

Genetic assimilation can occur in the absence of selection for the assimilating phenotype, suggesting a role for the canalization heuristic

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Abstract

Genetic assimilation occurs when an acquired trait loses dependency on its environmental trigger and becomes an inherited trait. According to the standard quantitative genetic model for genetic assimilation, the trait is determined by the contributions of multiple genes. Trait expression occurs at a lower threshold with the trigger. Selection for the trait in the presence of the trigger increases the frequency of the trait-determining alleles. Eventually these alleles become frequent enough to breach the higher threshold for expression in the absence of the trigger. This loss of dependence on the trigger signifies genetic assimilation. Here I show that genetic assimilation can occur in the absence of selection for the trait in an evolutionary simulation of a gene network model. This contradicts the prediction of the standard quantitative genetic model, but is consistent with an explanation in terms of the canalization heuristic.

Introduction

In a classic experiment in 1953, *Drosophila* pupae were exposed to heat shock, inducing a *cross-veinless* phenocopy with a frequency of 40%. After selection for *cross-veinless* under heat shock conditions, the *cross-veinless* phenotype continued to appear even when heat shock was no longer applied (Waddington, 1953). Waddington called this loss of dependency on an environmental trigger 'genetic assimilation', and argued that it was a consequence of canalization during the period of selection (Waddington, 1953, 1961). He envisaged development as a ball rolling down a high-dimensional epigenetic landscape, and canalization as the deepening of pathways down this slope, making the developmental outcome less sensitive to perturbations. Once the *cross-veinless* canal has become deep enough, the wild-type developmental canal can no longer be found and genetic assimilation has occurred.

Others have proposed a simpler model, illustrated in Fig. 1, to explain the data (Stern, 1958; Bateman, 1959; Scharloo, 1991; Falconer & Mackay, 1996). The appearance of *cross-veinless* is assumed to be determined by the contributions of multiple genes and to occur at a lower threshold with heat shock. Selection under heat shock conditions increases the frequency of *cross-veinless* alleles, eventually breaching the higher threshold for appearance in the absence of heat shock. No reference to canalization is required to explain the phenomenon of genetic assimilation.

This second model predicts that if selection for the assimilating phenotype *cross-veinless* were relaxed, genetic assimilation would not occur. Here I test this prediction in an *in silico* model of development and evolution. This model allows selection for the assimilating phenotype to be relaxed while still allowing canalization to occur (Siegal & Bergman, 2002).

The motivation for and details of similar models of development have been described elsewhere (Wagner, 1996; Siegal & Bergman, 2002). Briefly, development is modelled as the dynamic interaction of a network of transcription factors. Each individual is defined by its genotype matrix **W**, which specifies which transcription factors activate or repress each other. A vector **S** specifies

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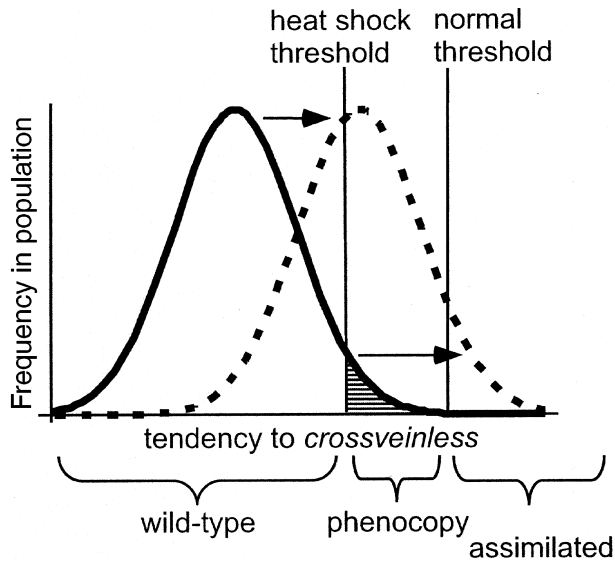


Fig. 1 Standard model to explain genetic assimilation (Falconer & Mackay, 1996). The solid curve shows the frequency distribution of genetic variance in the tendency to produce *cross-veinless*. The threshold for *cross-veinless* is normally too high for the phenotype to be seen. A *cross-veinless* phenocopy is revealed when the threshold is lowered by heat shock. After selection for the phenocopy (shaded area), the curve shifts to the right (dashed curve), eventually breaching the higher threshold for the expression of *cross-veinless* in the absence of heat shock. In this way, an acquired phenocopy becomes an inherited phenotype.

which transcription factors are present at any point in time. Development is modelled as a nonlinear, iterative function of \mathbf{W} on \mathbf{S} describing the interaction of the transcription factors, as shown in Fig. 2. If this iteration reaches equilibrium, then the final state $\hat{\mathbf{S}}$ represents that individual's phenotype. Perturbation, analogous to heat shock, can be modelled as an increase in the level of stochastic noise in the system. During evolution, segregation and mutation act on the set of \mathbf{W} genotypes.

The product of development is subject to two distinct and separable forms of selection. First, there is selection for developmental equilibrium, defined as the requirement that developmental iteration reaches some stable equilibrium of \mathbf{S} within 100 iterations, irrespective of the value of the phenotype $\hat{\mathbf{S}}$. Developmental equilibrium implies that the nonlinear process of development avoids oscillations and chaotic behaviour. Secondly and optionally, there may be additional selection for a particular value of the phenotype $\hat{\mathbf{S}}$.

When selection for a particular value of $\hat{\mathbf{S}}$ is absent, then there is no selection for the contribution of genes towards $\hat{\mathbf{S}}$. The standard model therefore predicts that genetic assimilation will not occur. Canalization, defined as decreasing sensitivity of phenotypes to perturbation by mutation, still occurs in these circumstances (Siegal & Bergman, 2002). If canalization rather than the standard

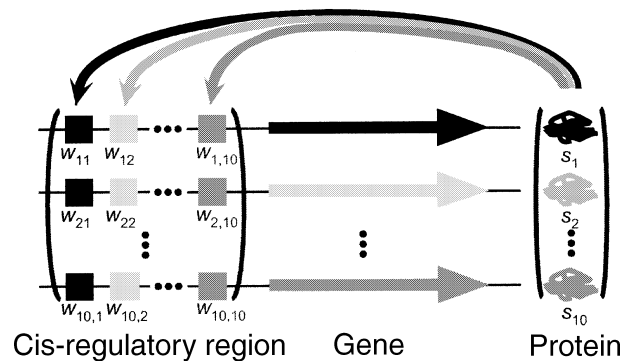


Fig. 2 Outline of developmental process. Shown on the right, a vector \mathbf{S} , consisting of ones and zeros, indicates which transcription factors are present at a given developmental time point. These transcription factors then act on each other's cis-regulatory regions, shown on the left, forming a gene network. These interactions are described by the matrix \mathbf{W} . Each element $w_{i,j}$ in this matrix describes the effect of transcription factor j on the expression of transcription factor i . Positive matrix elements indicate activation and negative elements indicate repression. The product of the \mathbf{W} matrix and the \mathbf{S} vector is added to a random noise term and fed through a step function to calculate the new vector \mathbf{S} representing which transcription factors will be produced at the next developmental time step. This process is repeated until a stable value $\hat{\mathbf{S}}$ is obtained for four time steps in a row. The matrix \mathbf{W} represents the genotype of an individual and the value of \mathbf{S} represents the phenotype of that individual. If no stable value of \mathbf{S} is obtained within 100 time steps, the developmental process is terminated. Developmental equilibrium has not been achieved, and the individual is assumed to be unviable.

model is at least partly responsible for genetic assimilation in this system, then genetic assimilation should still occur when selection for the assimilating phenotype is absent.

Materials and methods

Development

Development is illustrated in Fig. 2. The vector $\mathbf{S}(t) = [s_1 \dots s_{10}]$ describes the expression levels of 10 transcription factors at developmental time t . $s_i = 1$ indicates that gene i is 'on' and $s_i = 0$ indicates that it is 'off'. For each developmental time step, $s(t)_i = f(\mathbf{W}\mathbf{S}(t-1))_i + \varepsilon$ where ε is drawn from the normal distribution $N(0, \sigma^2)$, $f(x) = 0$ for $x < 0$ and $f(x) = 1$ for $x \geq 0$. If \mathbf{S} does not remain at some stable value $\hat{\mathbf{S}}$ for four time steps in a row within 100 time steps, then the individual represented by the matrix \mathbf{W} is assumed to be unviable as development does not reach equilibrium, and \mathbf{W} is discarded.

An environmental perturbation, analogous to heat shock or other treatment, was simulated by increasing the noise parameter σ from 0.01 to 0.5. Other models of perturbation were also investigated, including the inactivation of a single gene. Similar results were generated

using all models of perturbation tested (data not shown). Indeed, genetic assimilation can occur *in vivo* under a range of perturbations, both environmental (Waddington, 1953; Bateman, 1959; Ho *et al.*, 1983) and genetic (Rutherford & Lindquist, 1998; Sollars *et al.*, 2003). The perturbation selected here has the advantages of being very general in its effects and of being asymmetric i.e. it is clear which condition is the perturbation and which is normality.

Segregation, mutation and selection of next generation

Five hundred individuals are selected for each generation. Each potential individual is generated by segregation and mutation from the \mathbf{W} matrices of two parents drawn at random from the preceding generation. A row represents a set of cis-regulatory elements regulating the same gene, and so offspring inherit each row of the \mathbf{W} matrix as a single block. Rows are assumed to segregate randomly from each other, and so each row is inherited at random from either parent. Around half the matrix elements are active, while the other half are permanently set to zero. After segregation, each nonzero matrix element mutates with probability 0.002 (an average of 0.1 mutations per reproduction) to a new value drawn randomly from the distribution $N(0,1)$. Development of the new genotype was then simulated to calculate developmental equilibrium. If equilibrium was achieved then phenotype could also be calculated. Choice of two parents, segregation and mutation were simulated until 500 \mathbf{W} matrices were found whose development met the selection criteria, and these 500 individuals formed the next generation. Developmental equilibrium was always required for inclusion in the next generation. At some stages of the experiment, a particular phenotype was also required.

Initialization of experiment

An outline of the experiment in evolutionary time is shown in Fig. 3. An initial vector $\mathbf{S}(0)$ was generated randomly, with equal probabilities that $\mathbf{S}(0)_i = 1$ or 0. The same initial vector was used for all individuals throughout evolution. Evolution was initiated with 500 genetically identical individuals. The initial genotype was generated by setting each element of \mathbf{W} to zero with probability 0.5, otherwise sampling it from the normal distribution $N(0,1)$. At least one element in each row was required to be nonzero, and the initial individual was required to reach developmental equilibrium.

Segregation, mutation and selection were simulated for 500 generations. For each generation, new \mathbf{W} matrices were generated until 500 were found that achieved some developmental equilibrium with the low noise parameter $\sigma = 0.01$. After these 500 generations, the most frequent phenotype in the population was determined and was labelled \mathbf{S}_{old} for the remainder of the simulation. An additional 500 generations of selection for the particular phenotype $\mathbf{S} = \mathbf{S}_{old}$ followed, still at low noise. This resulted in a genetically diverse population, canalized around the phenotype \mathbf{S}_{old} .

Requirement for a phenocopy

Classical experiments on genetic assimilation can be performed *in vivo* only when a phenocopy is seen. A phenocopy is defined as a variant that is produced in response to environmental perturbation and that mimics a variant phenotype that can be produced by mutation. Mirroring this bias *in vivo*, I performed genetic assimilation experiments only on populations that exhibited a phenocopy. To test for the presence of a phenocopy, the population was subjected to perturbation for one generation. If high noise generated a variant phenotype

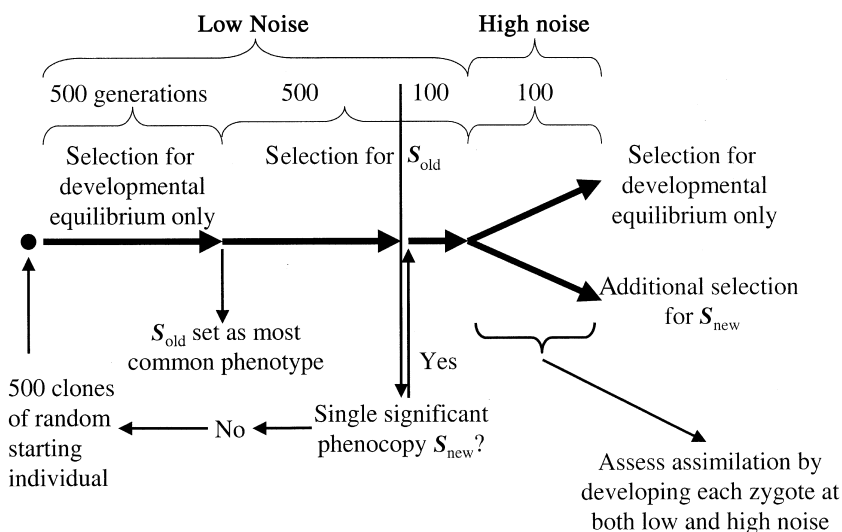


Fig. 3 Outline of experimental protocol on the time scale of evolutionary processes, indicating noise level, number of generations and selection type at each stage. A diverse population was generated by exposing a clonal population to 500 generations of selection for developmental equilibrium only at low noise. The most common phenotype at this point was then labelled as \mathbf{S}_{old} . Five hundred generations of selection for \mathbf{S}_{old} at low noise followed, resulting in a diverse population, canalized around \mathbf{S}_{old} . The population was then tested for the presence of a phenocopy. If one was present, it was labelled \mathbf{S}_{new} and an assimilation experiment was performed on the population 100 generations later. In the assimilation experiment, evolution takes place in the presence of high noise, under the two selection conditions of interest.

$S_{\text{new}} \neq S_{\text{old}}$ with a frequency of at least 10% while S_{new} appeared under low noise at <10% of this frequency, then a phenocopy was deemed to be present. Genetic assimilation experiments were performed only when no third phenotype other than S_{new} and S_{old} was generated by high noise at a frequency of more than 20% of that of S_{new} . Phenocopies meeting these requirements were seen in around 10% of tested populations. Otherwise, a new random individual was generated and the process of evolution began again.

Before the assimilation experiment, an additional 100 generations of selection for $\hat{S} = S_{\text{old}}$ with $\sigma = 0.01$ were performed. This removed any bias based on drift that might have been introduced by the group selection criteria for the presence of a phenocopy.

Genetic assimilation

Assimilation experiments were performed on 100 populations produced as described above. In the assimilation experiment, 100 generations of evolution with the high noise parameter $\sigma = 0.5$ were performed. Both developmental equilibrium selection alone and additional selection for phenotype $\hat{S} = S_{\text{new}}$ were simulated, in order to test whether selection for a specific phenotype was necessary for genetic assimilation.

Genetic assimilation was assessed at regular intervals by developing each W matrix at both low noise and high noise. Genetic assimilation is defined as the loss of dependency of a phenocopy's expression on the mechanism by which it was originally revealed. It can therefore be measured as the conditional probability that a W matrix will exhibit S_{new} at low noise, given that it has the phenotype S_{new} at high noise.

Statistics

Means and standard errors are shown in Fig. 4 for the proportion of individuals exhibiting phenotype S_{new} .

These were generated by the SAS glimmix macro, acting on PROC MIXED with a logistic link function and a binomial error function. An independent analysis was carried out for each time point. One hundred experiments were carried out, and the experiment number was modelled as a random variable. The selection condition was modelled as a fixed variable. Figure 4c shows the conditional probability that an individual will exhibit phenotype S_{new} at low noise, given that it exhibits S_{new} at high noise. To avoid bias produced by unequal numbers of data points between experiments, additional individuals were generated for each assimilation measurement until 500 were found that exhibited S_{new} at high noise. These additional individuals were used only for analysis, and did not contribute to the next generation.

Results

Both development and evolution were modelled, as described in Materials and methods. An outline of the experiment in evolutionary time is shown in Fig. 3. A starting population was generated as described in Materials and methods, canalized around a phenotype S_{old} and exhibiting a phenocopy S_{new} in the presence of perturbation. Genetic assimilation experiments were conducted on 100 such populations.

Genetic assimilation was assessed by developing each W matrix at both low noise and high noise. There is no spontaneous increase in the phenocopy S_{new} at high noise in the absence of selection for S_{new} , as shown in Fig. 4a. There is, however, a modest increase in the assimilated phenotype S_{new} at low noise in the absence of selection for S_{new} , as shown in Fig. 4b. We can calculate the probability that a W matrix will exhibit S_{new} at low noise, given that it has the phenotype S_{new} at high noise, and this conditional probability is shown in Fig. 4c. Genetic assimilation is defined as the loss of dependency of a phenocopy's expression on the

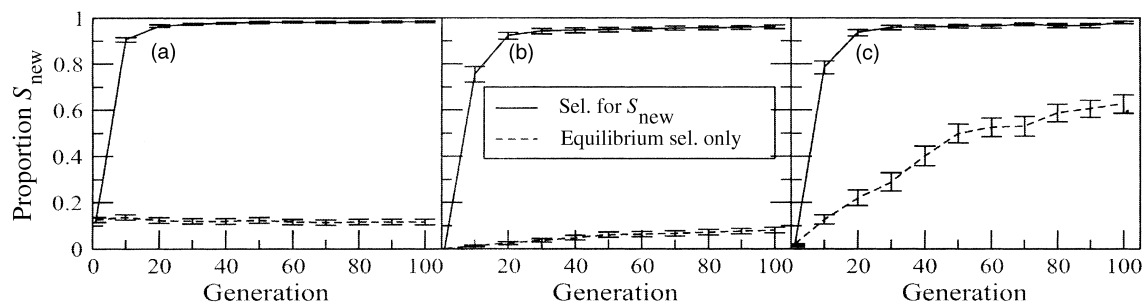


Fig. 4 Frequency of the assimilating phenotype S_{new} amongst W matrices reaching developmental equilibrium assessed at high noise (a) and low noise (b). The conditional probability of a W matrix exhibiting S_{new} at low noise, given that it exhibits S_{new} at high noise, is shown in (c). Selection for the phenotype S_{new} is shown by the upper curve (solid). Selection for developmental equilibrium only is shown by the lower curve (dashed). Mean and standard errors were estimated from 100 experiments using the SAS glimmix macro, as described in Materials and methods.

mechanism by which it was originally revealed. This conditional probability therefore assesses the extent of genetic assimilation of the phenocopy.

We see that there is a consistent increase in genetic assimilation, even when there is no selection for the assimilating phenocopy S_{new} . Genetic assimilation occurs faster when there is additional selection for S_{new} , as can be seen by the upper curve in Fig. 4c. This is not surprising, as selection intensity is greater in this case. The surprising result is that genetic assimilation continues to occur at all when selection for the assimilating phenotype is excluded by the experimental design. This result is in contradiction to the standard model for genetic assimilation (Falconer & Mackay, 1996).

Discussion

In the *in silico* model of development studied here, selection for developmental equilibrium is sufficient for canalization, defined as a reduction in the sensitivity of phenotype to mutation (Siegal & Bergman, 2002). Here I have shown that selection for developmental equilibrium is also sufficient for genetic assimilation. According to Waddington's heuristic of the epigenetic landscape, assimilation is expected whenever canalization occurs. My findings therefore support the utility of Waddington's heuristic of canalization as an explanation for genetic assimilation in this system.

Note that when additional selection for the assimilating phenocopy is present, both the threshold model and more general processes of canalization may occur. It is not clear how to assess the relative significances of the two mechanisms when they work together. What I have shown here is that when the threshold model is excluded by the experimental design, a certain level of genetic assimilation continues to occur. The canalization heuristic has been largely discarded in favour of the threshold model as an explanation for genetic assimilation: my results suggest that this is premature, and that both models should be considered.

The system studied here is of course only a numerical simulation, and the true question is how best to explain genetic assimilation *in vivo*. Such studies are far more difficult. Nevertheless, one *in vivo* experiment suggests that it is at least possible that my result may apply *in vivo* (Ho *et al.*, 1983). In a variation on Waddington's experiment, a *Drosophila* line exhibiting the *bithorax* phenocopy in the presence of ether was treated with ether over multiple generations, without artificial selection for *bithorax* (Ho *et al.*, 1983). The frequency of *bithorax* in the presence of ether increased (Ho *et al.*, 1983). When ether treatment was relaxed, the *bithorax* phenotype

continued to be seen, at least initially, showing that genetic assimilation had occurred (Ho *et al.*, 1983). *Bithorax* is likely to be deleterious, so there is no obvious reason why *bithorax* should spontaneously increase in the presence of ether. This makes the experiment difficult to interpret. My *in silico* experiment did not suffer from this complication, and so I was able to test more directly the hypothesis that genetic assimilation requires selection for the assimilating phenotype. I found that genetic assimilation is possible without such selection, and that both the threshold model and the canalization concept should therefore be retained as useful heuristics to understand genetic assimilation.

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