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A scaling law of multilevel evolution: how the balance between within- and among-collective evolution is determined

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Abstract

Numerous living systems are hierarchically organized, whereby replicating components are grouped into reproducing collectives—e.g., organelles are grouped into cells, and cells are grouped into multicellular organisms. In such systems, evolution can operate at two levels: evolution among collectives, which tends to promote selfless cooperation among components within collectives (called altruism), and evolution within collectives, which tends to promote cheating among components within collectives. The balance between within- and among-collective evolution thus exerts profound impacts on the fitness of these systems. Here, we investigate how this balance depends on the size of a collective (denoted by $N$) and the mutation rate of components ($m$) through mathematical analyses and computer simulations of multiple population genetics models. We first confirm a previous result that increasing $N$ or $m$ accelerates within-collective evolution relative to among-collective evolution, thus promoting the evolution of cheating. Moreover, we show that when within- and among-collective evolution exactly balance each other out, the following scaling relation generally holds: $Nm^a$ is a constant, where scaling exponent $a$ depends on multiple parameters, such as the strength of selection and whether altruism is a binary or quantitative trait. This relation indicates that although $N$ and $m$ have quantitatively distinct impacts on the balance between within- and among-collective evolution, their impacts become identical if $m$ is scaled with a proper exponent. Our results thus provide a novel insight into conditions under which cheating or altruism evolves in hierarchically organized replicating systems.

Keywords: major evolutionary transitions; multilevel selection; group selection; power law; Price equation; quantitative genetics

Introduction

A fundamental feature of living systems is hierarchical organization, in which replicating components are grouped into reproducing collectives (Maynard Smith and Szathmáry 1995). For example, replicating molecules are grouped into protocells (Joyce and Szostak 2018), organelles such as mitochondria are grouped into cells (Burt and Trivers 2006), cells are grouped into multicellular organisms (Buss 1987), and multicellular organisms are grouped into eusocial colonies (Davies et al. 2012; Gershwin et al. 2014).

Such hierarchical organization hinges on altruism among replicating components (Bourke 2011), the selfless action that increases collective-level fitness at the cost of self-replication of individual components (West et al. 2007). For example, molecules in a protocell catalyze chemical reactions to facilitate the growth of the protocell at the cost of self-replication of the molecules, a cost that arises from a trade-off between serving as catalysts and serving as templates (Durand and Michod 2010; Iwata et al. 2013). Likewise, cells in a multicellular organism perform somatic functions beneficial to the whole organism, such as defence and locomotion, at the cost of cell proliferation due to different trade-offs (Bell 1985; Buss 1987; Kirk 1998).

Altruism, however, entails the risk of invasion by cheaters—selfish components that avoid altruism and instead replicate themselves to the detriment of a collective. For example, parasitic templates replicate to the detriment of a protocell (Maynard Smith 1979; Bansho et al. 2016), selfish organelles multiply to the detriment of a cell (Burt and Trivers 2006), and cancer cells proliferate to the detriment of a multicellular organism (Greaves and Maley 2012; Aktipis et al. 2015). Since cheaters replicate faster than altruists within a collective, they can out-compete the altruists, causing the decline of collective-level fitness—within-collective evolution, for short. However, collectives containing many altruists can reproduce faster than those containing many cheaters, so that altruists can be selected through competition among collectives—among-collective evolution. Evolution thus operates at multiple levels of the biological hierarchy in conflicting directions—conflicting multilevel evolution. Whether within- or
Materials and methods

Model

Our model is an extension of the Wright–Fisher model to incorporate conflicting multilevel evolution (Ewens 2004). The model consists of a population of $M$ replicators grouped into collectives, each consisting of at most $N$ replicators (Figure 1 and Table 1). The number of replicators in a collective can increase or decrease, and if this number exceeds $N$, the collective randomly divides into two.

Replicator $j$ in collective $i$ is assigned a heritable quantitative trait (denoted by $k_j$) representing the degree of altruism it performs within collective $i$ (e.g., $k_j$ represents the amount of chemical catalysis a replicating molecule provides in a protocell or the amount of somatic work a cell performs in a multicellular organism). Replicators are assumed to face a trade-off between performing altruism and undergoing self-replication. Thus, the fitness of individual replicators (denoted by $w_i$) decreases with individual trait $k_j$, whereas the collective-level fitness of replicators ($w_{ij}$) increases with collective-level trait ($k_{ij}$), where ($x_i$) represents $x_i$ averaged over replicators in collective $i$ (i.e., $x_i$ is averaged over the index marked with a tilde; see also Table 1). For simplicity, we assume that the strengths of selection within and among collectives, defined as

$$s_w = -\frac{\partial \ln w_{ij}}{\partial k_j}, \quad s_a = \frac{\partial \ln (w_{ij})}{\partial (k_{ij})},$$

respectively, depend only very weakly on $k_j$ and $k_{ij}$ (i.e., $\partial s_w / \partial k_j \approx 0$ and $\partial s_a / \partial (k_{ij}) \approx 0$). This assumption implies that the relative fitness of replicators and collectives is translationally invariant with respect to $k_j$ and $k_{ij}$, respectively—that is, $(w_{ij} + \Delta w_{ij})/w_{ij} \approx 1 + s_w \Delta k_j$, and $(w_{ij} + \Delta (w_{ij}))/(w_{ij}) \approx 1 + s_a \Delta (k_{ij})$. Owing to this assumption, our model informs only about short-term evolution. For computer simulations, we used the following fitness function:

$$w_{ij} = e^{s_w (k_j)} e^{-s_a k_{ij}} / (e^{-s_a k_{ij}}),$$

where $s_w$ and $s_a$ are constant so that Equation (2) satisfies the above assumption. This particular form of fitness function, however, does not affect our main conclusion, as will be seen from

![Figure 1](https://academic.oup.com/genetics/article/220/2/iyab182/6409194)
Table 1 Symbol list

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M$</td>
<td>Total number of replicators.</td>
</tr>
<tr>
<td>$N$</td>
<td>Maximum number of replicators per collective.</td>
</tr>
<tr>
<td>$L$</td>
<td>Total number of collectives.</td>
</tr>
<tr>
<td>$n_i$</td>
<td>Number of replicators in collective $i$.</td>
</tr>
<tr>
<td>$k_{ij}$</td>
<td>Degree of altruism performed by replicator $j$ in collective $i$.</td>
</tr>
<tr>
<td>$w_{ij}$</td>
<td>Fitness of replicator $j$ in collective $i$.</td>
</tr>
<tr>
<td>$s_{w}$</td>
<td>Within-collective selection strength: $-\frac{\partial}{\partial (k_{ij})} \ln(w_{ij})$.</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Variance of $\epsilon$ (only in continuous-trait model).</td>
</tr>
<tr>
<td>$\langle x_i \rangle$</td>
<td>Within-collective average: $\frac{n_i^{-1} \sum_{j=1}^{n_i} x_{ij}}{n_i}$.</td>
</tr>
<tr>
<td>$\langle \langle x_{ij} \rangle \rangle$</td>
<td>Global average: $M^{-1} \sum_{i=1}^{M} \sum_{j=1}^{n_i} x_{ij} = \text{ave}[\langle x_{ij} \rangle]$.</td>
</tr>
<tr>
<td>$\mathbb{E}[X]$</td>
<td>Expected $X$ after one iteration of Wright–Fisher process.</td>
</tr>
<tr>
<td>$\text{cov}_{ij}([x_i, y_j])$</td>
<td>Within-collective covariance: $\frac{n_i^{-1} \sum_{j=1}^{n_i} ((x_{ij} - \langle x_{ij} \rangle)(y_{ij} - \langle y_{ij} \rangle))}{n_i}$.</td>
</tr>
<tr>
<td>$\text{cov}_{ij}([x_j, y_j])$</td>
<td>Among-collective covariance: $M^{-1} \sum_{i=1}^{M} n_i (x_{ij} - \text{ave}[x_i])(y_{ij} - \text{ave}[y_j])$.</td>
</tr>
<tr>
<td>$v_w$</td>
<td>Within-collective variance of $k_{ij}$: $\text{ave}[[{(k_{ij} - \langle k_{ij} \rangle)}^2]]$.</td>
</tr>
<tr>
<td>$v_t$</td>
<td>Total variance of $k_{ij}$: $\langle{(k_{ij} - \langle k_{ij} \rangle)}^2\rangle = v_w + v_a$.</td>
</tr>
<tr>
<td>$c_w$</td>
<td>Within-collective third central moment of $k_{ij}$: $\text{ave}[[{(k_{ij} - \langle k_{ij} \rangle)}^3]]$.</td>
</tr>
<tr>
<td>$c_a$</td>
<td>Among-collective third central moment of $k_{ij}$: $\text{ave}[[{(k_{ij} - \langle k_{ij} \rangle)}^3]]$.</td>
</tr>
</tbody>
</table>

The state of the model is updated in discrete time (Figure 1). In each generation, $M$ replicators are sampled with replacement from replicators of the previous generation with probabilities proportional to $w_{ij}$, as in the Wright–Fisher process (Ewens 2004). During the above sampling, a replicator inherits group identity $i$ and trait $k_{ij}$ from its parental replicator with potential mutation (no migration among collectives is allowed). More precisely, the $k_{ij}$ value of a replicator is set to $k_{ij} + \epsilon$, where $k_{ij}$ is the trait of the parental replicator, and $\epsilon$ takes a value of zero with a probability of 1 − $m$ or a value sampled from a Gaussian distribution with mean zero and variance $\sigma$ (determining mutation step size) with a probability of $m$ (representing a genetic or epigenetic mutation rate). The assumption that the mean of $\epsilon$ is zero is made on the following premise: evolution is mainly driven by selection or random genetic drift, and the direction of evolution is not directly determined by mutation. Although this premise often approximates reality, it can be wrong if a mutation rate is so high as to dictate the direction of evolution as in the error catastrophe (Domingo et al. 2005), a situation that is ignored in this study. The assumption that $\epsilon$ is independent of $k_{ij}$ is made for simplicity and implies that our model informs only about short-term evolution. Although we could reduce the number of parameters by aggregating $m$ and $\sigma$ into $ma$ (which is the variance of $\epsilon$), we keep them separate so that the mutation rate as usually defined is discernible.

After the above sampling, collectives containing more than $N$ replicators are randomly divided into two, and those with no replicators removed (Figure 1).

Parameter-sweep diagram

The values of $\Delta(\langle k_{ij} \rangle)$ used to create Figure 2 were estimated from slopes of the least squares regression of $\langle (k_{ij}) \rangle$ against time. The estimates were unreliable if $|\Delta(\langle k_{ij} \rangle)| < 3 \times 10^{-7}$ owing to a limitation of simulations, in which case the right-hand side (RHS) of Equation (3) was used as a proxy for $\Delta(\langle k_{ij} \rangle)$. Parameter-region boundaries across which $\Delta(\langle k_{ij} \rangle)$ changes sign were estimated as follows. The value of $N$ for which the RHS of Equation (3) becomes zero was estimated for each selected value of $m$ with linear interpolation from the values of the RHS of Equation (3) (or $\Delta(\langle k_{ij} \rangle)$) if $s_a \geq 10$ measured through simulations for two smallest values of $N$ for which the RHS of Equation (3) (or $\Delta(\langle k_{ij} \rangle)$) if $s_a \geq 10$ has different signs (the values of $m$ and $V$ for which simulations were run were selected as shown in Figure 2). The resulting estimates of $N$ were then used to estimate the value of $x$ through the least squares regression of $N \propto m^{-e}$. Ancestor tracking

Ancestor tracking is a method that provides novel information about evolutionary dynamics by tracking the genealogy of individuals backwards in time. In our study, individuals whose genealogy was tracked were collectives. Since collectives undergo binary fission, their genealogy can be pictured as a binary tree, where an event of binary fission is represented by the coalescence of two branches of the tree. As the tree is traversed from the tips to the root (i.e., from the present to the past), all branches eventually coalesce to a single branch, the stem of the tree, which represents the lineage of common ancestors of all collectives present at a particular point in time. Information about common ancestors can be visualized as time-series data along their line of descent, i.e., along the stem of the tree. In Figure 5D, $n_i$ and $(k_{ij})$ of the common ancestors are plotted.

Results

Demonstration of a scaling relation by computer simulations

By simulating the above model, we measured the rate of change of $\langle (k_{ij}) \rangle$, where $\langle (k_{ij}) \rangle$ is $x_0$ averaged over all replicators, at steady
states as a function of $m$ and $N$, assuming $s_w = s_a$. The result indicates the existence of two distinct parameter regions, where $\langle k_j \rangle$ either increases or decreases through evolution (Figure 2; the section Parameter-sweep diagram). (Note that although the model displays an unlimited increase or decrease of $\langle k_j \rangle$ over time, the model is intended to inform about short-term evolution as described above; therefore, its result should be considered as providing information about an instantaneous rate of evolution in a steady state for given parameters.)

The two parameter regions mentioned above are demarcated by scaling relation $N \propto m^{-a}$, for $a \geq 0 \geq s \geq 0$ (Figure 3A)—i.e., the evolution of $\langle k_j \rangle$ becomes increasingly independent of $m$ as $s$ decreases. Similar scaling relations hold also when $s_w = 10 s_a$ or $s_w = 0.1 s_a$ (Figure 3A). These results generalize those previously obtained with specific models of protocells (Takeuchi et al. 2016, 2017).

Mathematical analysis of the scaling relation

Next, we present a theory that can account for $N \propto m^{-a}$ under the assumptions that $s_a$ and $s_w$ are sufficiently small. Although such a theory could in principle be built by calculating the dynamics of the frequency distribution of $k_j$, for simplicity, we instead calculate the dynamics of the moments of this distribution. The expected change of $\langle k_j \rangle$ per generation is expressed by Price’s equation as follows (Price 1972; Hamilton 1975) [see Supplementary Text S1 “Derivation of Equation (3)”:]

$$E[A(\langle k_j \rangle)] = \langle |w_j| \rangle \{ \text{cov}_{ij}[w_{ij}, (k_{ij})] + \text{ave}_{ij}[\text{cov}_{ij}[w_{ij}, k_{ij}]] \},$$

where $E[x]$ is the expected value of $x$ after one iteration of the Wright-Fisher process, $\text{cov}_{ij}[x_i, y_j]$ is the covariance between $x_i$ and $y_j$ over replicates, $\text{cov}_{ij}[x_i, y_j]$ is the covariance between $x_i$ and $y_j$ over replicators in collective $i$, and $\text{ave}_{ij}[x_i]$ is $x_i$ averaged over replicates (see Table 1 for precise definitions). Note that the RHS of Equation (3) is divided by $\langle |w_j| \rangle$, so that $E[A(\langle k_j \rangle)]$ depends on relative rather than absolute fitness (note also that relative fitness is independent of the absolute values of $k_j$ and $\langle k_j \rangle$, as described in the section Model).

Expanding $w_j$ in Equation (3) as a Taylor series around $\langle k_j \rangle$ and $k_j = \langle k_j \rangle$ (Iwasa et al. 1991), we obtain [see Supplementary Text S1 “Derivation of Equation (4)”,]

$$E[A(\langle k_j \rangle)] = s_a v_a - s_w v_w + O(s_a^2) + O(s_w^2),$$

where $v_a$ is the variance of $\langle k_j \rangle$ among collectives, and $v_w$ is the average variance of $k_j$ among replicators within a collective (Table 1). Equation (4) implies that if $s_a$ and $s_w$ are sufficiently small, the boundary of the parameter regions, on which $E[A(\langle k_j \rangle)] = 0$, is given by the following equation: $s_a v_a = s_w v_w$. Since this equation is expected to imply scaling relation $N \propto m^{-a}$, we need to calculate $v_w$ and $v_a$ to calculate $a$.

To calculate $v_w$ and $v_a$, we first consider a neutral case where $s_a = s_w = 0$. Let the total variance be $v_t = v_a + v_w$. In each generation, $M$ replicators are randomly sampled from replicators of the previous generation with mutation. The mutation increases $v_t$ to the variance of $k_j + \epsilon$, which is $v_t + 2 \sigma$, since $k_j$ and $\epsilon$ are uncorrelated (the variance of $\epsilon$ is $m \sigma$). Moreover, the sampling decreases the variance by a factor of $1 - M^{-1}$ (in general, sample variance of sample size $M$ is smaller than population variance by a factor of $1 - M^{-1}$). Therefore, the expected total variance of the next generation is

$$E[v_t'] = (1 - M^{-1})(v_t + m \sigma).$$

Likewise, the expected within-collective variance of the next generation can be calculated as follows. To enable this calculation, we assume that all collectives always consist of $\beta^{-1} N$ replicators, where $\beta$ is a constant (as will be described later, this approximation becomes invalid for $s \ll 1$, however, its validity for $s \ll 1$ is suggested by the fact that it enables us to calculate scaling exponent $a$ correctly). Randomly sampling $\beta^{-1} N$ replicators from a collective with mutation is expected to change $v_w$ to

$$E[v_w'] = (1 - \beta N^{-1})(v_w + m \sigma).$$

Since $E[v_t'] = E[v_t] - E[v_w']$, we obtain

$$E[v_t'] = (1 - M^{-1})v_a + (\beta N^{-1} - M^{-1})(v_w + m \sigma),$$

where the first term on the RHS indicates a decrease due to random genetic drift, and the second term indicates an increase due...
to random walks of \( \langle k_j \rangle \) through within-collective neutral evolution. Note that Equations (6) and (7) partially incorporate the collective-level division-removal process implicitly through the assumption of a constant collective size.

Next, we incorporate the effect of selection on \( v_w \) and \( v_a \). Allowing for the fact that replicators are sampled with probabilities proportional to fitness \( w_i \), we can use Price's equation to express the expected values of \( v_w \) and \( v_a \) after one iteration of the Wright–Fisher process as follows [see Supplementary Text S1 "Derivation of Equation (8)", Zhang and Hill 2010]:

\[
E[v'_w] = (1 - \beta N^{-1})[v_w + m\sigma - s_w w_o + O(s_w^2)]
\]

\[
E[v'_a] = (1 - M^{-1})[v_a + s_a c_a + O(s_a^2) + O(s_a c_a)]
\]

\[
+ (\beta N^{-1} - M^{-1})[v_w + m\sigma - s_w w_o + O(s_w^2)],
\]

where \( c_w \) is the average third central moments of \( k_j \) within a collective, and \( c_a \) is the third central moment of \( \langle k_j \rangle \). Besides the assumption of a constant collective size, the derivation of Equation (8) involves the additional assumption that the variance of \( k_j \) within collective \( i \) is statistically independent of \( \langle k_j \rangle \) as it varies.

Given that the dimension of \( c_w \) and \( c_a \) is equivalent to that of \( v_w^{3/2} \) and \( v_a^{3/2} \), we make a postulate, which we verify later by simulations, that

\[
c_w = -\gamma_4 v_w^{3/2},
\]

\[
c_a = -\gamma_4 v_a^{3/2},
\]

where \( \gamma_4 \) and \( \gamma_6 \) are positive constants. An intuitive reason for postulating that \( c_w < 0 \) is due to the finiteness of \( M \), as follows (Figure 4). The distribution of \( \langle k_j \rangle \) has a finite range since \( M \) is finite. The right tail of this distribution, the one with greater \( k_j \), is exponentially amplified by selection among collectives; however, the right tail cannot be extended because its length is finite (Tsimring et al. 1996; Hallatschek 2011). By contrast, the left tail is contracted by among-collective selection, and this contraction is unaffected by the finiteness of the tail length. Likewise, the finiteness of tail lengths does not affect the rightward shift of the mean of the distribution due to among-collective selection. Consequently, asymmetry builds up such that the right tail becomes shorter than the left tail, hence \( c_w < 0 \). The same argument can be applied to \( c_a \), but the direction of selection is opposite, hence the opposite sign: \( c_a > 0 \).

**Figure 4** Mechanism by which trait distribution becomes skewed owing to selection and finiteness of population. Drawing depicts frequency distributions of collective-level trait \( \langle k_j \rangle \) (orange) and effect of among-collective selection (blue arrows; for simplicity, within-collective selection is not depicted). Distribution is initially assumed to be symmetric (left), so that its third central moment \( c_w \) is zero. Tails of distribution have finite lengths due to finiteness of total population size \( M \) (red arrows). Because of finite lengths, left and right tails react differently to selection depending on whether they are amplified or reduced (red cross; see also main text). Consequently, distribution gets skewed (right), and \( c_a \) becomes negative. It is postulated based on dimension that \( c_a \propto -v_a^{3/2} \) at steady state, where \( v_a \) is variance of \( \langle k_j \rangle \).

Combining Equations (8) and (9), we obtain

\[
E[v'_w] = (1 - \beta N^{-1})[v_w + m\sigma - s_w w_o v_w^{3/2} + O(s_w^2)]
\]

\[
E[v'_a] = (1 - M^{-1})[v_a + s_a c_a + O(s_a^2) + O(s_a c_a)]
\]

\[
+ (\beta N^{-1} - M^{-1})[v_w + m\sigma - s_w w_o v_w^{3/2} + O(s_w^2)],
\]

from which we make a postulate, which we verify later by simulation:

\[
E[v'_w] = (1 - \beta N^{-1})[v_w + m\sigma - s_w w_o v_w^{3/2} + O(s_w^2)]
\]

\[
E[v'_a] = (1 - M^{-1})[v_a + s_a c_a + O(s_a^2) + O(s_a c_a)]
\]

\[
+ (\beta N^{-1} - M^{-1})[v_w + m\sigma - s_w w_o v_w^{3/2} + O(s_w^2)],
\]

Equations (10) and (11) enable us to calculate \( v_w \) and \( v_a \) at a steady state if \( s_w \) and \( s_a \) are sufficiently small (a steady state is defined as \( E[v'_w] = v_w \) and \( E[v'_a] = v_a \)). For illustration, let us consider extreme conditions in which the expressions of \( v_w \) and \( v_a \) become simple. Specifically, if \( \beta^{-1} N >> 1 \) and \( s_w \ll |v_w\sqrt{\sigma} (\beta^{-1} N)^{3/2}|^{-1} \), Equation (10) implies that

\[
v_w \approx \beta^{-1} N m\sigma.
\]

Moreover, Equation (11) implies that

\[
M^{-1} v_a + s_a v_a^{3/2} \approx (\beta^{-1} N - M^{-1}) v_w,
\]

where the term involving \( s_a M^{-1} \) is ignored under the assumption that both \( s_a \) and \( M^{-1} \) are sufficiently small (and the assumption that \( \beta^{-1} N \gg 1 \) and \( s_w \ll |v_w\sqrt{\sigma} (\beta^{-1} N)^{3/2}|^{-1} \) are used again). Substituting Equation (12) into Equation (13), we obtain

\[
v_a \approx \frac{M m\sigma}{s_a} \left( \frac{1 - \beta^{-1} N}{M} \right)^{3/2} \text{ if } s_a \ll |v_w\sqrt{\sigma} (\beta^{-1} N)^{3/2}|^{-1},
\]

\[
v_a \approx \frac{M m\sigma}{s_a} \left( \frac{1 - \beta^{-1} N}{s_a} \right)^{3/2} \text{ if } s_a \gg |v_w\sqrt{\sigma} (\beta^{-1} N)^{3/2}|^{-1}.
\]

Figure (14) shows that \( v_a \) at a steady state is independent of \( N \) if \( \beta^{-1} N \ll M \), a result that might be contrary to one’s intuition since the law of large number, increasing \( N \) reduces random
genetic drift within collectives and thus deaccelerates the growth of \(v_s\). Indeed, the increase of \(v_s\) per generation is approximately proportional to \(N^{-1/2}\), according to the second term of Equation (11). However, since \(v_w \propto M N\) according to Equation (12), \(N\) cancels out, so that \(v_s\) is independent of \(N\) (see Supplementary Figure S1 for simulation results). This cancelation resembles that occurring in the rate of neutral molecular evolution, which is also independent of population size (Kimura 1968).

To examine the validity of Equations (10) and (11), we measured \(v_s\), \(v_w\), \(c_w\), and \(c_w\) through simulations, assuming \(v_w = s_w = s\) (Figure 5). The results show that \(v_s \propto M\) for a very small value of \(s\) (viz., \(10^{-6}\)) in agreement with Equation (14) (Figure 5A). Moreover, \(v_w \propto M N\) as predicted by Equation (12) (Figure 5B), except for cases where \(\Delta(k_{ji}) < 0\) (this deviation will be discussed later). Finally, \(c_s = \gamma_s v_s^{1/2}\) if \(s < 1\) (Figure 5C), and \(c_w = \gamma_w v_w^{1/2}\) if \(s < 1\) and \(\Delta(k_{ji}) < 0\) (Supplementary Figure S2), as postulated in Equation (9). Taken together, these results support the validity of Equations (10) and (11) when \(s_w\) and \(s_s\) are sufficiently small, and \(m\) and \(N\) are close to the boundary of the parameter regions (i.e., \(\Delta(k_{ji}) < 0\)).

Using Equations (10) and (11), we can calculate the scaling exponent \(x\) of the boundary of the parameter regions for sufficiently small \(s_s\) and \(s_w\). Since \(\Delta(k_{ji}) = 0\) on the parameter boundary, Equation (4) implies that \(v_s/v_w = s_w/s_s\). Thus, for extreme parameter conditions (viz., \(1 < \beta^{-1}N < M\), and \(s_w = \sqrt{\gamma_s s_a M^{1/2}}\), Equations (12) and (14) imply that

\[
x \approx 0 \quad \text{if} \quad s_a < \left(\frac{\gamma_s \sqrt{\gamma_s s_a M^{1/2}}}{1}\right)^{-1}
\]

\[
x \approx 1/3 \quad \text{if} \quad s_a > \left(\frac{\gamma_s \sqrt{\gamma_s s_a M^{1/2}}}{1}\right)^{-1}.
\]

For \(s_a = \left(\frac{\gamma_s \sqrt{\gamma_s s_a M^{1/2}}}{1}\right)^{-1}\), Equations (12) and (13) imply that

\[
r M^{-\beta^{-1}} (N + \Gamma s_a \Gamma N)^{1/2} = 1,
\]

where \(r = s_w/s_s\) and \(\Gamma = \gamma_s \sqrt{\gamma_s \beta M}\), and Equation (16) implies that \(x\) increases from zero to one-third as \(\gamma s_a\) increases from zero.

We also numerically obtained \(x\) by calculating the values of \(N\) and \(M\) (in \(10^{-10}, 10^{-11}\)) that satisfy \(v_s/v_w = s_w/s_s\) at a steady state using Equations (10) and (11), and the values of \(\beta\), \(\gamma_s\), and \(\gamma_w\) estimated from Figure 5B and Supplementary Figure S2, respectively (viz., \(\beta = 1.45\) and \(\gamma_w = 0.26\) through least squares regression of Equation (12) and (9)) for \(s = 10^{-6}\) and \(10^{-7}\), respectively; \(\gamma_s = 0.25\) through least squares regression of Equation (9) for \(\Delta(k_{ji}) < 0\). The results agree with the simulation results for \(s_a < 1\) when \(r \approx 1\) and 10, and for \(s_a > 10^{-3}\) when \(r = 0.1\) (Figure 3A). We do not know why the validity of analytical prediction is restricted when \(r\) is small. Overall, the above results support the validity of Equations (10) and (11) for sufficiently small values of \(s_s\) and \(s_w\).

In addition, we note that the postulate in Equation (9) is also supported by previous studies calculating the evolution of a quantitative trait (viz., fitness) subject to single-level selection (Tsimring et al. 1996; Hallatschek 2011). These studies show that fitness increases through evolution at a rate proportional to the two-thirds power of the mutation rate. That result is consistent with Equations (10) and (11) and, hence, also with Equation (9), as follows. Since Tsimring et al. (1996) assume single-level selection and a very large population, let us also assume that \(s_w = 0\) and \(M \to \infty\), respectively, in our model. Then, Equations (4) and (14) imply that logarithmic fitness, \(\ln((w_i)) \times (k_{ji}))\), increases at a rate proportional to \(m^{1/3}\) (Supplementary Figure S1). Reversing the argument, we can also use the model of Tsimring et al. (1996) to estimate the value of \(\gamma_a\) as about 0.25 (Supplementary Text S1 under “Estimation of \(\gamma_a\)”), which matches the value measured in our model (viz., 0.26). Moreover, the model of Tsimring et al. (1996) can also be applied to estimate \(\gamma_w\), and the value of \(\gamma_w\) measured in our model is about 0.25 (Supplementary Figure S2). Taken together, these agreements corroborate the validity of Equation (9).

Finally, to clarify why Equations (10) and (11) deviate from the simulation results for \(s > 1\) or \(\Delta(k_{ji}) < 0\), we tracked the genealogy of collectives backwards in time to observe the common ancestors of all collectives (the section Ancestor tracking). Figure 5D displays the dynamics of \(k_{ji}\) and \(n_i\) (the per-collective number of replicators) in these ancestors along their single line of descent. The results indicate that the model displays a phenomenon previously described as evolutionarily stable disequilibrium (ESD, for short; Takeuchi et al. 2016). Briefly, the collectives constantly oscillate between growing and shrinking phases (Figure 5D). During the growing phase, the collectives continually grow and divide, and their \(k_{ji}\) values gradually decline through within-collective evolution, a decline that eventually puts the collectives to a shrinking phase. In the shrinking phase, the collectives steadily decrease in the number of constituent replicators; however, their \(k_{ji}\) values abruptly jump at the end of the shrinking phase, a transition that brings the collectives back to the growing phase. This sudden increase of \(k_{ji}\) is due to random genetic drift induced by very severe within-collective population bottlenecks. Although such an increase of \(k_{ji}\) is an extremely rare event, it is always observed in the lineage of common ancestors because these ancestors are the survivors of among-collective selection, which favors high \(k_{ji}\) values (Takeuchi et al. 2016, 2017).

ESD breaks the assumption—involved in Equations (10) and (11)—that all collectives always consist of \(\beta^{-1}N\) replicators because ESD allows extremely small collectives to regrow and contribute significantly to \(v_w\) and \(v_s\) (note that the contributions of collectives to \(v_w\) and \(v_s\) are proportional to the number of replicators they contain, as defined in Table 1). We found that ESD occurs for \(s \approx 1\) (Figure 5D) or \(\Delta(k_{ji}) < 0\) (Supplementary Figure S3). Thus, ESD might be responsible for the failure of Equations (10) and (11) to predict \(x\) for \(s \approx 1\) (Figure 3) as well as the fact that \(c_s\) is not proportional to \(mN\) when \(\Delta(k_{ji}) < 0\) (Figure 5B).

Another potential reason for the failure of Equations (10) and (11) for \(s \geq 1\) is that the fact that \(s_a\) and \(c_s\) constantly oscillate with a periodic sign change of \(c_s\) (Figure 6). This oscillation not only invalidates the assumption that \(c_a = \gamma_s v_s^{1/3}\) but also makes it questionable to consider a steady-state solution of Equations (10) and (11). Finally, we add that this oscillation is distinct from ESD, in that it is observed in terms of \(v_u\) and \(v_s\), which are properties of an entire population of collectives, whereas ESD is observed in terms of the properties of common ancestors of collectives.

Comparison to a binary-trait model

To examine further the generality of the scaling relation described above, we next consider a study by Kimura (1984, 1986). Kimura has investigated a binary-trait (i.e., two allele) model of multilevel evolution formulated based on a diffusion equation. Using this model, Kimura has revealed the following scaling relation that holds when within- and among-collective evolution exactly balances each other out.
The results presented above suggest that scaling relation $N \propto m^{-\alpha}$ is a general feature of conflicting multilevel evolution. Scaling exponent $\alpha$, however, depends in a nontrivial manner on the strength of selection and whether altruism is a quantitative or binary trait.

Although we have assumed that the parameters involved in the scaling relation—the mutation rate, selection strength, and the distinction between quantitative and binary traits—are independent of each other, these parameters are potentially correlated in reality. While such correlations are not well understood (Thompson et al. 2013; Kasper et al. 2017), discussing them can...
illustrate the utility of the findings of this study. For this illustration, we first note that whether altruism is a quantitative or binary trait can be translated into the number of loci involved in altruism: a quantitative trait involves many loci, whereas a binary trait involves one. The number of loci is likely to be positively correlated with the mutation rate of the trait, and it is possibly negatively correlated with the effect size of mutation (e.g., a single locus with large effects vs many loci with small effects). The effect size of mutation, in turn, is possibly positively correlated with the strength of selection. These correlations, which we assume here for the sake of illustration, would imply a spectrum of altruism ranging from a strongly selected, binary trait with a low mutation rate to a weakly selected, quantitative trait with a high mutation rate (we are ignoring the possibility that mutations have highly heterogeneous effects). Such correlations would be conducive to the evolution of altruism, an inference that is enabled by the following findings of this study: binary-trait altruism is susceptible to the invasion by cheaters for a high mutation rate, but this susceptibility decreases with selection strength (α decreases with s); by contrast, quantitative-trait altruism is relatively insensitive to mutation for weak selection (α decreases to zero as s decreases to zero).

Although the results of this study are phrased in the language of multilevel selection (Wilson 1975; Slatkin and Wade 1978; Aoki 1982; Crow and Aoki 1982; Leigh 1983; Kimura 1984, 1986; Frank 1994; Rispe and Moran 2000; Goodnight 2005; Traulsen and Nowak 2006; Bijma et al. 2007; Chuang et al. 2009; Leigh 2010; Frank 2012; Simon et al. 2013; Tarnita et al. 2013; Fontanari and Serva 2014; Luo 2014; Takeuchi et al. 2016, 2017; Blokhuis et al. 2018; Cooney DB. 2019; Takeuchi and Kaneko 2019; van Vliet and Doebeli 2019), they can easily be rephrased, mutatis mutandis, in the language of kin selection (Cheverud 1985; Queller 1985; Queller 1992; Wolf et al. 2018; Wolf et al. 1999; Frank 1997; Gardner et al. 2007; Bijma and Wade 2008; Chuang et al. 2010; McGlothlin et al. 2010; Queller 2011; Akçay and van Cleve 2012; McGlothlin et al. 2014). To do this, we define the relatedness of replicators as the regression coefficient (kij) on kj (Hamilton 1970; Rice 2004), i.e., R = uij/(ujs + uis), and express all the results in terms of R instead of uij/uis. Therefore, our results are compatible with the kin selection theory.

An important issue to address for future research is to test whether the scaling relation is observed in reality. Such tests could in principle be conducted through evolutionary experiments.

Capturing the essence of multilevel selection, the models and analyses presented above are likely to have broad utility. They are generally relevant for the evolution of altruism in replicators grouped into reproducing collectives, e.g., symbionts, organelles, or genetic elements grouped into cells (Burt and Trivers 2006), cells grouped into multicellular organisms (Buss 1987), or other systems that have emerged through major evolutionary transitions (Maynard Smith and Szathmáry 1995).

**Data availability**

Supplementary Texts and Figures can be found in Supplementary File S1. C++ source code implementing the models can be found in Supplementary File S2. Supplementary material is available at figshare: https://doi.org/10.25386/genetics.14337251.

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**Conflicts of interest**

The authors declare that there is no conflict of interest.

**Literature cited**
