryotes, and epigenetic phenomena are known for plants, animals as well as fungi[37] and protists[38]. Therefore, the present model, incorporating the cumulative selection mechanism, should be applicable to a wide range of eukaryotic organisms including plants and animals. Among the assumption examined above, perhaps the most difficult one is that of the existence of adaptive epigenetic changes. The cooperative model does not work unless this assumption holds (even if all the others are valid). The variety of eukaryotic organisms and the time-scale of their evolution may suggest that there might have been many occasions where the cooperative mechanism had worked. In order to estimate the frequency of evolution by the cooperative mechanism, we need to accumulate more knowledge about the relationship between epigenetic changes at molecular level and phenotypic changes at macroscopic level[39].

Phenotype-driven mechanism of evolution

Many researchers have argued the possibility of "phenotype-driven mechanism of evolution" which is much faster than gradual evolution suggested by the conventional theory of evolutionary synthesis[40–46]. In the era of classical biology, the Baldwin effect and Schmalhausen and Waddington's genetic assimilation have been proposed (Ref. 23, Chap. 10). More recently, the book by Gilbert and Epel[23] and the theory of facilitated phenotypic variation by Kirschner and Gerhart[47] argue similar ideas. Their basic idea that "the phenotype proceeds the genotype"[24] seems plausible, but none of them has succeeded in proposing a concrete evolutionary mechanism that implements the idea. So far, there is no model of evolution that overcomes the modern evolutionary synthesis.

The cooperative model, however, can explain all the phenomena implied by the phenotype-driven evolution. We have already seen that the genetic assimilation well corresponds to the simulation of the cooperative model. The conventional theory of evolutionary synthesis was not able to logically explain this phenomenon[22]. By incorporating the epigenetic effect on the phenotype, the cooperative model provides a ratchet mechanism to promote the cumulative selection.

Besides the genetic assimilation, Kirschner and Gerhart have proposed the "theory of facilitated phenotypic variation"[47]. They start by asking how the accumulation of random mutations leads to new, adaptive traits. Based on the latest knowledge of molecular and cellular biology as well as developmental biology, they argue that new traits are obtained when fundamental processes in development are "deconstrained" and that deconstraining is facilitated by ran-

dom genetic mutations. However, this hypothesis is logically flawed, as it states that random genetic mutations cause adaptive traits through ontogenesis, which is too opportunistic (if random mutations affect ontogenesis, it can be also harmful). To correct their hypothesis, we should first abandon the causal relationship between random genetic mutations and deconstraining (or plasticity) of ontogenesis. Then, we should assume that the cooperation between ontogenetic plasticity and genetic mutations induces a phenotypic change that is naturally selected (i.e., the cooperative model).

There have been many studies on possible roles of epigenetics in evolution. In particular, it has been reported that some epigenomic marks in germ cells are directly transmitted to the next generation (i.e., transgenerational epigenetic inheritance as described in the first section). This phenomenon is often observed in plants[48], and examples in animals are reviewed by Jablonka[49] and summarized by Gilbert and Epel (Ref. 23, pp. 450–453). However, epigenomic marks transmitted to the next generation do not change the genotype (or DNA sequence). For evolution to occur, epigenetic changes must be replaced with a change in genotype. The observation of transmitted epigenomic marks do not explain how such replacement is possible. The epigenomic marks should be regarded as an element constituting a phenotypic change, whether they are transmitted to the next generation or not. Thus, they may be represented as the F term in Eq. (2) and be made related to evolution.

Conclusion

We have constructed the cooperative model of evolution by shifting the object of natural selection from the phenotype caused solely by genetic factors and environmental deviation (modern evolutionary synthesis) to the one caused by both genetic and epigenetic factors. Simulations based on the cooperative model exhibited much faster evolution (fixation of adaptive genetic mutations) than those based on the modern evolutionary synthesis. It was found that this accelerated evolution was made possible by the cumulative selection and that the cumulative selection was made possible by the cooperation of genetic and epigenetic factors. In the context of theories of evolution, the cooperative model belongs to the family of "phenotype-driven evolution." For example, the genetic assimilation is very closely related with the cooperative model. Curiously, although there are many researchers who advocate the advantages of phenotype-driven evolution, no one seems to have succeeded in formulating a concrete example of the phenotype-driven mechanism of evolution. The cooperative model may be a first such example, and it automatically leads to the cumulative selection. The cumulative selection is not only incompatible with the conventional theory of modern evolutionary synthesis, but also has been ignored by those who support the phenotype-driven evolution. The cooperative model has demonstrated that the cumulative selection is logically possible based on plausible assumptions and a simple equation.

Note added in proof

While this paper was being reviewed, a comment appeared in *Nature*, provocatively entitled "Does evolutionary theory need a rethink?"[50] In this Comment, a group of people, saying "Yes, urgently," argue that the standard evolutionary theory is too much gene-centric and advocate the "extended evolutionary synthesis" that incorporates the effects of development and environment, while the other group, saying "No, all is well," argue that the present evolutionary theory is comprehensive enough to explain a wide range of evolutionary phenomena. The Comment vividly shows us that the conflict between the two groups, having persisted for more than 10 years with ever increasing intensity, is about to explode. It appears to us that only a new theory that truly overcomes the standard theory can resolve the conflict. We believe that the cooperative model that we have proposed in this paper is such a theory as it embodies the mechanism of cumulative selection that overcomes the limitation of the standard (conventional) theory. We therefore believe it is an "extended evolutionary synthesis" in its true sense. We sincerely invite the reader to verify our claim.

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