Theory Biosci. (2002) 121: 1–80

http://www.urbanfischer.de/journals/theorybiosc

Modularity and the units of evolution

Gerhard Schlosser

Brain Research Institute, University of Bremen, FB 2, PO Box 33 04 40, 28334 Bremen, Germany

Address of correspondence: Dr. Gerhard Schlosser, Brain Research Institute, University of Bremen, FB 2, PO Box 33 04 40, 28334 Bremen, Germany,
phone: +49 421 218 3867, fax: +049 421 218 4549, e-mail: gschloss@uni-bremen.de

Received: January 7, 2001; accepted: October 20, 2001

Key words: unit of selection, epistasis, constraints, coevolution, development, evolvability, mosaic evolution

Summary: While many developmental processes (e. g., gene networks or signaling pathways) are astonishingly conserved during evolution, they may be employed differently in different metazoan taxa or may be used multiply in different contexts of development. This suggests that these processes belong to building blocks or modules, viz., highly integrated parts of the organism, which develop and/or function relatively independent from other parts. Such modules may be relatively easy to dissociate from other modules and, therefore, could also serve as units of evolution. However, in order to further explore the implications of modularity for evolution, the vague notion of "modularity" as well as its relation to concepts like "unit of evolution" need to be more precisely specified. Here, a *module* is characterized as a certain type of dynamic pattern of couplings among the constituents of a process. It may or may not form a spatially contiguous unit. A *unit of selection* is defined as a unit of those constituents of a reproducing process/system, which exists in different variants and acts as a nondecomposable unit of fitness and variant reproduction during a particular selection process. The more general notion of a *unit of evolution* is characterized as a nondecomposable unit of constituents with reciprocal fitness dependence, be it due to fitness epistasis or due to the lack of independent variability. Because such fitness dependence may only be observed for some combinations of variants, several constituents may act as a unit of evolution only with a certain probability (coevolution probability). It is argued, that under certain conditions modules are likely to act as units of evolution with high coevolution probabilities, because there is likely to be a close tie between the pattern of couplings of the constituents of a reproducing system and their interdependent fitness contributions. Moreover and contrary to the traditional dichotomy of genes versus organisms as units of selection, modules tend to be more important in delimiting actual units of selection than either organisms or genes, because they are less easily disrupted by recombination than organisms, while having less contextsensitive fitness values than genes. Finally, it is suggested that the evolution of modularity is self-reinforcing, because the flexibility of intermodular connections facilitates the recombination among modules and their multiple employment in new contexts.

1431-7613/02/121/01–1/$ 15.00/0

1. Introduction

In the last decade, research in developmental genetics has revealed an astonishing conservation of many developmental processes in a wide range of metazoan taxa (for review see Zuckerkandl 1994; Raff 1996; Gerhart and Kirschner 1997; Gilbert 2000). These findings rekindled the interest of developmental biologists in evolution and raised the question of how the evolutionary divergence of the body plans of different taxa (e. g., insects and vertebrates), which are very obvious at a morphological level, can be reconciled with the surprising conservation of molecular pathways (e. g., Wray 1994; Gerhart and Kirschner 1997). An answer is suggested by the observation that many of these processes are not only found in many different taxa, but also tend to be employed repeatedly and in different contexts in development (e. g., Raff 1996; Gerhart and Kirschner 1997; Duboule and Wilkins 1998; Gilbert 2000). This indicates that these conserved processes belong to building blocks or *modules*, i. e., highly integrated parts of the organism, which develop and/or function relatively independent from other parts. Such modules may be relatively easy to dissociate from other modules and, therefore could also serve as building blocks of evolution. This idea that modules are the units of development and/or function as well as of evolution is at present a very promising working hypothesis. It was already suggested by Riedl (1975) and Bonner (1988) and is now thought to play a key role for new attempts to resynthesize evolutionary and developmental biology (see e. g., Wagner 1995, 1996; Wagner and Altenberg 1996; García-Bellido 1996; Raff 1996; Gilbert et al. 1996; Gerhart and Kirschner 1997; Gilbert 1998, 2000; Kirschner and Gerhart 1998; Hartwell et al. 1999; von Dassow and Munro 1999; Niehrs and Pollet 1999; Brandon 1999; Schlosser and Thieffry 2000; Schank and Wimsatt, 2001; Schlosser, in press a).

However, before the implications of modularity for development and evolution can be fully explored, several important questions need to be addressed. First and foremost is the question, how the rather vague notion of a module can be made more precise. Second, the current discussion in this field is dominated by developmental biologists and insights from evolutionary biology (in particular population genetics) still need to be integrated (Arthur 1997; Wilkins 1998) in order to clarify in what sense modules can be "units" of evolution. The aim of this paper is to provide a general conceptual framework for addressing these questions and to show how the idea that modules are units of evolution can be understood in a conceptually coherent way. Its focus is on concepts, but I will try to make clear how they can be mapped on empirical examples, without going into too much detail (but see Schlosser, 2001 in press a, b). These examples suggest that modularity indeed plays a role in the evolution of life, but much empirical work remains to be done, before its importance and prevalence

can be established. My argument will proceed in three steps. First, I will develop a general concept of modularity (section 2). Second, I will analyze, what units of evolution are (section 3). And third, I will argue that modules are, in fact, likely to act as units of evolution under certain conditions (section 4).

2. What are modules?

The modularity of living beings has always attracted attention. Throughout the centuries and in many different cultures, human fantasies have produced monsters, composed of familiar parts, which were put together in new, fictitious combinations, giving rise to angels, winged horses, mermaids etc. Importantly, these new creations do not only reflect the limitations of the human imagination. Very often, the recombined parts are indeed units of function and/or development (wings, tails etc.), i. e., modules which were employed in new contexts to serve new, imaginary uses (see also Raff 1996).

Intuitively, it is clear that when we talk about modules here, we talk about highly integrated parts of the organism, which are relatively independent from other parts. Biologists have always recognized such parts and have distinguished units such as the different organs (heart, lung, liver etc.) by their separable function as well as by their origin from distinct primordia (limb bud, ear placode etc.), that tend to develop relatively independent from their surroundings in a highly coordinated fashion and often with striking regulatory capacities ("morphogenetic fields"; Gilbert et al. 1996). Some of these units, such as hair, feathers, scales, teeth, are used as "standard parts" (Riedl 1975) over and over again in one and the same organism. This is particularly obvious for such large scale units as segments on the one hand and for such small scale units as cell types (muscle cells, neurons etc.) on the other hand. Moreover, many networks of regulatory gene interactions and signaling pathways act as developmental and functional units that seem to be largely conserved in evolution although they have been repeatedly reused for the development of different structures. These include, to name only a few (see Gerhart and Kirschner 1997; Gilbert 2000 for overviews): (1) the network of *Hox* genes, repeatedly used in positional specification; (2) signaling cascades involving the hedgehog, wingless and TGF (transforming growth factor)-β proteins, employed in a variety of inductive interactions and spatial patterning; (3) Delta-Notch mediated mechanisms of lateral inhibition, involved in a great variety of cell fate choices (e. g., neural-nonneural); (4) selector genes of the basic helix-loop-helix gene family (e. g., *MyoD*, *Neurogenin*, *NeuroD*) and their downstream targets, regulating determination and differentiation of various cell fates.

While we are sometimes able to recognize modules intuitively, we need to go beyond intuition in order to explore the implications of modularity in a theoretically satisfying manner. I will in the following paragraphs attempt to abstractly define a module as a certain type of dynamic pattern, i. e., a certain type of process that may or may not form a spatially contiguous unit. Such a concept of modularity is widely applicable to a variety of different systems, but is narrow enough to serve as a basis for models, in which the consequences of modularity for development and evolution of complex systems can be explored.

2.1 Processes and constituents

A set-theoretical framework for the analysis of processes and their constituents

Let me start by clarifying what a process is. A process of type **P** is a set (throughout the text, I will always denote sets in bold type) of events, i. e., a set of input-output transformations. Typically, a process can be analyzed into causally connected sequences of events or states $\mathbf{S_{i-1}}$, $\mathbf{S_i}$, $\mathbf{S_{i+1}}$ etc. in time, where a token of state $\mathbf{S_i}$ at time t_i is a causally necessary and sufficient condition for the occurrence of a token of state $\mathbf{S_{i+1}}$ at time t_{i+1}[1]. For Markov processes (in which each state does only depend on the immediately preceding one), each state $\mathbf{S_i}$ of a process can be characterized as a set of transformations from input to output values (which serve as input values for the next state), i. e., from preceding into successive states (Fig. 1). For simplicity, I will here only discuss Markov processes with discrete states but the argument should be generalizable to continuous processes as well.

This set $\mathbf{S_i}$ is defined by

1) a general transformation rule (that may be deterministic or probabilistic), that determines the value of a state variable s at time t_i as a function of the value of s at time t_{i-1}; s at time t_{i-1} is the value of the state variable at the previous state $\mathbf{S_{i-1}}$;
2) a range $\mathbf{s_{iP}}$ of permitted values for s: only if s falls within this range, will a token of $\mathbf{S_i}$ form part of a token of process **P**.

Each state $\mathbf{S_i}$ at time t_i may be analyzed into a number of element states. Element states will be called *constituents* $\mathbf{A_i}$, $\mathbf{B_i}$, $\mathbf{C_i}$, **…** of $\mathbf{S_i}$ (also constituents of **P**) when tokens of $\mathbf{A_i}$, $\mathbf{B_i}$, $\mathbf{C_i}$, **…** are singly necessary and jointly sufficient for the realization of tokens of $\mathbf{S_i}$. I will call each constituent of $\mathbf{S_i}$ *compositionally necessary*[2] for $\mathbf{S_i}$ at the same time t_i and *causally neces-*

[1] Although strictly speaking "being a necessary or sufficient condition" refers only to a relation between the truth values of two propositions, I will here adopt a looser usage of this phrase and talk about events or states (described by a proposition) as being necessary or sufficient conditions for other events or states (described by another proposition).

[2] Previously termed "constitutively necessary" (Schlosser 1998), but the latter term is dropped here, because it may lead to confusion with constitutive coupling (see below).

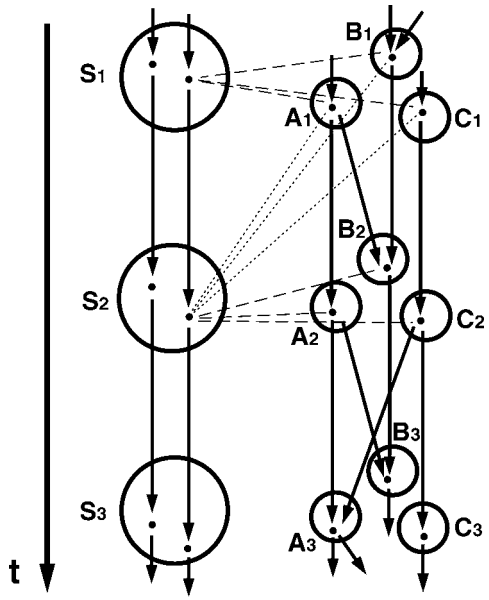


Fig. 1. Three successive states S_1, S_2 and S_3 of a process **P** during time t are illustrated as sets. Each state is a set of transformations (represented by dots) from inputs to outputs (arrows) defined by a general transformation rule and a range of permitted values. Each state may be analyzed into a number of constituents **A**, **B** and **C**. A_1, B_1 and C_1 are the constituents of S_1. Each of these constituents is compositionally necessary (dashed lines) for S_1 and causally necessary (dotted lines) for S_2, for which A_2, B_2 and C_2 are compositionally necessary. Jointly, A_1, B_1 and C_1 are compositionally sufficient for S_1 and causally sufficient for S_2. For simplicity, only two tokens (dots) of each state and one token of each constituent are shown. For further explanation see text.

sary for S_{i+1} at time t_{i+1}. There may also be some other nonconstitutive elements of S_i, which are present as inadvertent byproducts of S_{i-1}, but are not compositionally necessary for S_i (nor causally necessary for S_{i+1})[3]. All constituents that are singly necessary and jointly sufficient for all state transitions of a process **P** may be defined as a *unit* with respect to **P**.

[3] Several concepts introduced here require further comments. I will keep these brief in order to avoid straying too far from the main topic of this paper. First, it could be spelt out precisely what it means for one state to be more elementary than another. I will not do this here as the notion is intuitively clear. Second, I wish to point out that "necessity" is meant here in a completely naturalistic way (see also Schlosser 1998); when A_i is said to be necessary for S_i, I refer to a testable relationship between non-empty sets of real-world phenomena. Third, I should also concede that for processes, that can be instantiated by several alternative routes depending on the circumstances (the description of the process would then involve a disjunctive description of state transition sequences), constituents need to be only *conditionally* necessary (necessary under certain circumstances, i. e., for one of the alternative routes). Fourth, constituents have been defined here as elements of states of processes. Such processes can occur as very complex systems. Organisms, for instance, are complex selfmaintaining or self-reproducing processes or systems, i. e., processes that recurrently construct their own constituents. Constituents of such a system are all those elements, that are under certain circumstances necessary for the selfmaintenance of the system (involving their own self-re-production; see also Schlosser 1998).

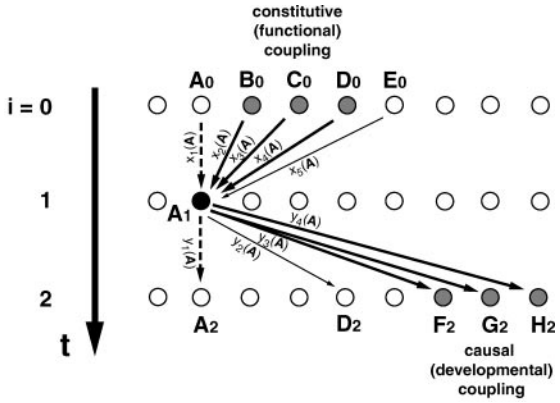


Fig. 2. Open circles in each row represent different elements **A**, **B**, **C** etc. of a process. Successive rows of circles represent successors of these elements at successive points along the time axis t. Filled circles indicate that elements adopt a particular range of values at t = i and so act as constituents $\mathbf{A_i}$, $\mathbf{B_i}$; … of a state $\mathbf{S_i}$. Arrows between circles indicate dependences between constituents. A constituent $\mathbf{A_1}$ (black circle) of a state $\mathbf{S_1}$ is coupled to a number of other constituents (grey circles). The input variables ($x_1(\mathbf{A})$, $x_2(\mathbf{A})$ etc.) of $\mathbf{A_1}$ are among the output variables of constituents $\mathbf{A_0}$, $\mathbf{B_0}$, $\mathbf{C_0}$, $\mathbf{D_0}$ and $\mathbf{E_0}$ of a a previous state $\mathbf{S_0}$ as indicated by arrows. Only $\mathbf{B_0}$, $\mathbf{C_0}$, $\mathbf{D_0}$ are strictly constitutively or functionally coupled (grey circles) in $\mathbf{A_1}$ because only they are causally necessary (thick solid arrows) for its realization. Conversely, the output variables $y_1(\mathbf{A})$, $y_2(\mathbf{A})$ etc. of $\mathbf{A_1}$ are among the input variables of $\mathbf{A_2}$, $\mathbf{D_2}$, $\mathbf{F_2}$, $\mathbf{G_2}$ and $\mathbf{H_2}$. $\mathbf{F_2}$, $\mathbf{G_2}$ and $\mathbf{H_2}$ are strictly causally or developmentally coupled by $\mathbf{A_1}$, because the latter is causally necessary (thick solid arrows) for the realization of each of them. Arrows originating from a single root indicate that the same output variable $y_3(\mathbf{A})$ serves as input variable to several constituents. The broken arrows between $\mathbf{A_0}$, $\mathbf{A_1}$ and $\mathbf{A_2}$ indicate that an output variable of **A** itself may or may not be among its input variables. For further explanation see text.

A constituent $\mathbf{A_i}$ of a state $\mathbf{S_i}$ of a process **P** can be characterized as a set of transformations defined by

1) a general transformation rule, that determines the value of several variables at time t_i, which may include a state variable A and/or several other output-variables such as $y_1(\mathbf{A})$ and $y_2(\mathbf{A})$, as a function of several variables at time t_{i-1}, which may include the state variable A and/or other input-variables such as $x_1(\mathbf{A})$ and $x_2(\mathbf{A})$. Constituents are coupled (Figs. 1, 2; see below), because the input variables of a constituent $\mathbf{A_i}$ of a state $\mathbf{S_i}$ are among the output variables of constituents of the preceding state $\mathbf{S_{i-1}}$, e. g., $\mathbf{B_{i-1}}$ and $\mathbf{C_{i-1}}$, whereas the output-variables of $\mathbf{A_i}$ are among the input-variables of constituents of the successive state $\mathbf{S_{i+1}}$, e. g., $\mathbf{D_{i+1}}$ and $\mathbf{G_{i+1}}$ (Fig. 2). One output variable may also be input variable of several successive constituents, e. g., $\mathbf{F_{i+1}}$ and $\mathbf{G_{i+1}}$ (Fig. 2).
2) ranges of permitted values for the state variable A and the output variables $y_1(\mathbf{A})$ and $y_2(\mathbf{A})$, i. e., values that allow s to be within the bounds of $\mathbf{s_{iP}}$ (these ranges may be conditional on the presence of particular values of other constituents).

It should be noted that these definitions are also applicable to inhibitors of a state transition. The constitutive absence of inhibitors can be expressed

by defining an inhibitor as a constituent, whose range of permitted values excludes the range of values required for inhibition. The definition also allows for the possibility of positive or negative feedback, resulting in nonlinear dynamics: Nonlinear transformations from state variable A or input-variables $x_1(\mathbf{A})$ and $x_2(\mathbf{A})$ at t_{i-1} into A or output-variables $y_1(\mathbf{A})$ and $y_2(\mathbf{A})$ at t_i are possible. This has important consequences for dynamics (see e. g., Thomas 1978 1991; Thomas et al. 1995; Thieffry and Romero 1999; Bhalla and Iyengar 1999; Freeman 2000), which can, however, not be discussed here in detail.

Attention should also be drawn to the fact that the general definition of constituent presented here, allows constituents of a process to be recognized at many different levels, i. e., there may be a hierarchy of constituents, constituents of constituents and so forth. Lower level constituents of a constituent **A** may either be constituents that are realized at the same time as **A** and are *compositionally necessary* for **A** or constituents realized at an earlier point in time being *causally necessary* for **A**. Constituents may be thought of as characters, which can be analyzed into lower level characters depending on the purpose of the analysis. In analysing the behavior of an organism, for example, we may focus on interactions among complex organs, among cell types or among genes and proteins depending on the question we are asking.

Application to network models

The various concepts introduced so far can be easily illustrated by considering patterns of activity in neural networks (see Hertz et al. 1991 for review). A state $\mathbf{S_i}$ of a certain pattern of activity changes **P** in a network is a set of transformations defined by 1) the architecture of the network, which embodies a general rule of transformation and 2) a range $\mathbf{s_{iP}}$ of activity patterns which supports **P**. The nodes of the network with their links and their state-specific activity patterns are constituents $\mathbf{A_i}$, $\mathbf{B_i}$, $\mathbf{C_i}$, **…** of the state $\mathbf{S_i}$. In addition, for all real networks certain events external to the network but influencing its activity at certain interfaces have to be considered as constituents; for the sake of argument, however, we will only consider the nodes of the network here. The role of each node in the realization of $\mathbf{S_i}$ can be represented as a set of transformations given by 1) the general function which determines its activity and the activity of its output links to other nodes from the activity of its input links from other nodes and 2) a range of node activity values which contributes to keeping the activity of the network within the range $\mathbf{s_{iP}}$. In order to be useful for the representation of biological processes this network analogy has to be taken a bit further. So far, we have only considered the possibility that the activity of nodes a time t_{i+1} depends on the activity of other nodes at time t_i, assuming that the architecture of the network itself is hardwired and is not altered in the process. However, the organization of living systems is charac-

terized by the fact that it is self-constructing, self-maintaining or self-reproducing (e. g., Varela et al. 1974; Maturana and Varela 1975; Schlosser 1993, 1998; Fontana and Buss 1994 a, b; Fontana et al. 1995; here as in Schlosser (1998) "re-production" indicates the mere re-establishment of a state in distinction to "reproduction", which stands for its *multiplicative* re-production). Therefore, in a biologically more plausible network model, we should allow for higher order (constructional) dynamics, in which not only the activity of the nodes is changed, but the architecture of the network itself is constructed and reconstructed. The variables cited in the transformation rule of a constituent node activity would in this case not only include descriptors of activity patterns of other nodes, but also parameters of the transformation rules of other nodes, for instance parameters defining the kind of transformation function or the kind and strength of its in- and outputs.

Network models of organisms attempt for instance to model certain processes occuring in living systems as networks of interacting cells or molecules such as DNA-sequences, proteins etc. (e. g., Mjolsness et al. 1991; Kauffman 1993, 1995; Burstein 1995; Bray 1995; McAdams and Shapiro 1995; Somogyi and Sniegowski 1996; Sharp and Reinitz 1998; Thieffry and Thomas 1995; Thieffry et al. 1998; Mendoza et al. 1999; Thieffry and Romero 1999; von Dassow et al. 2000). It needs to be emphasized that such models are possible only because molecules can be conceived as processes or patterns of interactions rather than as substances. A regulatory gene **A**, for instance, can be activated by the binding of certain transcription factors to its response elements and may itself code for a DNA-binding protein, which is able to activate other genes. Hence, **A** can be a constituent of certain processes because it can be represented as a set of transformations defined by a general transformation rule – embodied, for instance, by the structure of its binding sites – and a certain range of values for the intensity of its effects on other molecules. When we say that a gene **A** is constitutive for a some developmental or physiological process **P** (often disguised in the "gene for" **P** metaphor), this can only mean that a certain pattern of **A**s activity is one out of several necessary factors for the occurrence of this process. Therefore, it is the pattern of gene **A**'s interactions, not the nucleotide sequence of **A** per se, which is a constituent of **P** and the realization of these patterns is typically contextdependent, i. e., it requires the instantiation of other constituents. The same is true for other molecules and for all kinds of structures in general: They act as constituents of processes only because they define certain contextdependent classes of interactions, i. e., sets of transformation. Nonetheless, it will be difficult to avoid speaking of structures as constituents. We may call teeth constituents of the feeding apparatus, or DNA-polymerases constituents of replication. Such formulations are, however, to be seen as a shorthand for the respective claims that certain types of interactions of teeth contri-

bute to feeding and, similarly, that there are certain interaction patterns of DNA-polymerase, without which there would be no replication.

2.2 Coupling of constituents

Functional and developmental couplings

It was mentioned already that constituents of successive states of a process are coupled. These couplings can be of two kinds, constitutive or causal (Fig. 2). First, the input-variables of a constituent **A** of state $\mathbf{S_i}$ are identical with the output variables of one or several constituents of the previous state $\mathbf{S_{i-1}}$ at time t_{i-1} such as **B**, **C**, **D** and **E** (and possibly **A** as well). Therefore the realization of all or of a subset of these constituents, e. g., **B**, **C** and **D** maybe causally required in a certain context at time t_{i-1} for the realization of **A** at t_i. In this case, I will call **B**, **C** and **D** of $\mathbf{S_{i-1}}$ *constitutively coupled* in the realization of **A** of $\mathbf{S_i}$. Moreover, I will call **A**, **B**, **C** and **D** *constitutively connected*. In self-constructing and selfmaintaining systems such as organisms, in which **A** of $\mathbf{S_i}$ is in turn involved in the re-establishment of **B**, **C** and **D** in a future instantiation of state $\mathbf{S_{i-1}}$, constitutive dependence is usually characterized by calling **A** of $\mathbf{S_i}$ the function of **B**, **C** and **D** of $\mathbf{S_{i-1}}$ (see Schlosser 1998); constitutively coupled constituents are then said to be *functionally coupled* in the realization of function **A** (and **A**, **B**, **C** and **D** are termed *functionally connected*). Because this paper will mainly deal with living systems, I will use both terms interchangably here[4]. For instance, the presence of certain levels of insulin, the insulin receptor and several other factors are functionally coupled in the maintenance of a balanced blood sugar level. Similarly, the actions of teeth, jaw bones and jaw muscles are functionally coupled in feeding.

Second, the output variables of a constituent **A** of a state $\mathbf{S_i}$ at time t_i will be among the input variables of a number of elements such as **D**, **F**, **G** and **H** (and possibly **A** as well) of the successive state $\mathbf{S_{i+1}}$ at time t_{i+1} and the

[4] Sometimes the relation between constitutively or functionally coupled constituents is referred to as *epistatic*, when their effects on the constituent, in which they are coupled, are interdependent, i. e., not merely additive or multiplicative (e. g., Cheverud and Routman 1995; Wagner et al. 1998). However, in order to avoid confusion, I will use the term epistasis here exclusively to refer to *fitness* relations between constituents (see below). While fitness epistasis often reflects the interdependence of effects due to functional coupling among constituents, under certain conditions the former may exist even with merely additive or multiplicative effects (e. g., with selection for intermediate character values determined by additive polygenic effects; Futuyma 1986). Readers should also be alerted to the fact that the term epistasis is used in still another sense in quantitative genetics to characterize socalled interaction variance, a nonheritable component of the variance of character measures or fitness values (Falconer 1960; Maynard Smith 1989; Ridley 1993). Using the term epistasis in the latter sense is potentially misleading and will not be adopted here, because fitness epistasis (as characterized above) contributes to additive variance as well as to interaction variance (e. g., Cheverud and Routman 1995; Wagner et al. 1998). For a discussion of different concepts of epistasis see also Hedrick et al. (1978), Wade (1992), Whitlock et al. (1995) and Fenster et al. (1997).

realization of **A** in a certain context at t_i may be causally required for the realization of all or of a subset of these elements, e. g., **F**, **G**, **H** at t_{i+1}. In this case, I will call **F**, **G**, **H** *causally or developmentally coupled* by the realization of **A** of $\mathbf{S_i}$. Moreover, I will call **A**, **F**, **G** and **H** *causally or developmentally connected*. While elements **F**, **G**, **H** may themselves be constituents of $\mathbf{S_{i+1}}$, this need not be the case. An example for developmental coupling would be transcription from various thyroid hormone response genes. The transcription of these genes requires that the thyroid hormone receptor binds to a conserved thyroid hormone response element in the regulatory region of each of them (e. g., Yen and Chin 1994; Tsai and O'Malley 1994).

Pleiotropy

Some patterns of coupling among constituents imply that a constituent **C** plays several different roles for process **P**. In this case there are said to be *pleiotropic roles* of **C** (in short: pleiotropy of **C**)[5]. Pleiotropy of constituents can be of different kinds. In the simplest kind, which may be called *conjunctive pleiotropy* (Fig. 3 A, B), a constituent is involved in the realization of several strictly developmentally coupled constituents, i. e., none of them can be realized without the other. Conjunctive pleiotropy of **C** is reflected in the form of the transformation rule of the constituents, which are developmentally dependent upon it. The latter may even in some cases all share the same input variables. For instance, the opening of unspecific cation channels in the cell membrane, as they are found in vertebrate photoreceptors (see e. g., Gerhart and Kirschner 1997) will lead to the inflow of several types of cations (e. g., K^+, Na^+) into the cell, because each of them responds to the change in membrane permeability. Or several genes regulated by the same transcription factor (e. g., thyroid hormone receptor) may share the same binding sites (e. g., thyroid hormone response elements, Yen and Chin 1994; Tsai and O'Malley 1994) in their promotors or enhancers (see also Lewin 2000). In more complex kinds of pleiotropy (Fig. 3 C–F, G), which shall be labeled *disjunctive pleiotropy*, a constituent may be either alternatively dependent on several clusters of singly necessary and jointly sufficient constituents that are strictly functionally coupled within but not between themselves (Fig. 3 C, E, F, G); or it may give rise to several clusters of developmentally coupled constituents, each of which depends on different additional constituents for its realization so

[5] In order to understand the role of pleiotropy for evolution (see below) it is important to bear in mind that pleiotropy (pleiotropic roles) of a constituent does not imply that any variations of that constituent have pleiotropic effects (see 4.1). Moreover, the term "pleiotropy", strictly speaking, should be reserved for functional constituents (constituents of functional, self-re-producing systems). Constituents are functional, when they are under certain circumstances necessary for their own re-establishment (self-re-production; see Schlosser 1998). Functional constituents are pleiotropic, when they have several functions, i. e., when they play several independent roles for their re-production.

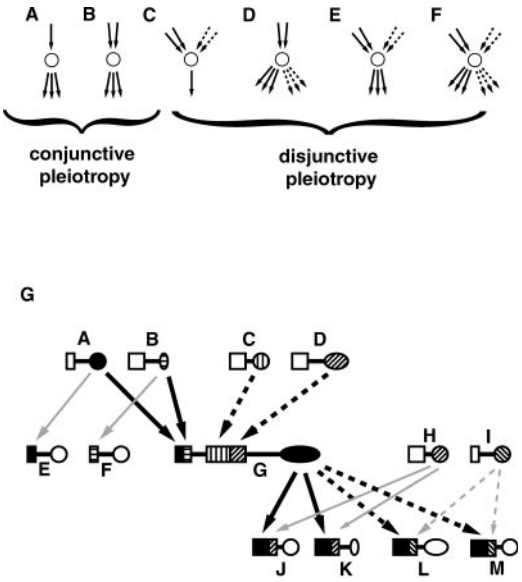


Fig. 3. A–F) Conjunctive and disjunctive pleiotropy of a constituent (circle). Arrows indicate the pattern of dependences on other constituents as shown in detail in Fig. 2. Pleiotropic roles of a constituent may exist even when there are no multiple output variables, because a single output variable can be input variable to several other constituents (see e. g., y_3(**A**) in Fig. 2). In cases C), E), and F), the constituent can be realized either by the joint action of one group of constituents (solid arrows) or by the joint action of another group of constituents (dashed arrows). In cases D) and F), the constituent will either jointly activate one group (solid arrows) or another group of constituents (dashed arrows) depending on the circumstances. G) illustrates how very complex modes of pleiotropy as illustrated in F) can be realized by genes with composite regulatory regions. Response elements of the regulatory regions are shown as rectangles, coding regions as ellipses. The pattern of each coding region corresponds to the pattern of the response element that it activates (e. g., by coding for a transcription factor binding to it). Response elements are drawn as clusters (enhancers), when their joint activation is necessary for gene expression. Expression of gene **G** may be activated either by the joint binding of **A** and **B** (which also coactivate **E** and **F**, respectively) or by the joint binding of **C** and **D**. **G** in turn activates genes **J** and **K** in conjunction with **H**, but genes **L** and **M** in conjunction with **I**. The pleiotropy of **G** action is facilitated by the modularity of gene regulation itself at two different levels. First, transcription of the same coding region may be regulated by different enhancers – combinations of response elements – in different contexts (e. g., **AB** or **CD** respectively). Second, the same response element may operate in different contexts and may contribute to enhancers of different genes. For further explanation see text.

that there is strict developmental coupling within but not between the clusters (Fig. 3 D, F, G). Examples for the former would be genes which can be activated either by the binding of a complex of various transcription factors to a regulatory region R_1 or by the binding of a different complex to a regulatory region R_2 (see e. g., Kirchhamer et al. 1996; Arnone and Davidson 1997; Yuh et al. 1998; Lewin 2000). Examples for the latter would be genes, which activate or repress different downstream genes depending on which other genes are coactivated (this may typically be the case for homeodomain genes, e. g., *Hox* genes in the vertebrate limb vs.

nervous system; see Gerhart and Kirschner 1997) or alternatively spliced transcripts, which after translation may act as trancription factors for different batteries of genes (see Gilbert 2000). This kind of pleiotropy is called disjunctive, because it implies that the transformation rule of a constituent **C** can be written as a disjunction of several component transformation rules, each of which cites only a subset of the input- or output variables of **C**s transformation rule.

Identity of constituents through state transitions

The functional and developmental couplings of constituents raise the question if it makes sense to assume that constituents retain their identity through state transitions. In other words: is it possible to identify for a constituent **C** of state $\mathbf{S_i}$ a *precursor* in state $\mathbf{S_{i-1}}$ (among all constituents constitutively coupled in **C**) and a *successor* in state $\mathbf{S_{i+1}}$ (among all elements developmentally coupled by **C**)? In systems that can be represented as networks with first-order, non-constructional dynamics this question is easier to answer in the affirmative. Here, an element of a state $\mathbf{S_{i+1}}$ is the successor of the constituent of state $\mathbf{S_i}$, which is among its causally necessary conditions and with which it shares the same state variable (and transformation rule). In a network it is the same node at different times. The successor of a constituent of $\mathbf{S_i}$ may be a constituent of $\mathbf{S_{i+1}}$, but this need not be the case. Consider, for example, the process describing activity changes of a gene **A**. The activity of gene **A** may be regulated by a transcription factor **B** at time t_i, but may depend on another transcription factor **C** at time t_{i+1}. In this case, **B** is a constituent of state $\mathbf{S_i}$, but its successor is not a constituent of state $\mathbf{S_{i+1}}$.

As soon as higher order, constructional dynamics are permitted, things get much more difficult, because then constituents (nodes) can be constructed and destructed and their links and transformation rules can be altered. Examples are to be found in chemical reactions (see Fontana and Buss 1994 a, b; Fontana et al. 1995) where several reactants (e. g., HCl, NaOH) may combine to form different products (NaCl, H_2O). Transcription, translation and replication of genes are also among these processes. In DNA replication, the interaction of a DNA sequence with several other molecules including nucleotides and enzymes such as polymerases results in the duplication of the sequence and the depletion of the nucleotides. In these cases it is on the one hand not always possible to unambiguously identify constituents across state transitions: It does not make sense to identify NaCl rather than H_2O as the successor of HCl. On the other hand, not all constituents will necessarily have a precursor and/or successor, while others may have several precursors and/or successors (which are capable of independent interactions and hence have different state variables): Nucleotides are used up during replication, but a second DNA-sequence is constructed. With respect to the latter example, I will call the copies of the

DNA-sequence descendants rather than successors of the original DNA-sequence. More generally, I will call **C′** a *descendant* of **C** and **C** an *progenitor* of **C′** whenever **C′** is connected to **C** by an uninterrupted chain of either direct successors of **C** or by constituents, that arose by *copying* of **C** or its successors and descendants. The descendence relation is more general than the successorship relation, because it permits copying and hence the multiplication of **C**, i. e., the generation of several elements with the same transformation rule as **C** but with distinct state variables (multiplication may, however, also result from other processes than copying, such as in collectively autocatalytic networks). Entities that are capable of reiterated copying, i. e., whose descendants themselves serve as templates in a copying process, are usually called "replicators" (see 3.1).
By calling **C′** and **C″** the descendants of **C**, we single out **C** from a number of constituents which are all constitutively coupled in **C′**and **C″** by attributing a special status to copying relations. The replication of a DNA-sequence **C** requires polymerases and nucleotides in addition to **C** as template, but only **C** is considered the progenitor of DNA-sequences **C′** and **C″**. There are, however, cases, in which even descendence relations are not unambiguous and where a descendant has several progenitors, so for example, when DNA-sequences arise by the recombination of two DNA-sequences.

2.3 Modules

Modules are relatively autonomous subprocesses that are functionally and/or developmentally integrated

Due to the interdependence of different constituents of a process not only sequences of descendants can be identified but also *cascades* of all elements, which are constitutively (functionally) and causally (developmentally) connected, without including only descendants of each other. When feedback is involved these cascades may also form loops or cycles. On the one hand, a constituent **E** which is dependent on the functionally coupled constituents **D**, **E** and **F** may itself be functionally coupled with another constituent **G** in the realization of another constituent of a later state and so forth forming a *functional cascade* (Fig. 4). On the other hand, several elements may all be developmentally coupled by a constituent **D**, which itself is developmentally coupled with constituent **B** by constituent **C** and so forth forming a *developmental cascade* (Fig. 4). Such cascades of constitutive (functional) and causal (developmental) connections allow us to delimit different *modules* of a process. Restrictively, a module could be defined as a cascade of all developmentally or functionally connected constituents, i. e., as a subprocess (a higher level constituent of the process) that develops and functions completely independent from other subprocesses (Fig. 4). However, such a restrictive definition would severely con-

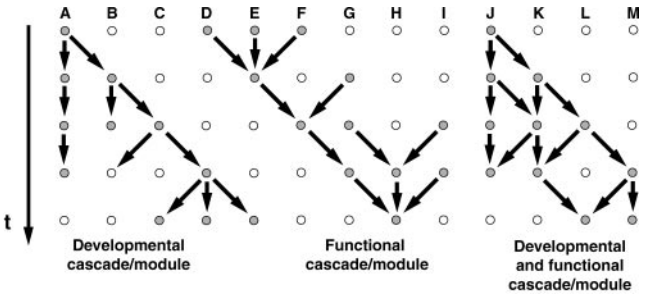


Fig. 4. Cascades of functionally or developmentally coupled constituents. For general notation see Fig. 2. Cascades of coupled constituents (grey), which are independent from other such cascades form modules. Modules may consist of purely functionally or developmentally coupled constituents or a combination of both. See text for further explanation.

strain the applicability of the concept and make it useless for our purposes. Therefore, a module will be characterized more generally as a subprocess (or constituent) of a process **P**, that is functionally and/or developmentally integrated and relatively autonomous (Fig. 5). All (lower level) constituents of a process that contribute in an integrated manner to a relatively contextinsensitive subprocess (a higher level constituent of the process **P**) constitute a module (as detailed below). All elements of the process which are not part of a module belong to the context or environment of the module. The spatial environment is formed by elements which exist at the same time as the constituents of the module; the temporal environment is formed by elements preceding or succeeding the constituents of the module in time.

The two characteristic features of a module – integration and relative autonomy – can be specified more precisely. Constituents contribute to a subprocess (or higher level constituent) in an *integrated* manner, when they do not independently contribute to its realization. This will be the case, when perturbations of one constituent that disrupt the subprocess (resulting in non-permitted input-output transformations) tend to be associated with coperturbation of other constituents (due to their causal coupling), or when the effect of perturbation of one constituent on the subprocess differs depending on the value of another constituent (due to their functional coupling). A subprocess is *relatively autonomous*, when its particular contribution to the larger scale process (i. e., its set of input-output transformations), is relatively insensitive to the context (i. e., other subprocesses), in which the subprocess operates. After perturbation of the context, there will tend to be no coperturbation of constituents of the subprocess which disrupts its permitted input-output transformations. Also, the effect of a context perturbation on the subprocess will usually not differ depending on the value of constituents of the subprocess.

The definition of a module given here implies, that the constituents of a module are strongly or multiply coupled with each other functionally, or

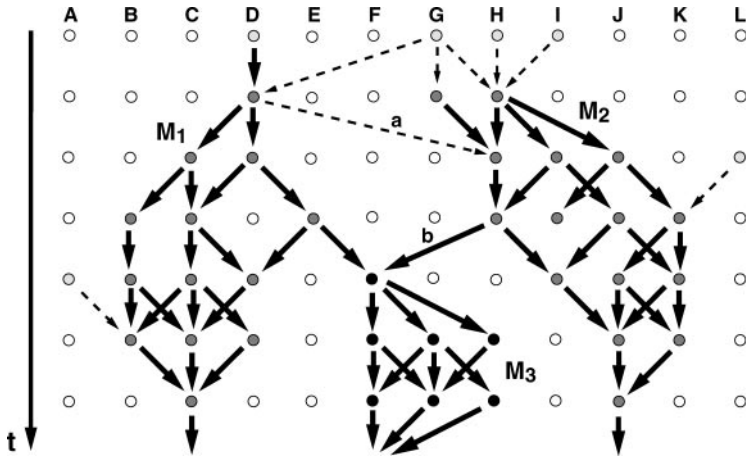


Fig. 5. Modules are integrated and relatively autonomous subprocesses. For general notation see Fig. 2. Strong connections are depicted by thick and solid arrows, weak connections by thin and broken arrows. Three modules are illustrated: $\mathbf{M_1}$ (dark grey circles, left), $\mathbf{M_2}$ (dark grey circles, right), and $\mathbf{M_3}$ (black circles). The integration of modules is reflected in multiple and strong developmental and/or functional couplings among their constituents (arrows). The relative autonomy or contextinsensitivity of modules is reflected in the fact that the input output transformation of modules is relatively independent from their environment, although weak dependences (broken arrows) may exist. For instance, constituent **G** of module $\mathbf{M_2}$ is only weakly functionally coupled (broken arrow a) with the context (e. g., **D** of $\mathbf{M_1}$) in the realization of another constituent of the module (**H** of $\mathbf{M_2}$). As a corollary, constituent **H** of $\mathbf{M_2}$ is also only weakly developmentally coupled to **C** and **D** of M_1, by **D** of $\mathbf{M_2}$. However, while modules are relatively contextinsensitive (i. e, there are no strong inputs from the context after initiation of the module), they may nonetheless be indispensable for the context, i. e., there may be strong outputs from the module to the context (e. g., arrow b). These may, for instance, be important for the initiation (e. g., induction) of a new module $\mathbf{M_3}$ by constituents of $\mathbf{M_2}$ and $\mathbf{M_3}$ as illustrated. See text for further explanation.

developmentally or both (integration), whereas neither strong functional couplings with the context in the realization of a constituent of the module, nor strong developmental couplings with the context by a constituent of the context are permitted (relative autonomy). The converse, however, does not need to be true: a module may well be indispensable for the context to which it is insensitive. In other words: while there should be no strong inputs from the context to the module, there may be strong outputs from the module to the context (Fig. 5).

In extreme cases, a module may consist of simple cascades of constituents, without reciprocal connections. On the one hand, there may be *functional modules*, i. e., cascades of exclusively converging functional couplings (Fig. 4), on the other hand, *developmental modules* or cascades of exclusively diverging developmental couplings (Fig. 4). Typically, however, there is a large degree of both convergence and divergence of processes in complex systems so that such systems can only be modular, when functional and developmental units at least partially coincide. Consequently, modules in complex systems will normally – but not necessarily – be

strongly coupled developmentally as well as functionally and as a consequence of this reciprocal coupling exhibit feedback loops of various kinds. The definition of module developed here, is broadly applicable, for instance to the different organs and "standard parts" (hair, feather etc.) of organisms, as well as to the signalling cascades or regulatory gene networks introduced above (see 2.1). Moreover, this concept of module is also applicable to units which are not spatially contiguous. Hormones, for instance, selectively affect and orchestrate a subset of processes throughout the organism and these hormonally interdependent processes (for instance in sexual differentiation or metamorphosis) should be considered elements of the same module.

Spatiotemporal embedding and overlap of modules

It should be emphasized again that the definition of modules is deliberately fuzzy in that only relative autonomy is required. Relative independence of a module from its environment increases with 1) decreasing strength and number of inputs from elements of its environment and 2) the degree to which there are regulatory capacities (allowing its dependence on environmental elements to be distributed over different alternatives). For the purpose of this paper it can be left open where exactly the boundary between sufficiently and insufficiently weak connections between a module and its environment shall be drawn. Maybe computer models will be helpful in defining this notion more precisely (e. g., by identifying thresholds in the degree of independence, beyond which coupled elements begin to behave coherently).

The relative autonomy of modules from their spatial environment may be primarily due to weak influences from the latter. However strong interactions with the environment may be permitted, if the module possesses regulatory capacities. These may take one of two forms: plasticity or canalization. Plasticity involves "exploratory mechanisms" (Gerhart and Kirschner 1997), which allow the accomodation of a module to different environments (e. g., matching the size of a population of neurons to the size of its target via neurotrophic factors; see Reichardt and Fariñas 1997). Canalization, (e. g., Waddington 1957; Hall 1992; Wagner et al. 1997; Wilkins 1997; Rutherford 2000; Gibson and Wagner 2000) involves the existence of several alternative cascades of couplings, which are contingent on different environmental conditions but all lead to the same outcome, thereby allowing modules to retain their integrity and autonomy despite environmental perturbations.

In the temporal dimension, a module $\mathbf{M_3}$ may be relatively autonomous from cascades such as $\mathbf{M_1}$ and $\mathbf{M_2}$ from which it arises or to which it gives rise, when elements of the module are not strongly and/or multiply connected to elements of these cascades. Several possibilities exist for the determination of temporal boundaries of a module. This may be

illustrated considering, for example, the origin of $\mathbf{M_3}$ (Fig. 5). First, $\mathbf{M_3}$ may be tightly coupled to $\mathbf{M_1}$ (and/or $\mathbf{M_2}$) but there may be a "bottleneck" of relations between cascades $\mathbf{M_1}$ (and/or $\mathbf{M_2}$) and $\mathbf{M_3}$ in the sense that after initiation of $\mathbf{M_3}$, none of its successive constituents is coupled to $\mathbf{M_1}$ and/or $\mathbf{M_2}$ (as in many embryonic inductions, e. g., the induction of the limb module by FGF proteins; see Xu et al. 1999; Gilbert 2000). Second, $\mathbf{M_3}$ may be tightly coupled to input cascades, but there may be redundancy with respect to the initiation of $\mathbf{M_3}$; for instance elements of $\mathbf{M_1}$ and $\mathbf{M_2}$ may be able to substitute for each other in the initiation of $\mathbf{M_3}$ (e. g., the induction of the nervous system can be initiated by various factors such as noggin or chordin; see Sasai and de Robertis 1997; Gilbert 2000). Third, $\mathbf{M_3}$ may be weakly connected ("weak linkage", see Conrad 1990; Gerhart and Kirschner 1997; Kirschner and Gerhart 1998) to a multitude of input cascades, which may act in various combinations and none of which may by itself be necessary for the initiation of $\mathbf{M_3}$ (e. g., an action potential of a nerve cell may result from various combinations of inputs).

The fact that modules need to be only relatively, but not absolutely independent from other modules has the important implication that modules can be *embedded* in larger scale modules both in the spatial and temporal dimension. Spatially, modules may be part of more comprehensive modules in a hierarchical fashion, temporally, modules may split and unite (Figs. 5, 6). The cooperation of cells in the formation of tissues and organs (e. g., muscle cells constituting the heart) is an example for the former, while the subdivision into relatively independent subunits in the development of organ primordia (e. g., the formation of chondrogenic condensations during limb development) or the functional integration of different developmental units (e. g., the formation of skeletal and muscular connections between the limbs and the head or axial skeleton), are examples for the latter.

Besides the embedding of modules into higher units, modules can also be related in a second way, viz. by sharing constituents or their descendants. I will call this *overlap* of modules. The reason for it is pleiotropy. Pleiotropy as introduced above means that a constituent plays several roles during a process, either by being involved in the realization of several other constituents or by being alternatively dependent on several clusters of singly necessary and jointly sufficient constituents or by both (Fig. 3). A transcription factor that activates several genes in a cell has pleiotropic roles as does a transcription factor that activates the same gene in response to different signals. The pleiotropic relations (in this narrow sense) of constituents of a module $\mathbf{M_1}$ may be restricted to other constituents of $\mathbf{M_1}$ or they may extend to constituents of another module $\mathbf{M_2}$. If the latter are crucial for the realization of $\mathbf{M_2}$, the constituent can be counted as part of both modules and there will be *overlap* of modules.

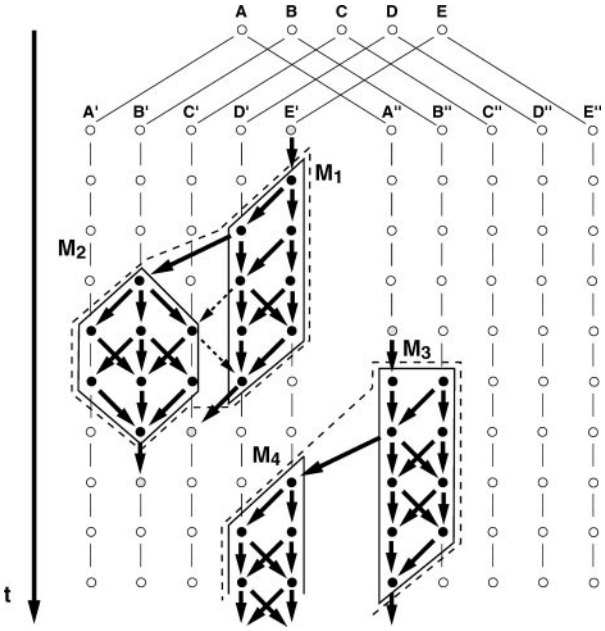


Fig. 6. Spatiotemporal embedding and overlap of modules. For general notation see Fig. 2. Constituents **A′**, **B′**, **C′** etc. as well as **A″**, **B″**, **C″** etc. are descendants, which originated as copies of **A**, **B**, **C** etc., respectively. Modules are surrounded by a thin line; their constituents are drawn as black circles. While modules are relatively independent from each other, there are some interactions between them. Therefore, modules are spatiotemporally embedded into higher order modules (dashed lines) in a hierarchical fashion. Some constituents have pleiotropic roles in several modules (e. g., **C′** for $\mathbf{M_1}$ and $\mathbf{M_2}$). As in Fig. 3, dashed arrows here indicate input output relations of a constituent that can be realized alternatively and independently of the input output relations indicated by solid arrows. Pleiotropic constituents may be counted as parts of both modules and modules (e. g., $\mathbf{M_1}$ and $\mathbf{M_2}$) may accordingly be considered overlapping. There will also be overlap in a broader sense of modules (e. g., $\mathbf{M_2}$ and $\mathbf{M_3}$) that are not spatiotemporally integrated, when two descendants (e. g., **A′** and **A″** or **B′** and **B″**) of the same constituent (**A** or **B**) participate in different modules ($\mathbf{M_2}$ and $\mathbf{M_3}$). See text for further explanations.

There is overlap of modules in a strict sense, when there are one or several pleiotropic constituents that are *compositionally* necessary for both of them. For instance, the secretion of the diffusible signalling molecule sonic hedgehog is important for specification of neural cell fates in the neural tube as well as for somite development (reviewed in Hammerschmidt et al. 1997). Overlap of modules due to pleiotropy in this strict sense can be considered a special case of spatiotemporal embedding of modules. However, modules may also be said to overlap in a wider sense, when each of them includes different descendants of the same progenitor constituents. In that case, the overlap of modules is due to the pleiotropy of constituents that are *causally* necessary for both of them and does not involve direct spatiotemporal embedding (Fig. 6). This kind of overlap is particularly relevant in multicellular organisms. For example, in two cells **C′** and **C″** of

a multicellular organism, which are descendants of the same zygote **C**, transcription factor **T′** may or may not be dependent on different regulators and may or may not activate a different gene in cell **C′** than the "same" (because also descended from **T**) transcription factor **T″** in cell **C″**. The development of neural tube and somites on the one side is overlapping with limb development in this sense, because sonic hedgehog expressed by cells in the limb is involved in anteroposterior limb patterning, while sonic hedgehog expressed by a different set of cells in the notochord has the effects on neural cell fates and somites already mentioned (Hammerschmidt et al. 1997).

2.4 Comparison of different concepts of modularity

The meanings of "modularity"

The idea of modularity is not new (see e. g., Simon 1962; Riedl 1975; Bonner 1988), but has recently attracted renewed attention among biologists and philosophers of biology (e. g., Wagner 1995, 1996; Wagner and Altenberg 1996; Raff 1996; Gilbert et al. 1996; Kirchhamer et al. 1996; Gerhart and Kirschner 1997; Arnone and Davidson 1997; Gilbert 1998, 2000; Kirschner and Gerhart 1998; Yuh et al. 1998; Hartwell et al. 1999; von Dassow and Munro 1999; Niehrs and Pollet 1999; Brandon 1999; Thieffry and Romero 1999; von Dassow et al. 2000; Schlosser and Thieffry 2000; Schank and Wimsatt, 2001; Schlosser, in press a, b). However, different authors understand modularity differently. The term "modularity" is usually used in two senses. It either refers to the independence of several units from each other or to the repeated use of the same unit in a system. I use the term in the first sense, because it is more general and the possibility of modularity in the second sense depends on it[6]. Modularity as I have defined it refers to the fact that there are relatively independent subprocesses of a process. It may therefore also be called "process modularity". The idea of structural modularity can be derived from this general notion, in analogy to the argument presented above concerning structural constituents (2.1): If a structure is termed a module, this is to be seen as shorthand for a claim that certain processes, in which the structure plays a role, act as integrated and autonomous subprocesses of a containing process. For example, complexes of different transcription factor binding sites or enhancers in the cis-regulatory regions of genes have been termed "modules" (Kirchhamer et al. 1996; Arnone and Davidson 1997; Yuh et al. 1998), because cooperative binding of transcription factors to one enhancer contributes in an integrated manner to gene regulation, independent from the binding of transcription factors to another enhancer.

[6] Sometimes modules are defined much more narrowly and in the second sense of the term as "iterated multicellular units of growth and organization" (Andrews 1998; p.107; see also Harper et al. 1986).

Process modularity and network models

Process modularity is important in several network models. In the randomly connected Boolean networks (where each element can only take one of two values) of S. Kauffman (1993, 1995), different modules appear as different attractors, i. e., different state cycles. These models assume networks with first-order, non-constructional dynamics. Their typical or generic behavior depends on their connectivity K. When K is very small (K = 1), the system will behave very predictably: There will be a large number of attractors, each of which consists of a very small state cycle involving few nodes. When K is large, the system will behave chaotic: There will be a relatively large number of attractors with very long state cycles. Only when K is around 2 or – in case of higher connectivity – when certain additional conditions are met (homogeneity clusters or canalyzing functions), the system will tend to exhibit behavior reminiscent of complex systems such as organisms: There will be a relatively small number of attractors of reasonably small, but not too small length that tend to be stable with respect to perturbations. All connectivity conditions that lead to complex behavior have in common that they partition network activity into several attractors or modules, that are simultaneously realized in adjacent parts of the network. In Kauffman's models, attractors often form islands of oscillating elements that are separated by a "frozen component" from each other preventing the spread of perturbations from one island to the next. Other network models have also shown that attractors may be composed of relatively independent subattractors which are confined to different parts of the network (see Somogyi and Sniegoski 1996; Mendoza et al. 1999). Moreover, Thieffry and coworkers argued – using an approach pioneered by Thomas (e. g., Thomas 1978; 1991; Thomas et al. 1995) – that this modularity of attractors results from the partitioning of the underlying networks into subnetworks of relatively independent feedback circuits (circular patterns of connections) or groups of circuits (Mendoza et al. 1999; Thieffry and Romero 1999). These authors formally define a "module" as a set of feedback circuits sharing some elements. There are different modules in a single network when there are different quasi-independent subnetworks, each of which may support various activity patterns or attractors. According to my terminology, however, each of these attractors would be called a different module.

The notion of modularity introduced here is, however, more general than the notion of a cyclic attractor of network activities, because it also includes non-cyclic cascades of events and is applicable to constructional dynamics. Such non-cyclic cascades have been modeled, for example, as so-called "synfire chains" in neural networks (e. g., Abeles et al. 1994). Briefly, synfire chains are such subnetworks of a network in which the activity of a group of neurons (nodes) determines the activity of the next group of neurons in a strongly reciprocally coupled way. Nonlinear effects

are permitted, i. e., the same neuron can (but does not necessarily) participate repeatedly in the same chain. Consequently feedback circuits can be modeled as a special case of synfire chains. Under certain conditions the neurons, which form the elements of such synfire chains, cooperate in a coordinated fashion, e. g., by synchronizing their activity. This phenomenon is also observed when synfire chains are weakly coupled to other synfire chains as long as the coupling is not too strong. In the latter case, synfire chains loose their character of an independent module and integrate their activity with neighboring synfire chains. Beyond their applications in neuroscience, synfire chain models may serve as a more general tool for analyzing modularity because they provide a framework for representing modules which do form state cycles as well as modules which form non-cyclic cascades of events. Moreover, constructional dynamics can be easily implemented in synfire chain models, e. g., by allowing the reorganization of synfire chains using a Hebbian learning rule to structurally reinforce coincident activities. However, in order to make models useful for representing a variety of biological processes including developmental processes, additional constructive processes, e. g., processes allowing for metabolic reactions, gene replication and cell proliferation have to be accounted for in the models (see e. g., Mjolsness et al. 1991). Unless models allow for such constructional dynamics they will not allow us to capture the most important aspects of the organization of living systems (Fontana and Buss 1994 a, b; Fontana et al. 1995).

My concept of (process) modularity is intended to be very general. It has many affinities to H. Simon's (1962) concept of a "nearly decomposable system", in which there are strong interactions within and weak interactions among subsystems (see also Wimsatt 1986; Schank and Wimsatt 1986, 2001). Simon (1962) has shown that such systems exhibit a couple of remarkable properties. Frequently the short run behavior of a module can be predicted by treating it as independent from other modules, while its long run behavior will depend on other modules only in an aggregate way and with lower frequency dynamics (Simon 1962)[7]. Recently, Wagner has developed a related notion of modularity (Wagner 1995, 1996; Wagner and Altenberg 1996). He considers modularity to be a property of the "genotype-phenotype map", and a module as a "complex of characters that 1) collectively serve a primary functional role, 2) are tightly integrated

[7] Simon (1962, p. 474) illustrates this general property with a very simple example: "Consider a building whose outside walls provide perfect thermal insulation from the environment (…) The building is divided into a large number of rooms, the walls between them being good, but not perfect, insulators (…) Each room is divided by partitions into a number of cubicles, but the partitions are poor insulators (…) Suppose that at the time of our first observation (…) there is a wide variation in temperature from cubicle to cubicle and from room to room (…) several hours later (…) there will be very little variation in temperature among the cubicles within each room, but there may still be large temperature variations *among* rooms. (…) several days later, we find an almost uniform temperature throughout the building".

by strong pleiotropic effects … and 3) are relatively independent from other such units" (Wagner 1996, p. 38). The general outlook of this approach is very similar to mine, in particular when it comes to modules as units of selection (see below). However, I should point out two main differences between Wagner's concept of modularity and mine. First, modules as defined here do not need to be a unit of function. As argued above, modules will typically be both developmental and functional units, but may sometimes be purely (or mainly) functional or purely (or mainly) developmental units (as example of the latter consider the thyroxine-governed reorganization of many organs during metamorphosis). Second, and more importantly, modules are defined here without reference to a genotype-phenotype map, thus allowing for the possibility that genes may contribute to multiple modules. I suspect that the metaphor of a genotype-phenotype map may blind us to the possibly important role of overlap of modules and the pleiotropy of their constituents. As I will argue in more detail below, selection for modularity does not necessarily reduce pleiotropic roles of constituents as often assumed (Riedl 1975; Cheverud 1984, 1996; Wagner 1996; Wagner and Altenberg 1996). In contrast, certain kinds of pleiotropy may be in fact selectively favored in modular systems, where flexible intermodular connections facilitate the redeployment of optimized modules in novel contexts.

3. What are units of evolution?

Units of evolution are entities, which evolve more or less independent of other entities. But in order to be useful, this notion has to be made more precise. While evolution does not necessarily involve selection, I will in the following discussion initially focus on the question, how entities can be units of *selection*. Tackling this highly contentious question first, will lead us to insights which also bear on the more general problem.

3.1. Selection processes and units of selection

The debate about the unit of selection was triggered by an influential paper by Lewontin (1970), in which he pointed out that selection is a rather general process, which will take place whenever three conditions are met: there has to be variation, it has to be reflected in differences in fitness (reproductive rates) and fitness differences have to be heritable. Entities, which fulfill these criteria can be called the "units of selection". It is uncontroversial that these conditions can be met in principle by entities at very different levels of organization, for instance by single genes or by entire organisms. They can also be met by coevolving organisms or by groups of organisms (group selection). Controversy sets in, when it comes

to establish the relative importance of selection at these different levels of organization.

The "unit of selection" debate

Gene selectionists such as Williams (1966) and Dawkins (1976, 1978, 1982) argue for the gene as the principal (or most parsimonious) unit of selection (see also Maynard Smith 1987; Sterelny and Kitcher 1988; Waters 1991), while many other authors subscribe to a view, where units of selection can occur at several hierarchically nested levels, but where typically either the organism or the group are most important (e. g., Mayr 1963; Wimsatt 1980, 1981; Sober 1981, 1984, 1987; Sober and Lewontin 1982; Gould 1982; Wilson 1983; Eldredge 1985; Lloyd 1988; Brandon 1990; Roth 1991; Sober and Wilson 1998). Although I want to support some kind of hierarchical view here, I also will argue that organisms are likely candidates for the unit of selection only under special conditions and that modules may often be more important and prevalent in delimiting units of selection than either genes or organisms.

It has been attempted to clarify the differences between these two camps by distinguishing units of transmission or "replicators" from units of function – "interactors" (Hull 1980, 1981) or "vehicles" (Dawkins 1978, 1982) – in selection processes. A replicator is "an entity that passes on its structure directly in replication" (Hull 1980, p. 318), usually via a copying process, while an interactor or a vehicle is "an entity that directly interacts as a cohesive whole with its environment in such a way that replication is differential" (Hull 1980, p. 318)[8]. While gene selectionists focus on replicators, the other camp emphasizes interactors. Dawkins (1978, 1982), for instance, recognizes that good replicators are typically not good interactors: They exert their phenotypic effects only in cooperation with other genes, which may reside in the same or even in another organism (the "extended phenotype"). However, he claims that only replicators, such as genes, can be units of selection because only they possess "longevity, fecundity and fidelity": In contrast to gene complexes or organisms they are not reshuffled at the generation boundary (longevity); they make copies of themselves (fecundity); and they have heredity, i. e., they faithfully transmit variations to their copies (fidelity). The critics of gene selectionism point out that something important is missing here: The fitness (fecundity) of a single replicator is not determined independently from the fitness of other replicators. Rather replicators give rise to entites which cooperate with each other and with other entities and thereby jointly determine viability and reproductive success. From here the argument may take one of two turns. Either, it is emphasized that interactors rather than replicators are the

[8] A related distinction is the distinction between "units of selection" and "levels of selection" (Brandon 1990, 1999), where the former correspond to replicators, the latter to interactors.

units of selection, because the environment "sees" only phenotypes (e. g., Mayr 1963; Brandon 1990): the fitness of genotypes is "screened off" (Brandon 1990) by the fitness of the former. Or it is concluded that complexes of replicators (gene complexes) which cooperatively determine a contextindependent fitness value are the units of selection (e. g., Mayr 1963; Lewontin 1974; Wimsatt 1980, 1981; Sober and Lewontin 1982; Sober 1984; Lloyd 1988). The latter view emphasizes that the fitness of an allele may depend on which other alleles at the same and other loci it is associated with. A famous example is heterozygote superiority in sickle cell anemia (Sober and Lewontin 1982). The recessive allele a of the sickle cell gene causes severe anemia and consequently low fitness in the homozygous state aa. However, the heterozygotes Aa do not develop the disease. In addition, heterozygotes are actually favored in malaria infected areas, because they are less susceptible to malaria. This shows that the fitness of the a allele is contextsensitive, it depends on whether it is paired with a or with A. Sober and Lewontin argued that here the pairs AA, Aa and aa rather than the single alleles a and A are the units of selection, because their fitness values are contextinsensitive and hence are the real causes of the selection processes. The same holds when several genes cooperate in the realization of a given function: In this case the coselected gene complex rather than the single genes are the units of selection because only they can be assigned contextindependent fitness values (e. g., Wimsatt 1980, 1981; Sober 1984; Lloyd 1988).

General requirements for selection

I do not think that the conflict between the two camps has yet been resolved. In fact, the apparently uncontroversial distinction between replicators and interactors itself is misleading (see also Griffiths and Gray 1994) and may unnecessarily have polarized the discussion. As I will argue below, units of selections are neither replicators nor interactors, although they have properties of both. Both gene selectionists and hierarchy theorists have contributed important criteria for units of selection, but unfortunately neither of them are comprehensive. Rather the virtues of one camp are the vices of the other. While gene selectionists unduly neglect the contextdependence of fitness, hierarchy theorists downplay the problems that arise from the disruption of units due to recombination at the generation boundary. In addition, there is a second problem that has not yet been well addressed in the unit of selection debate. Even when the fitness of a unit is jointly determined by several cooperating subunits, contextdependence of subunit fitness will only be observed, if at least some of the other subunits exist in several variants. This raises the important question (akin to the "absent value problem" of Sober 1984), whether subunits that are important for determining the fitness of the unit, but which are fixed in the population should be considered part of the unit of selection or not.

Before these questions can be answered (in sections 3.2 and 3.3), the general conditions for selection processes need to be reconsidered. I suggest that selection takes place (Fig. 7)

(1) when variants of an entity with different fitness (expected reproductive rates) exist in the population (*variation*);
(2) when each variant reliably (and potentially multiplicatively) reproduces a variant of the same type (*reliable reproduction of variants*)[9];
(3) when the reproduced variants have the same (or similar) fitness values (or at least a restricted range of fitness values) relative to other variants of the same type (*reliable fitness reproduction* of variants).

In summary, selection takes place among several variants of entities, whenever these variants as well as their fitness differences are recurrently reproduced. Direct and sustainable selection of an entity, i. e., the recurrent introduction of variants into a entity and its participation in a sequence of selection processes, is, however, only possible when an additional criterion is met, viz.

(4) when the entity has the property that variants meeting criteria (2) and (3) can be generated recurrently by direct variation of the entities themselves or their constituents (*heredity*)[10].

Selection so defined is typically observed among living systems or their constituents but it is not confined to them[11]. There can also be selection among

---

[9] This could be made more precise to allow for the possibility that the reproductive rate for one or several of the variants is smaller than one or that selection may occur in declining populations. In general, selection occurs when for at least one of several variants there is a non-zero probability of reproducing entities of the same type in non-average frequency.

[10] General criteria for selection processes have been given by several authors and while they usually address similar points, their emphasis differs. Lewontin (1970) suggests that selection requires 1) phenotypic variation, 2) which has to be reflected in differences in fitness (reproductive rates) and 3) fitness differences have to be heritable. His first criterion corresponds to my criterion (1), while his second criterion is implied by my (2) and (3). His third criterion, which requires fitness heritability, is also covered by my criterion (3). Maynard Smith (1987) requires variation and multiplication (my criteria (1) and (2)/(3), respectively) for selection (his "unit of selection"), while he additionally requires heredity (my criterion (4)) for evolution by selection (his "unit of evolution"). An entity has heredity, in the sense intended by Maynard Smith, when it has the property that reliably reproducing variants of the entity can be generated by direct variation of the entities themselves (see below). Dawkins (1978, 1982) argues similarly: His "longevity, fecundity, fidelity" correspond to my criteria (2), (3) and (4) respectively.

While competition is not regarded as a generally necessary condition for selection (e. g., Lewontin 1970), it nonetheless is often important. In many actual selection processes, there is an upper capacity limit for population size, which may result from direct competition or from density-dependent adverse effects (e. g., predator pressure). In these cases selection is additionally constrained in that the frequency of a variant is inversely proportional to the frequency of other variants.

[11] The organization of living systems makes them particularly prone to be involved in selection processes because it facilitates multiplicative reproduction. The organization of living systems involves the continuous maintenance or construction of an organized state far from equilibrium under a variety of environmental conditions. Hence, living systems can be characterized as autopoietic (Varela et al. 1974; Maturana and Varela 1975), self-maintaining (Fontana and Buss 1994 a, b; Fontana et al. 1995) or complex self-re-producing systems (Schlosser

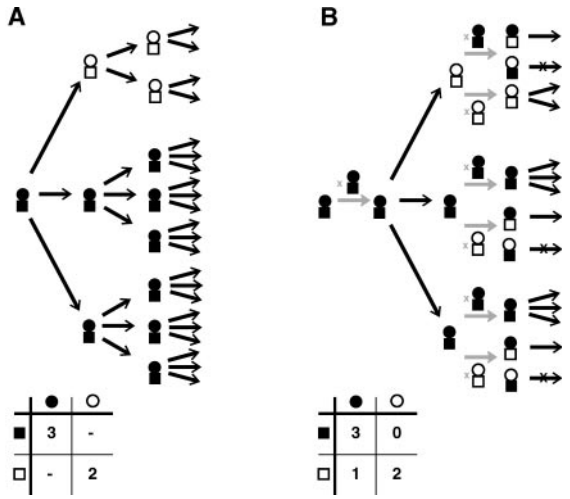


Fig. 7. Schematic diagrams illustrating selection processes. A) Selection among two elements (circle **A** and square **B**), that are numerically coupled in reproduction and tightly linked. Selection occurs among circles as well as among squares, because (1) squares and circles each exist in different variants (black and white); (2) each variant tends to reliably reproduce variants of the same type (black variants reproduce black variants; white variants reproduce white variants; the origin of different colored variants by mutation is a rare event); (3) reproduced variants retain the same fitness value (black variants tend to have three progeny, white variants two). The little table tabulates the fitness values for the different types of reproducing variants. Because no recombination exists, the fitness of circles (**A .**) and squares (**. B**) considered in isolation is identical to the fitness of the composite unit (**AB**) of circles and squares, i. e., each unit satisfies requirements (2) and (3) and acts as a unit of selection, of which **AB** may be considered decomposable into **A .** and **. B**. B) Selection process involving two elements (circle **A** and square **B**), that are numerically coupled in reproduction and not tightly linked, i. e., there may be recombination between them during reproduction. Grey arrows indicate possible outcomes of recombination events after crosses with particular other variants (indicated above or below grey arrows) in the population. Due to non-linkage, all possible combinations of variants of circles and squares will be generated. Under these conditions and assuming that there is fitness epistasis between squares and circles as indicated in the fitness table, units of selection cannot be unambiguously identified, because the composite unit of square and circle **AB** will tend to satisfy requirement (3) but not (2) (it will be a fitness unit, but no unit of variant reproduction), whereas circles (**A .**) and squares (**. B**) will tend to satisfy (2) but not (3) (they will be a unit of variant reproduction, but no fitness unit). See text for further explanation.

computer programs (e. g., genetic algorithms; see Holland 1975; Goldberg 1989; Coveney and Highfield 1995) among patterns in cellular automata (e. g., Reggia et al. 1993) or among RNA molecules, which are replicating in vitro (e. g., Eigen et al. 1981; Maynard Smith and Szathmáry 1995; Ancel and Fontana 2000) to give only a few examples (see also Bernstein et al. 1983).

1998; see also Schlosser 1993). Self-maintenance is usually assumed to be realized by collectively autocatalytic networks of reactions (e. g., Fontana and Buss 1994; Szathmáry 1995). The re-production of a state in such networks goes along with the multiplicative reproduction of their constituents. This facilitates multiplicative reproduction of the entire system, when supplemented with mechanisms of spatial compartmentation (Szathmáry 1995; Maynard Smith and Szathmáry 1995; Szathmáry and Maynard Smith 1995, 1997).

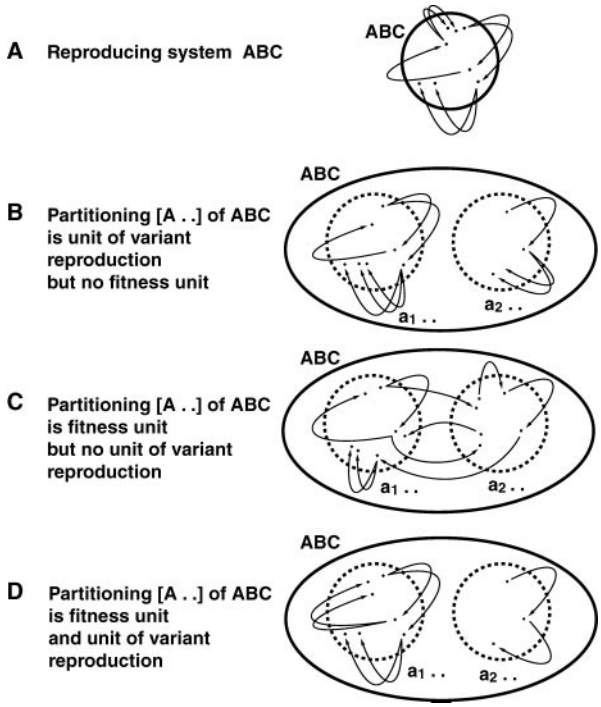


Fig. 8. A) A reproducing system can be depicted as a set of transformations during which tokens (dots) of **ABC** give rise to other tokens of **ABC**. Reproduction may be multiplicative, i. e., a token may give rise to several other tokens. B–D) Variants of **ABC** are subsets (dashed lines) of this set, such as $\mathbf{a_1..}$ (= $\mathbf{a_1BC}$) and $\mathbf{a_2..}$ (= $\mathbf{a_2BC}$). B) These variants may be units of variant reproduction, but no fitness unit in case that each variant reliably reproduces other tokens of the same variants, while the rate of reproduction (fitness) of $\mathbf{a_1..}$ relative to $\mathbf{a_2..}$ is highly variable under ceteris paribus comparisons. C) On the other hand, variants may be fitness units, but no units of variant reproduction in case that the rate of reproduction (fitness) of $\mathbf{a_1..}$ relative to $\mathbf{a_2..}$ is relatively constant (2 in the case depicted) under ceteris paribus comparisons, while variants do not reliably reproduce other tokens of the same variants. D) Finally, units of variant reproduction and fitness units may coincide (forming a unit of variant and fitness reproduction or multiplicator), when each variant reliably reproduces other tokens of the same variant and the fitness of $\mathbf{a_1..}$ relative to $\mathbf{a_2..}$ tends to be constant under ceteris paribus comparisons. See text for further explanations.

A set-theoretical framework for the analysis of selection processes

In order to analyze selection processes in some more detail, it will now be necessary to outline a settheoretical framework of reproducing systems (Figs. 8, 9). Only reproducable entities are selectable. Therefore, each type of selectable entity **A** will either be itself a constituent of a reproducing process or system **R** or will be strictly causally coupled to its constituents. The reproducing system, to which **A** belongs, will be a type of entity whose constituents (e. g., **A**, **B** and **C**) are singly necessary and jointly sufficient for their (multiplicative) reproduction including the reproduction of

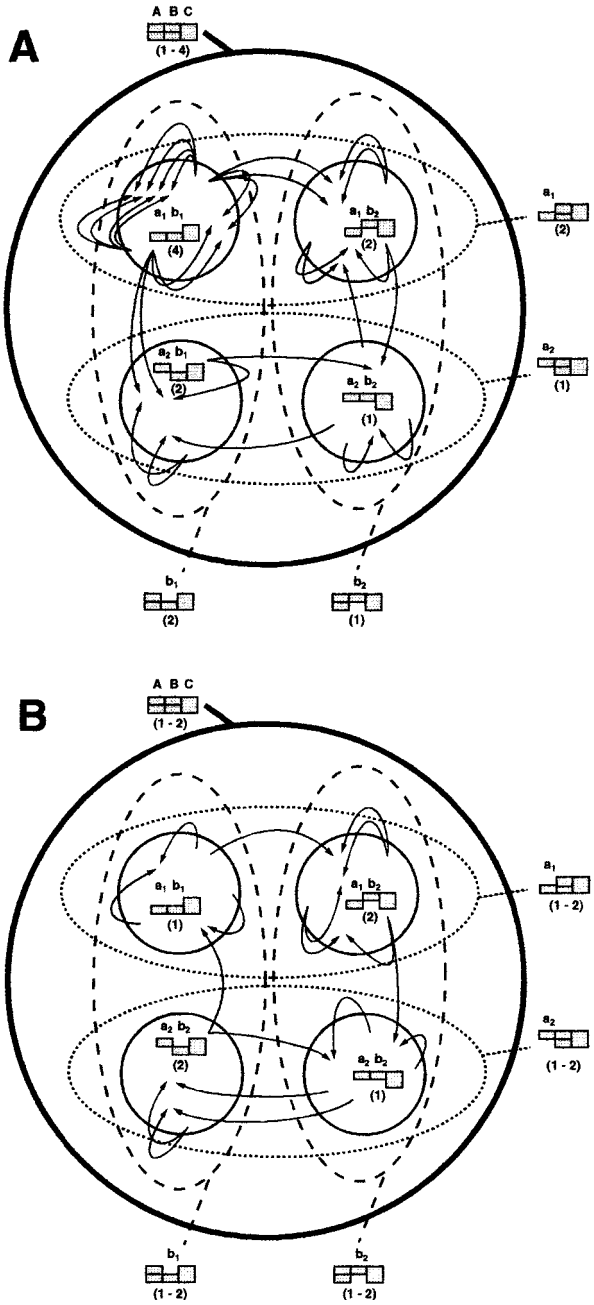
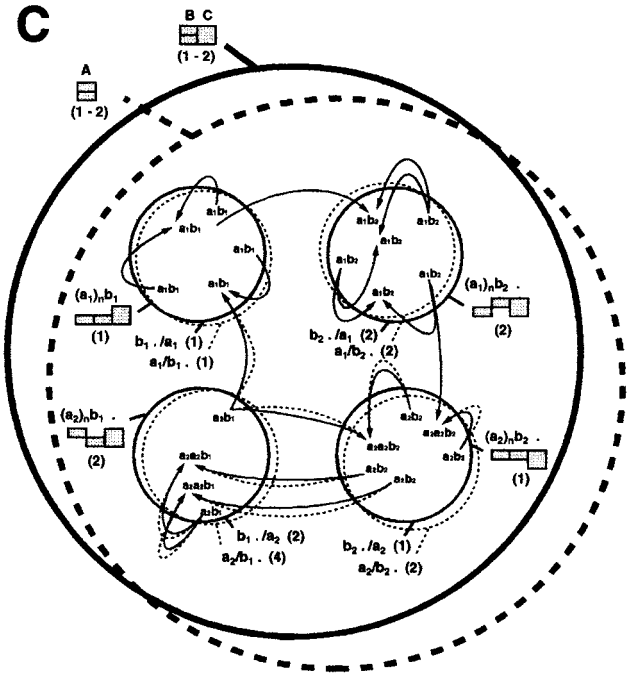


Fig. 9. A) and B) Reproducing system **ABC** (thick black line), where constituents **A** and **B** exist in different variants $\mathbf{a_1}$ (lower left box), $\mathbf{a_2}$ (upper left box) and $\mathbf{b_1}$ (lower middle box), $\mathbf{b_2}$ (upper middle box), respectively. It is assumed that reproduction of variants (arrows) involves mating between two haploid parents followed by recombination and reduction to the haploid state by discarding one of the two combinations of recombined constituents with equal probability. Three different partitionings (thin lines) of the entire set **ABC** (thick black line) are indicated: (1) partitioning [$\mathbf{AB}.$] (thin solid lines) with the 4 subsets $\mathbf{a_1b_1}.$, $\mathbf{a_1b_2}.$, $\mathbf{a_2b_1}.$ and $\mathbf{a_2b_2}.$, (2) partitioning



[**A . .**] (dotted lines) with the 2 subsets $\mathbf{a_1}$ . . and $\mathbf{a_2}$ . ., and (3) partitioning [**. B .**] (hatched lines) with the two subsets . $\mathbf{b_1}$. and . $\mathbf{b_2}$ .. The range of relative fitness values for each set (assuming ceteris paribus comparisons as in Fig. 8) is indicated in parentheses. In A) **A** and **B** make independent fitness contributions, i. e., there is no fitness epistasis between them. Fitness values of a variant of [**AB .**] can be calculated by simply multiplying fitness values for the orthogonal partitionings [**A . .**] and [**. B .**], i. e., the fitness of $\mathbf{a_1b_1}$. is the fitness of $\mathbf{a_1}$ . . multiplied with the fitness of . $\mathbf{b_1}$. etc.. Therefore all partitionings in A) are fitness units (and multiplicators if there is sufficient reliability of variant reproduction), but [**AB .**] is decomposable into [**A . .**] and [**. B .**]. In B) however, there is fitness epistasis between **A** and **B**. Contextinsensitive fitness values exist only for variants of the more comprehensive partitioning [**AB .**] and these cannot be calculated from the fitness values of the orthogonal partitionings [**A . .**] and [**. B .**]. Therefore in B) partitioning [**AB .**] is a nondecomposable fitness unit (and multiplicator if there is sufficient reliability of variant reproduction).

C) illustrates that a fitness unit [**AB .**] (thin solid lines) due to similar epistatic interactions between **A** and **B** may exist even in cases, where only **B** and **C** are numerically coupled in reproduction, whereas **A** is able to reproduce at a different rate (e. g., being a transposon). Solid arrows indicate co-reproduction of **A** and **BC**, while broken arrows indicates supernumerary reproduction of **A**. In the case depicted, variant $\mathbf{a_1}$ of **A** always coreproduces with **B** and **C**, whereas variant $\mathbf{a_2}$ always reproduces twice, when **B** and **C** reproduce once. The diagram also shows that when constituents are not numerically coupled, different fitness values can be determined (1) for each numerically uncoupled group of constituents, i. e., the fitness of variants of **A** in a particular context of **B** (denoted [**A/B .**], thin dashed lines), and the fitness of variants of **B** in a particular context of **A** (denoted [**B ./A**], thin solid lines); (2) for variants of reproducing units encompassing all constituents [**AB .**] (thin solid lines). Because **A** can reproduce independently, its number may vary relative to the number of **B** and **C**, so a more appropriate notation may be [$\mathbf{(A)_nB}$.]. Because only $\mathbf{a_2}$ is capable of independent reproduction in the case illustrated, the fitness of $\mathbf{b_1./a_2}$ is equal to the fitness of $\mathbf{(a_2)_nb_1}$. but differs from the fitness of $\mathbf{a_2/b_1}$ .. Units [**A/B .**] and [$\mathbf{(A)_nB}$.] are not in conflict in the case illustrated (conflict would arise, if the fitness of $\mathbf{(a_2)_nb_1}$. were less than the fitness of $\mathbf{(a_1)_nb_1}$.).

one or more other tokens of **A**[12]. Therefore, a reproducing system **R** may also be specified by a list of its constituents **ABC**. Each reproducing process/system defines sets of transformations for the constituents of the process/system as well as for all elements strictly causally dependent on them.
Two kinds of such reproducing systems can be distinguished. First, there are systems that reproduce their constituents, but do not reproduce other tokens of themselves. Examples would be collectively autocatalytic cycles of metabolic reactions (for instance the Calvin cycle, see Szathmáry 1995) or other self-maintaining but not self-reproducing organizations (Fontana and Buss 1994 a, b, Fontana et al. 1995). Second, there are reproducing systems that not only reproduce their constituents, but also reproduce other tokens of themselves. Such systems have been called "reproducers" (Griesemer, cited in Szathmáry and Maynard Smith 1997). A paradigmatic reproducer in the realm of living systems, is the entire "life cycle" of the organism. It includes all patterns of interactions among the parts of the organism as well as between the organism and its environment, which are reliably reproduced in subsequent generations (Uexküll 1928; Lewontin 1983; Oyama 1985; Wimsatt 1986; Gray 1992; Schlosser 1993, 1996; Griffiths and Gray 1994). This characterization allows for the possibility that not only elements, that are considered proper parts or "autoconstituents" (Schlosser 1993, 1996) of the organism are constituents of the reproducer, but also those elements of the environment, interactions with which are reliably reproduced and are themselves necessary factors of reproduction ("alloconstituents"; Schlosser 1993, 1996). The latter may include interactions with abiotic factors (e. g., absorption of photons from the sun in green plants) as well as with other organisms (e. g., pollination of plants by insects). Consequently, reproducers can be overlapping or may even be hierachically stacked like Russian dolls, because one element can be a constituent of several reproducers. Genes involved in the determination of flower traits which attract pollinating insects are, for example, at the same time autoconstituents of the flowering plant and alloconstituents of the pollinators. This implies that not all constituents of a reproducer necessarily reproduce at the same time and with the same absolute number of progeny. Only the autoconstituents of a reproducer, which always co-reproduce at the same time and by the same factor (e. g., all genes of a single

[12] Because reproduction (and re-production in general) involves quasi-cyclical sequences of state-transitions a reproducing system may be described by listing all elements of one of its states (e. g., the zygote or another propagule), which are either constituents of this state or whose descendants are constituents of successive states of the cycle. Elements of such a compressed description may be said to be coupled or connected, when there are couplings or connections either between them or between their descendant constituents during the state cycle. Because of the possibility that states may be reproduced by several alternative pathways (e. g., due to plasticity of state transitions to varying environmental conditions), it is often convenient to choose a state for description that is an obligatory bottleneck which is reliably reproduced in the state cycle (such as the zygote of sexually reproducing organisms).

organism), could be characterized as numerically coupled in reproduction (Fig. 7)[13].

It needs to be emphasized that neither a reproducing system in general nor a reproducer in particular is the same as a "replicator" (Dawkins 1976, 1978, 1982; Hull 1980, 1981). The term "replicator" should be confined to entities, for which the reproduction of all constituents involves direct copying from a template; the latter is necessary (although usually not sufficient) for replicator reproduction as a common progenitor giving rise to several descendants. Many reproducers, such as organisms are no replicators, because many of their constituents, e. g., proteins, membrane components and metabolic intermediates are reproduced without involving direct copying processes. Genes, however, would typically qualify as replicators, because they are copied from a template, but not as reproducers, because they depend on cooperation with other constituents (e. g., the proteins of the replication machinery as well as other genes and proteins subserving other vital functions of the organism in which they reside) for their reproduction.

Requirements for selection processes from a set-theoretical perspective

This set-theoretical framework of reproducing systems allows us to formulate the four requirements for selection processes listed above more precisely. First, in order for selection to occur, a reproducing system **R** has to occur in several variants. Generally, *variants* can be defined as different subtypes, e. g. $\mathbf{r_1}$ and $\mathbf{r_2}$, of **R**. Such subtypes may, for example, be characterized by a particular subset of the permitted range of values for one or several constituents of **R**. For instance, if **A**, **B** and **C** are constituents of **ABC**, we may recognize subtypes $\mathbf{a_1BC}$ and $\mathbf{a_2BC}$ (also denoted simply as $\mathbf{a_1..}$ and $\mathbf{a_2..}$; Fig. 8 B–D) of type **ABC**, where **A** may represent a gene, $\mathbf{a_1}$ and $\mathbf{a_2}$ two of its alleles. Typically, there are different ways (called "schema" in Holland 1975), how a type of reproducing system such as **R** can be completely partitioned into subtypes or variants (for a similar approach see Wagner and Laubichler 2000). These different partitionings of **R** may be defined by a variety of criteria, but in order to simplify the discussion, I will focus here on those partitionings that are defined by variants of one or a combination of several constituents of **R** (Fig. 9)[14]. Constituents,

---

[13] It is important to distinguish such numerical coupling of constituents from *linkage*! The latter term refers to the probability that a particular combination of variants of the constituents of a reproducer will not be disrupted, i. e., replaced by a different combination of variants of the constituents, during the process of reproduction. Thus, constituents that are tightly numerically coupled in reproduction might be either tightly linked (e. g., genes adjacent on the same chromosome) or completely unlinked (e. g., genes on different chromosomes that are randomly segregated).

[14] Partitionings are, however, not necessarily defined by a simple conjunction of variants of one or several of its constituents; some partitionings may rather be defined disjunctively: Constituents can be recognized at different hierarchical levels; constituent **A** (e. g., a type of

which "matter" for distinguishing different variants of a certain partitioning will be called *characterizing constituents*. I will denote a partitioning of **ABC** with a single characterizing constituent **A** as [**A..**]. This signifies that different tokens of **ABC**, which instantiate different variants $\mathbf{a_1}$ and $\mathbf{a_2}$ of constituent **A**, will belong to different subsets $\mathbf{a_1..}$ and $\mathbf{a_2..}$ of [**A..**], whereas the dots indicate that variants of other constituents (**B** and **C**) do not matter for defining subset membership in [**A..**]. Accordingly, tokens of types $\mathbf{a_1b_1c_1}$, $\mathbf{a_1b_2c_1}$, $\mathbf{a_2b_1c_1}$, $\mathbf{a_2b_2c_1}$, $\mathbf{a_2b_2c_2}$ etc. would be divided into two different subsets ($\mathbf{a_1..}$ and $\mathbf{a_2..}$) with respect to partitioning [**A..**], but into four different subsets ($\mathbf{a_1b_1.}$, $\mathbf{a_1b_2.}$, $\mathbf{a_2b_1.}$, $\mathbf{a_2b_2.}$) with respect to partitioning [**AB.**], where both **A** and **B** are characterizing constituents.

Second, selection can only take place among those variants of a reproducing system, which reproduce variants of the same type. I will call the unit of all characterizing constituents, which define a partitioning of reliably reproducing variants, a *unit of variant reproduction* (Fig. 8, B, D). Generally, I use the term "unit" to refer to the singly necessary and jointly sufficient constituents of a process (see above), in this case the process of reliable variant reproduction. Sometimes I will also use the term "unit" to refer to the partitioning itself. **AB** (or [**AB.**]) will be a unit of variant reproduction of a reproducing system **ABC**, if for instance variants of type $\mathbf{a_1b_1.}$ reproduce variants of $\mathbf{a_1b_1.}$, whereas variants of type $\mathbf{a_2b_2.}$ reproduce variants of type $\mathbf{a_2b_2.}$. In asexually reproducing organisms, all possible partitionings will be defined by units of highly reliable variant reproduction. With sexual recombination and high degrees of polymorphisms, however, reliability of variant reproduction declines for partitionings with increasing numbers of characterizing constituents. The implications of this will be discussed in detail in section 3.2.

Third, reliable reproduction of variants is not sufficient for selection. In addition it has to be true that reproducing variants have reliably recurrent fitness differences. I will call a unit of characterizing constituents a *fitness unit*, when they define a partitioning, all variants of which reproduce with an expected rate that is relatively constant (falling in a restricted range of fitness values) and conserved in subsequent generations (although gradual shifts due to frequency dependent effects on fitness may be permitted[15])

---

organ) of **ABC**, for example, may be analyzable into lower level constituents **X** and **Y** (e.g., two different cell types). Such an analysis may reveal that **A** can be multiply realized at a lower level and hence that a partitioning [**A..**] defined by subtypes of a particular constituent **A** may be defined by a disjunction of variants of lower level constituents: $\mathbf{a_1..}$ may for example be instantiated by $\mathbf{x_1y_1..}$ *or* $\mathbf{x_1y_2..}$, and $\mathbf{a_2..}$ by $\mathbf{x_2y_1..}$ *or* $\mathbf{x_2y_2..}$. Another possibility is that $\mathbf{a_1..}$ is instantiated by $\mathbf{x_1..}$ *or* $\mathbf{x_2..}$ and $\mathbf{a_2..}$ by $\mathbf{y_1..}$ *or* $\mathbf{y_2..}$. This allows for the possibility that a certain type of variant of a higher level structure like an organ may be represented by multiple combinations of genes.

[15] In cases of frequency dependent selection (see e.g., Sober 1984; Maynard Smith 1989; Ridley 1993; Michod 1999), variants will reproduce variants with slightly different fitness values in the next generation, due to the gradual increase or decrease of fitness with increasing frequency of a variant.

relative to other variants of the partitioning for a certain set of environments (Fig. 8 C, D). For instance, **AB** will be a fitness unit, if variants of one type (e. g., $\mathbf{a_1b_1.}$) tend to reproduce at an expected rate that is more or less fixed and conserved relative to all variants of another type (e. g., $\mathbf{a_2b_2.}$), where "expected rate" signifies rate under ceteris paribus comparisons (i. e., while reproductive rates may differ dramatically between say tokens of $\mathbf{a_1b_1c_1}$ and $\mathbf{a_1b_1c_2}$, tokens of $\mathbf{a_1b_1.}$ will tend to have a more or less constant fitness relative to tokens of $\mathbf{a_2b_2.}$, when tokens of $\mathbf{a_1b_1c_1}$ are only compared with tokens of $\mathbf{a_2b_2c_1}$, $\mathbf{a_1b_1c_2}$ with $\mathbf{a_2b_2c_2}$ etc. for a particular population at a given time and under similar environmental conditions). **AB** will be a *nondecomposable* fitness unit, if the rate of reproduction of $\mathbf{a_1b_1.}$ versus $\mathbf{a_2b_2.}$ will not merely be the sum or the product of the relative reproductive rates of $\mathbf{a_1..}$ vs. $\mathbf{a_2..}$ and $\mathbf{.b_1.}$ vs. $\mathbf{.b_2.}$ (Fig. 9).

Reliable reproduction of fitness differences will only take place, if the characterizing constituents of a partitioning are both a unit of variant reproduction *and* a fitness unit or, more precisely, when each variant of a partitioning tends to reliably reproduce variants of the same kind and with similar fitness differences relative to other variants. A unit fulfilling both of these requirements could be called a *unit of fitness and variant reproduction* or more shortly a *multiplicator* (Fig. 8 D). I will, here, adopt a restrictive use of the terms "unit of fitness and variant reproduction" or "multiplicator" to refer only to those partitionings that are at the same time a unit of variant reproduction and a *nondecomposable* fitness unit. A multiplicator, as defined here, has affinities to Hull's (1980, 1981) "interactor". Nonetheless, I will avoid the latter term. It is misleading, because it may be (and is often) used for entire reproducers as well as for multiplicators and therefore tends to blur these important distinctions.

A multiplicator is a unit of reproducing elements which cooperatively determine a relatively contextindependent rate of their reproduction (i. e., the latter remains sensitive to contingent factors, but is independent from the context of other reproducing elements). Unless the reproduction of all constituents of a reproducer is tightly linked (as in cases where there is no sexual recombination), there may be different multiplicators (e. g., **AB** and **C**) for a certain type of reproducing system (**ABC**), each of which reproduces and exhibits a contextindependent fitness value, which is determined by only a fraction of the constituents of the reproducing system **ABC**. Although as parts of the same reproducing system **A** and **B** are constitutive for the reproduction of **C**, **C** may be an independent multiplicator in case the relative fitness of variants of **C** remains the same for each variant of **A** or **B**. Therefore, each multiplicator has independent effects on the fitness of the reproducing system (Fig. 9). For instance, different character complexes of a type of organism (e. g., a moth) such as pigmentation on the one hand and locomotory structures on the other hand, may each exist in different variants (white and black color; fast and slow movement) with

independent effects on fitness (black moths may be three times fitter than white ones for each variant of the locomotory system; fast moths may be four times fitter than slow moths for each variant of pigmentation)[16]. Because different reproducing systems can be overlapping, the characterizing constituents of a multiplicator can also be parts of different reproducers. Examples are coevolving character complexes in mutualistic (e. g., flowers and feeding appendages of pollinators) or parasitic (e. g., "arms races" between host and parasite) interactions (see Dawkins 1982; Futuyma 1986; Ridley 1993) as well as "trait groups" (see Wilson 1983; Sober and Wilson 1998) of altruistically cooperating social behaviors (see also section 4.1).
All the characterizing constituents of a multiplicator as well as all elements strictly causally dependent on them are selectable entities, because they tend to produce other tokens of the same type at a reproducible rate. In other words, requirements (2) and (3) for a selection process ensure that the organization of an entity as well as its fitness are *heritable* and are in fact *inherited* by their progeny. It has been emphasized already that these requirements can be met by any entities that produce other entities of the same kind as part of a reproducing system and that it is not necessary that the entities are replicators in the narrow sense, i. e., that they directly serve

---

[16] Processes of reproduction in general and processes of selection in particular can only be understood, when it is always remembered that they are processes of the reproduction of different tokens of the same type. This may sound trivial, but it is easily forgotten leading to fallacious arguments. Sober and Wilson (1994), for example, use the argument of "common fate" to conclude that organisms can be units of selection but not their genes or even complexes of genes: "If the genes inside an organism are 'in the same boat', one gene cannot do better than other genes *in the same organism*." (p. 551, their italics). While this is certainly true for a single individual, it is – due to sexual recombination – not true for types, for instance organisms of the same genotype with respect to a certain gene or gene complex. After reproduction, a new token of an organism of the same genotype may contain another token of the gene, but in a different genetic background. Moreover, each individual organism is at the same time a token of several different genotypes. If we assume that one gene is constitutive for coloration and another gene for speed, a black and fast flying moth belongs at the same time to the genotype "black moths" that may be (ceteris paribus) three times fitter than the genotype "white moths" and to the type "fast moths" that may be (ceteris paribus) four times fitter than the type "slow moths". Under the further assumption that pigmentation and speed have independent fitness contributions (no epistatic effects), they can be separate units of selection, which spread with different rates in the population (black pigmentation slower than speed).
Because selection can take place only among types, the term "selection" can also not be used to characterise non-recurrent, singular events, where one entity has a reproductive advantage over another, because for such singular events the distinction between differential reproduction due to "chance" and differential reproduction due to "selection" is meaningless. If there is only a single black moth alive, which has more progeny than the average white moth in the population, it is principally impossible to attribute this excess to its particular coloration; it may have just been lucky. We only talk of "selection" when each variant typically (reliably, recurrently) gives rise to a variant with the same fitness, i. e., to a token of the same type with respect to fitness reproduction. In other words, the principle of the "survival of the fittest", often used to describe selection processes (although it is not applicable to all selection processes; see Michod 1999), is non-tautological only, if fitness is an attribute of types not of tokens.

as a template in a copying process (see also Roth 1991; Wagner 1995). Therefore, I agree with the proponents of the so-called "developmental systems view" that the concepts of inheritance and heritability are broadly applicable not only to genes, but to everything that is reliably reproduced including certain interactions with the environment (Oyama 1985; Wimsatt 1986; Gray 1992; Griffiths and Gray 1994). However, heritability or inheritance thus characterized does not guarantee that an entity can recurrently participate in selection processes. This is only the case for entities which additionally have *heredity*.

Heredity

Following Maynard Smith and Szathmáry (Maynard Smith and Szathmáry 1995; Szathmáry 1995; Szathmáry and Maynard Smith 1995, 1997), I define heredity as a property of only those entities, which will reliably reproduce any variation out of a more or less extensive set of variations resulting from the direct perturbation of these entities or their constituents. Entities with inheritance (reproducing entities) but without heredity are either not *directly* selectable, being strictly causally dependent on other selectable entities, or are not *sustainably* selectable, being not open for the repeated introduction of variants. This deserves some further comment, because the issues of inheritance and heredity are frequently confused (e. g., Oyama 1985; Gray 1992; Griffiths and Gray 1994; Sober and Wilson 1994).

First, not everything is *directly* selectable. Due to a mutation there might be two alleles of a gene, $\mathbf{a_1}$ and $\mathbf{a_2}$ in a population. Assuming that these two alleles will be transcribed and translated into proteins, there will also be two proteins $\mathbf{P(a_1)}$ and $\mathbf{P(a_2)}$. If there are fitness differences between the two, there will be selection. Both genes and proteins are inherited/heritable and selectable, because each is reproduced in the next generation in a certain number of copies. However, only the gene – more specifically only a gene residing in the germ line – has heredity, because when a new variant $\mathbf{a_3}$ is introduced by mutation of the gene it will be reproduced as well, whereas this will not be the case when a new variant $\mathbf{P(a_3)}$ of the protein is introduced by the manipulation of protein structure. Hence there may indeed be inheritance and heritability but there is no heredity of proteins! A protein is selectable only, insofar each of its variants is strictly causally dependent on a variant of a gene. However, the definition of heredity given, allows to attribute heredity not only to genes but also to higher level entities (such as the liver or other organs), when at least some of their lower level constituents (genes, proteins etc.) have heredity, thus allowing the recurrent introduction of variation.

Second, it is at least conceivable that in some cases there may be selection among reproducing entities which do neither have heredity nor are strictly dependent on hereditary factors (i. e., selection would be direct). For in-

stance, there may be selection between two versions of an autocatalytic cycle of metabolic reactions (such as the Calvin cycle, see Szathmáry 1995) with slightly different metabolic intermediates. However, such selection processes are singular events that are not open for further variation, because typically modifications of metabolic intermediates disrupt an autocatalytic cycle; they do not generally lead to another autocatalytic cycle in which they are reproduced (Szathmáry 1995; Maynard Smith and Szathmáry 1995). In other words, the selected entities in these cases are not *sustainably* selectable. In conclusion, it is certainly true that there is inheritance not only of genes, but also of cytoplasmic factors, membranes, traditions, some environmental factors and so forth (Oyama 1985; Gray 1992; Griffiths and Gray 1994). However, this alone implies nothing about heredity, which is the prerequisite for direct and/or sustainable selectability.

Of all the constituents of a reproducing system only some (often replicators residing in the germ line such as genes, but also traditions; moreover, higher level entities, containing such replicators as lower level constituents) will have heredity themselves, others, such as proteins, essential nutrients, membranes, many cytoplasmic factors or interactions with abiotic environmental factors, will have not, although they may be constitutive for survival and reproduction (e. g., the sun for green plants). In order to define variants involved in selection processes it is usually sufficient to describe all elements, which have heredity, because they comprise all directly and sustainably selectable elements and the reproduction of particular variants of all other selected elements is dependent on them. Therefore, a unit of fitness and variant reproduction that qualifies as a unit of selection should be typically definable exclusively in terms of characterizing constituents that have heredity – its "hereditary constituents". This does not preclude, however, that the same unit of selection may be identified by alternative, but equivalent descriptions that do not explicitly cite hereditary constituents[17].

The unit of selection

Before I summarize this section by giving a brief characterization of a unit of selection, one final point needs to be emphasized. For selection processes it is usually possible to distinguish between two different kinds of variants that are selected and can be attributed a fitness value. First, there may be different variants of hereditary (e. g., genes) or nonhereditary (e. g., proteins) constituents of a reproducer (i. e., elements which are under certain circumstances necessary for its reproduction) that differentially affect

[17] It also should be noted that a certain unit of selection may be multiply realized by its hereditary constituents (see footnote 14), i. e., a certain partitioning that acts as a unit of selection may have a disjunctive genetic basis.

their reproductive rates by affecting their connections to other constituents of the reproductive process (constitutive variants). Second, there may be variants of constituents exclusively affecting such other elements, which are only byproducts and not required for their reproduction, or variants of these neutral elements themselves. In Sober's terminology (1984) there is selection *of* each of the two kinds of variants, while there is only selection *for* the constitutive variants of a reproducer. For instance, a certain variant of a transmembrane receptor in the cell membrane may exhibit increased binding affinity for its ligand as well as for another transmembrane receptor. However, only ligand binding may be constitutive for reproduction, whereas dimerization of receptors may be a neutral side effect. In that case, there would be selection *of* ligand binding as well as of receptor dimerization, but only selection *for* the former[18]. Only constituents, for which constitutive variants exist, need to be considered part of the unit of selection.

In summary, then, a *unit of selection* is a unit of all those constituents of one or several reproducer(s), which exist in several constitutive variants, and which jointly define a partitioning into reliably reproducing variants (unit of variant reproduction) with reliable and nondecomposable fitness differences between them (fitness unit), in other words a nondecomposable unit of fitness and variant reproduction (or multiplicator) (Fig. 9). All constituents of a unit of selection may be said to be coselected. A unit of selection as defined here does not include nonconstitutive elements inadvertently selected as byproducts, and typically it will be definable exclusively in terms of hereditary constituents. In the biological context, a unit of selection may, for example, be represented by a complex of genes. However, because units of selection can span several reproducers, genes of such a complex may include constituents of different reproducers.

3.2. Units of selection at different levels

The last section broadly outlined the concept of a unit of selection. It is now necessary to have a closer look in order to clarify at what level of a hierarchy of organization units of selection may be found. For this purpose, I will try to restate some basic insights of population genetics in the

[18] Not always, however, is the distinction between variants, which affect other constituents for reproduction and have a function (see Schlosser 1998), and variants, which do not, so straightforward, because elements may be constitutive for reproduction (functional) in some environments, but not in others. To accomodate this, the concept of functional fitness has been introduced (Schlosser 1998), which measures the degree to which the fitness of a selected element is due to its constitutive involvement in its own reproduction (i. e., its functionality). In general, the functional fitness of an element is defined relative to a set of environments **E** as the fitness of the element weighed by a factor f_e, which ranges from 0 to 1 and indicates the probability that the element will be constitutive for its reproduction in **E**. When an element is under all circumstances necessary for its reproduction in **E**, its functional fitness equals its fitness.

settheoretical framework outlined above. I will first consider single selection processes among a particular given combination of variants of a reproducer (this section), and will then broaden my perspective and consider the implications for sets of various possible constellations of variants of a reproducer (section 3.3). Just for the sake of argument, I will always assume that reproducing organisms are haploid, because the possibility of dominance in diploid organisms additionally complicates matters (see e. g., Falconer 1960; Ridley 1993; Maynard Smith 1989), without affecting my principal conclusions (there can be sexual recombination in haploid organisms, when the meiotic division immediately follows the fusion of haploid gametes).

Units of selection may exist at several hierarchical levels

Let me introduce the problems with an imaginary example: the evolution of a reproducer **ABC** during a limited period of time T (Fig. 9). I assume that **A**, **B** and **C** are all its (hereditary) constituents. The reproducer can be represented as a set of all individuals of type **ABC** which are reproduced during T. During this period, the reproducer will exist in only a small number of variants, say $\mathbf{a_1b_1c_1}$, $\mathbf{a_2b_2c_2}$ and all recombinants ($\mathbf{a_1b_2c_2}$ etc.) between these two. I assume that each of these variants reproduces with a determinate fitness value (each token of the variant has the same fitness). Now there are several possibilities, how the set **ABC** can be completely partitioned into subsets, depending on which constituents of the reproducer are chosen as characterizing constituents for the definition of the partitioning: [**A . .**], [**. B .**], [**. . C**] , [**AB .**], [**. BC**] and [**ABC**]. In our case, 2 subsets ($\mathbf{a_1}$ **. .** and $\mathbf{a_2}$ **. .**) can be distinguished with respect to partitioning [**A . .**], 4 different subsets ($\mathbf{a_1b_1}$ **.**, $\mathbf{a_1b_2}$ **.**, $\mathbf{a_2b_1}$ **.**, $\mathbf{a_2b_2}$ **.**) with respect to partitioning [**AB .**], and 8 different subsets for partitioning [**ABC**] ($\mathbf{a_1b_1c_1}$, $\mathbf{a_1b_1c_2}$, etc.) (Fig. 9). Each variant of each partitioning can be assigned a range of relative fitness values, which is determined by ceteris paribus comparisons with other variants of the partitioning (e. g., the range of fitness values of $\mathbf{a_1b_1}$ **.** relative to $\mathbf{a_1b_2}$ **.** is determined by comparing the fitness of $\mathbf{a_1b_1c_1}$ with $\mathbf{a_1b_2c_1}$, $\mathbf{a_1b_1c_2}$ with $\mathbf{a_1b_2c_2}$ etc.).

The fact, that a reproducing system can be partitioned in many different ways implies that variants of the system can be recognized at many different levels. *Comprehensive partitionings* (e. g., [**ABC**]) of the set are defined by many characterizing constituents (high level entities such as a multigene complex) and allow the distinction of many (in our case eight) different variants, whereas *focal partitionings* (e. g., [**A . .**]) are defined by few characterizing constituents (low level entities such as a single gene) and, accordingly, allow the distinction of only few (in our case two) variants. I call a partitioning P_1 of a particular type (e. g., **ABC**) into subtypes *orthogonal* to another partioning P_2 of that type, if each subtype of P_1 overlaps with each subtype of P_2, i. e., when no subtype of P_1 does correspond

merely to the combination or splitting of subtypes of P_2 (see also Wagner and Laubichler 2000). For example, two partitionings are orthogonal, if they have no characterizing constituents in common ([**A . .**] and [**. B .**]). While there are few partitionings that are orthogonal to comprehensive partitionings, there are many partitionings that are orthogonal to focal partitionings (e. g., for an organism with a genome of 1000 genes, 1000 different orthogonal partitionings with single genes as characterizing constituents can be defined, but only one partitioning with all genes as characterizing constituents).

The possibility of partitioning a reproducing system into variants at many different levels, raises the question, whether comprehensive and focal partitionings are equally good candidates for units of selection. In order to qualify as a unit of selection, a partitioning needs to satisfy the criteria outlined above: Different variants of the partitioning need to differ in fitness and the partitioning has to be a multiplicator (unit of fitness and variant reproduction), i. e., it has to be (1) a unit of variant reproduction (different variants of the partitioning must reliably reproduce variants of the same type), and (2) a fitness unit (different variants of the partitioning need to exhibit a reliable – although possibly frequency dependent – and nondecomposable reproductive rate). To what extent are these requirements fulfilled by partitionings at different levels?

Let me focus on the second requirement first. It is ideally fulfilled whenever the fitness values of all the characterizing constituents of a partitioning ([**AB .**]) reciprocally depend on each other, but are contextindependent, i. e., do not depend on the realization of particular variants of the remaining constituents (**C**) of the reproducer (Fig. 9 B). This is the case when there is fitness *epistasis* for all characterizing constituents of the partitioning (nondecomposability), whereas there are nonepistatic – additive or multiplicative – fitness interactions with the context (contextinsensitivity). When these conditions are fulfilled, the fitness of a variant type of a comprehensive partitioning of a reproducer ($\mathbf{a_1b_1c_1}$) can be calculated by adding or multiplying the fitness of its variant type ($\mathbf{a_1b_1}$ **. .**) of a more focal partitioning ([**AB .**]) with the fitness of its variant type (**. .** $\mathbf{c_1}$) in a complementary orthogonal partitioning ([**. . C**]). The more comprehensive the partitioning is, the higher is the probability that variants of this partitioning will exhibit contextinsensitive fitness, in particular in tightly integrated reproducing systems such as organisms, where there is likely to be some degree of fitness epistasis between most of its constituents.

While such nondecomposable contextindependence of fitness has often been considered the hallmark of a unit of selection (e. g., Lewontin 1974; Wimsatt 1980, 1981; Sober 1981, 1984; Sober and Lewontin 1982; Lloyd 1988), others have argued that this is an unjustified requirement (Dawkins 1982; Maynard Smith 1987, 1998; Sterelny and Kitcher 1988; Waters 1991). The latter authors point out that if absolute contextinsen-

sitivity were required, nothing could ever be a unit of selection, because *every* selection process is relative to the presence of certain environmental conditions (as in frequencydependent selection and selection in spatially heterogeneous environments). In the case of sickle cell anemia, for example, heterozygote superiority will only be observed in areas where malaria is widespread. But if we accept the fact that fitness values of a gene or an organism are sensitive to specific environmental conditions, why should we not accept that the fitness values of a gene are also sensitive to the presence or absence of other genes of the same organism? From this inescapability of contextsensitivity often the pluralistic conclusion is drawn (Maynard Smith 1987; Sterelny and Kitcher 1988; Waters 1991) that while indeed entities at different levels (genes, organism etc.) can be viewed as units of selection, it is mainly a matter of preference which units of selection one emphasizes. But this pluralism throws out the baby with the bathwater, because it fails to make the important distinction of contexts which vary independently from the selected entity (contingent environmental conditions) and such contexts which are also subject to selection as part of a reproducer (constituents defining orthogonal partitionings) (for a similar argument see Lloyd 1988, Brandon 1990). Whereas in the former case, one can indeed do nothing but either restrict comparisons to similar contexts or average over different contexts (such as different environmental circumstances), in the latter case, one finds that selection processes are in principle more adequately represented and can be more accurately modeled, when a more comprehensive entity, which comprises coselected constituents, is recognized as fitness unit in a selection process[19]. In fact, gene selectionists resort to the same kind of reasoning when they argue that the gene

[19] It has been argued that contextsensitivity of fitness is merely a case of frequency dependent selection (e. g., Maynard Smith 1987), but this is potentially misleading. The term "frequency dependent selection" is usually reserved for cases where the fitness of one variant of a partitioning depends on the frequency of other variants of the partitioning. This may or may not be true for cases of contextsensitivity. When there is contextsensitivity (fitness epistasis between two loci), the accurate prediction of the frequency of a certain allele is only possible, when not only the frequency of other alleles at the same locus, but also the frequency of alleles at all other loci with epistatic fitness effects as well as linkage parameters are taken into account. The dimensionality of the problem quickly increases with the size of the coselected entities (Lewontin 1974; Wimsatt 1980, 1981). In the simplest case with fitness epistasis between 2 unlinked loci and 2 alleles each (a_1,a_2; b_1,b_2), 2 parameters p_{a1} (or p_{a2}) and p_{b1} (or p_{b2}) need to be known to describe changes in allele frequency (i. e., there are 2 degrees of freedom). Contextsensitivity can also be modeled by expressing the fitness of alleles at one locus (e. g., w_{a1}) as a function of the frequency of alleles at the coselected loci (e. g., p_{b1}). This is just another way to acknowledge the fact that the fitness values for the two orthogonal partitionings [**A . .**] and [**. B .**] cannot be calculated independently from each other, indicating that [**AB .**] rather than [**A . .**] or [**B . .**] is a fitness unit in this case. Importantly, while in the case of contextsensitivity, the fitness w_{a1} of an allele $\mathbf{a_1}$ is dependent on the frequency of alleles *at other loci*, e. g., **B**, this leaves completely open, if variants of the coselected entity [**AB .**], such as $\mathbf{a_1b_1}$**.** have a fixed fitness value or if their fitness is dependent on the frequency of other variants ($\mathbf{a_1b_2}$**.**, $\mathbf{a_2b_1}$**.**, $\mathbf{a_2b_2}$**.**) *of the same partitioning*, which is the hallmark of frequency de-

rather than the single nucleotide should be considered a unit of selection (e. g., Dawkins 1982).

However, nondecomposable contextinsensitivity of fitness is not enough. It is only one out of two requirements for a multiplicator. As discussed above, the other requirement is that variants of a partitioning also have to act as unit of variant reproduction, i. e., reliably reproduce variants of the same type. When constituents are reshuffled at the generation boundary due to sexual reproduction this is not trivial, in particular not for comprehensive partitionings. A particular combination of alleles that epistatically determines a certain fitness value, is likely to be disrupted in the progeny unless the mating partner happens to have the same combination. Nonetheless, even with random mating, allele combinations with high fitness will raise the probability of their future recurrence because they specifically increase the frequency of all constituent alleles in the population. However, the more alleles this unit comprises (the more comprehensive the partitioning is), the lower will this probability and as a consequence the selection coefficients for this unit be. It can be greatly increased by nonrandom mating (e. g., preferential mating of similar variants) between variants or by *linkage* (see e. g., Ridley 1993) among the alleles (e. g., due to clustering of loci on a chromososme). Generally, linkage of any type of combination of constituents can be defined as the probability of being not disrupted during the process of reproduction (e. g., due to sexual recombination).

In summary, only those combinations of characterizing constituents will qualify as multiplicators (units of fitness and variant reproduction) and hence as units of selection, which are at the same time (1) units of variant reproduction and (2) units of a nondecomposable contextinsensitive fitness value. Condition (1) is most likely met by partitionings with linkage between characterizing constituents and nonrandom mating among variant types, whereas condition (2) is most likely met by partitionings where epistatic fitness effects exist exclusively among characterizing constituents. Typically, these two requirements for a unit of fitness and variant reproduction are in conflict with each other (Fig. 7 B), because linkage tends to decrease for more comprehensive partitionings while contextinsensitivity

---

pendence. For instance, in single locus heterozygote superiority, the fitness of a_1 (w_{a1}) and a_2 (w_{a2}) depend on the frequency of these alleles p_{a1} and p_{a2}. If there are only 2 alleles, these two parameters are interdependent, because $p_{a1} = 1 - p_{a2}$. Therefore, in this case (one degree of freedom), the fitness w_{a1} of an allele is dependent on a single parameter p_{a1}. Analogously, in case of frequencydependence for [**AB.**] there would be four different variants with 2 degrees of freedom; hence, the fitness of each variant (e. g., w_{a1b1}) would be dependent on 2 parameters (e. g., p_{a1} and p_{b1}).

In conclusion, sensitivity of locus **A** on a particular context **B** ($w_{a1} = f(p_{b1})$; fitness expressable as function of the frequency of variants of the context) and frequencydependence of **A** or **AB** ($w_{a1} = f(p_{a1})$ or $w_{a1b1} = f(p_{a1},p_{b1})$; fitness dependent on the frequency of other variants) are two different phenomena that should be kept apart.

tends to increase[20]. This conflict arises particularly in case of sexually reproducing organisms, where comprehensive partitionings (e. g., variants of large gene complexes or entire organisms) tend to satisfy (2) but not (1), focal partitionings (e. g., variants of single genes) tend to satisfy (1) but not (2). Therefore, single genes are indeed unlikely candidates for a unit of selection, but the same is true for entire sexually reproducing organisms, contrary to the credo of many hierarchy theorists (e. g., Lloyd 1988; Brandon 1990). The paradox posed by these conflicting demands, can only be resolved, when the ideal requirements for a multiplicator are relaxed so that units qualify for a multiplicator already, when (1) variant types reproduce with above average probability in the chain of generations (despite frequent unfaithful reproduction due to recombination at the generation boundary), and when (2) each variant type is characterized by a limited range of fitness values rather than a particular fitness value identical for all its tokens.

Selection is most effective for intermediate level units of selection

In order to evaluate the relative importance of different partitionings as units of selection, we need to compare how different partitionings fulfill these two criteria. In order to do this, it is useful to introduce a general measure for the degree to which selection changes the frequency of the variant types of a certain partitioning. I will call this measure the *effectivity of selection*. The effectivity of selection E for a partitioning (e. g., [**A . .**]) may be defined as some function of the effectivities of selection E_i for each of its variant types (e. g., $\mathbf{a_1}$ **. .**), and the latter may be defined as a function of their average selection coefficient s (that may itself be a function of q in case of frequency-dependent selection) as well as of their frequency q in the population, each of which is weighed by a reliability factor r_s and r_q, respectively (Fig. 10):

$$E_i = f(r_{si}\, s_i,\ r_{qi}\, q_i)^{21}$$

The factor r_s (reliability of fitness reproduction of a variant) is supposed to measure to what degree the selection coefficient of a variant type changes in successive generations due to contextsensitivity of fitness (Fig. 10 A). In contrast the factor r_q (reliability of variant reproduction) is supposed to

[20] At present, there is little evidence to suggest that loci with epistatic fitness interactions tend to be generally clustered in the genome, although such clustering of strongly epistatic genes may sometimes occur (see e. g., Hurst 1998; Doolittle 1999; Smith et al. 1999).

[21] For certain purposes it may be useful, to account for additional factors, such as differences in generation time (see e. g., Lewontin 1970) between different constituents in a measure of selection effectivity. This will be important mainly, when constituents belong to different reproducers and their reproduction is not tightly numerically coupled. However, differences in generation time have often relatively little impact on selection, because small differences in reproductive rates (selection coefficients) can overcome large differences in generation time (Wimsatt 1980).

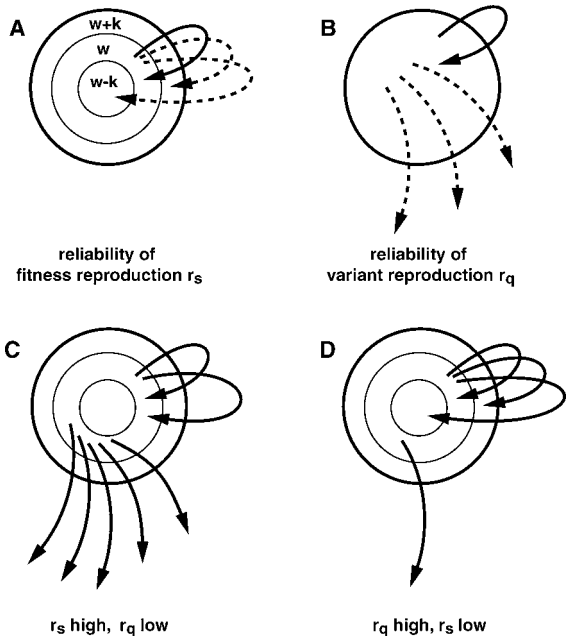


Fig. 10. Diagram illustrating reliability of variant reproduction r_q and reliability of fitness reproduction r_s for some variant of a partitioning (set bounded by thick solid line). A) r_s measures to what degree the selection coefficient of a variant type changes in successive generations due to contextsensitivity of fitness. Therefore, r_s is determined by the probabilities with which those variants of a partitioning that reproduce the same type of variant also reproduce the same relative fitness value w (or at least a fitness value falling within a restricted range of fitness values; subsets of the variant with different upper limits of fitness values w - k, w, w + k are indicated by thin lines). r_s depends on the proportion of reproduced variants that have the same fitness value (or a fitness value falling in the same range) as their ancestor (solid arrow) to reproduced variants with different fitness values (dashed arrows). B) r_q measures the probability with which a variant reproduces another variant of the same type. r_q depends on the proportion of reproduction of the same variant (solid arrow) to reproduction of other variants (dashed arrows) of a partitioning of a reproducing system. C) and D) illustrate patterns of reproduction for variants with high r_s and low r_q (C) and high r_q and low r_s (D), respectively. See text for further explanation.

measure the probability that the progeny of an individual that is member of a variant type will also be a member of that variant type; hence it measures the degree of variant reproduction (Fig. 10 B). A variant type may be termed more *relevant* for selection, the higher both its r_s as well as its r_q are. It would lead too far astray here, to develop this in more detail[22].

[22] Briefly, r_s may be defined, so that it ranges from 0 and 1. It may, for instance, be calculated as $1 - \Delta s$, where Δs measures the difference between selection coefficients in successive generations that can be expected due to the contextsensitivity of fitness for the unit rather than to the frequencydependence of its s (see footnote 19). Δs will be a function of three kinds of parameters: 1) the probabilities of recombination between the characterizing constituents and the remaining constituents, 2) the frequency of variants for each of the remaining constituents and 3) the degrees of epistasis between the characterizing constituents and the different variants of the remaining constituents. The factor r_q likewise may have values between 0 and 1

A partitioning will be a candidate for a unit of selection only, if it is a nondecomposable unit of fitness and variant reproduction with non-zero effectivity of selection for it. This requires that there are at least two relevant variant types, for which neither r_s nor r_q are zero. In the terminology of quantitative genetics, partitionings that have some relevant variants, which differ in fitness, are said to exhibit fitness heritability or additive variance for fitness[23]. Generally, additive variance for fitness for a partitioning is the variance of the average fitness of the progeny of its variant types; hence it is the reproducible fraction of the variance for fitness among its variant types. Given a certain average fitness difference between variant types of a partitioning, the additive variance for the partitioning will be the higher, (1) the less variance for fitness there is within its variant types (i. e., the larger r_s will tend to be) and (2) the higher the probability is that progeny of a variant type will belong to the same variant type (i. e., the larger r_q will tend to be). Therefore, the extent of nondecomposable additive variance for fitness of a partitioning P (i. e., additive variance that is not merely the sum or product of additive variance for fitness of all more focal partitionings of a certain level that are non-orthogonal to P), measures the degree to which the combination of characterizing constituents defining P is effective as a nondecomposable unit of selection. My reasoning here follows the analysis of Wimsatt (1980, 1981; see also Lloyd 1988), who also emphasized that units defining comprehensive ("higher lev-

---

and may be calculated as $1 - P_{\Delta V}$, where $P_{\Delta V}$ is the probability that the progeny of a member of that variant type will belong to a different variant type of the partitioning. $P_{\Delta V}$ will be a function of two kind of parameters: 1) the probabilities of recombination among the characterizing constituents, and 2) the frequency of variants for each of the characterizing constituents.

[23] Additive and epistatic fitness effects of constituents on the one hand and additive and epistatic *variance* for fitness on the other hand are sometimes confused (e. g., Sober and Wilson 1994), but need to be clearly distinguished. There are additive fitness effects of **A** and **B**, when the fitness of [**AB.**] can be calculated by the addition of fitness components for [**A..**] and for [**B..**]. When this is not the case, fitness effects of **A** and **B** are often called epistatic (e. g., Lewontin and Kojima 1960; Hedrick et al. 1978). A narrower and more biologically plausible concept of epistasis only terms those effects on fitness epistatic that are neither additive nor multiplicative (see e. g., Hedrick et al. 1978; Ridley 1993). Importantly, additive, multiplicative and epistatic effects on fitness can all contribute to the so-called additive variance for fitness (Whitlock et al. 1995; Cheverud and Routman 1995; Wagner et al. 1998). Additive variance for fitness is defined as the variance of the average effects of a certain allele or combination of alleles on the fitness of the progeny (assuming random mating for sexually reproducing species) in the population (e. g., Falconer 1960). It is a measure of the heritable variance component of fitness (including the heritable variance component of additive as well as of epistatic effects!), while epistatic or interaction variance meaures the non-heritable variance component that is due to epistatic fitness effects. Although the contribution of epistatic fitness effects to additive variance is often neglected in population genetic models in order to simplify mathematical treatment, it has been shown to be important in small populations (Bryant et al. 1986; Goodnight 1988; Cheverud et al. 1999).

It also needs to be noted that the fact that there is additive variance for the fitness of an element (gene or trait), does not allow one to conclude that this element is a constituent of a unit of selection; it may as well be just a nonfunctional byproduct that is coupled to such constituents (see also Sober 1984).

el units") as well as units defining focal partionings ("lower level units") can exhibit nondecomposable additive variance for fitness and, consequently, act as units of selection. However, Wimsatt (1981) as well as other authors promoting the variance approach tend to overlook that the probability of variant reproduction (r_q) is equally important for additive variance as contextinsensitivity of fitness (r_s) and that both demands are often conflicting[24]. In consequence, selection for more focal partitionings [**A..**] (e. g., selection for genes) may be more effective than selection for a more comprehensive partitioning [**ABC**] (e. g., selection for organisms), even when there is some contextsensitivity for the variants of [**A..**] but not for the variants of [**ABC**] ($r_{s[\mathbf{A..}]} < r_{s[\mathbf{ABC}]} = 1$), because the more reliable reproduction of variants of [**A..**] may more than overcompensate this (if $r_{q[\mathbf{A..}]} \gg r_{q[\mathbf{ABC}]}$).

In conclusion, an actual process of selection can be viewed as a parallel search for many different units (see e. g., Holland 1975). First, there will be a parallel search for units defining orthogonal partitionings (such as [**AB.**] and [**..C**]), Second, it is in principle possible that effective selection takes place for each of a variety of hierarchically nested partitionings (such as [**A..**], [**AB.**] and [**ABC**]) and that nondecomposable units of selection are to be found at different hierarchical levels (see also Wagner and Laubichler 2000), although the effectivity of selection for different partitionings may differ considerably. It is, therefore, inappropriate to consider it merely a matter of preference (Maynard Smith 1987; Sterelny and Kitcher 1988, Waters 1991), which unit is chosen to describe a selection process. Generally, it can be expected that in sexually reproducing organisms, the most effective selection will often take place for units at an intermediate level (e. g., small complexes of genes), rather than for high level units defining very comprehensive partitionings (e. g., large gene complexes or en-

---

[24] Wimsatt (1981), for instance, emphasizes the possibility for "segregation analogues" (i. e., variants with high r_q) at higher levels (comprehensive partitionings), and downplays the fact that for many biologically plausible conditions (in particular for low linkage among constituents with fitness epistasis) there will in fact be a conflict between the demands for reliability of variant reproduction (high r_q) and contextinsensitivity of fitness (high r_s). Similarly, it has been repeatedly suggested to use Price's covariance equation (Price 1972) to estimate the relative importance of units of selection at different levels (e. g., Wade 1985; Lloyd 1988; Michod 1997, 1999; Sober and Wilson 1998). However, the Price equation only describes selection in one generation (see e. g., Wade 1985) and cannot be used to adequately recognize levels of additive variance in recurrent selection processes without supplementing it with further assumptions about heritability. It can, therefore, be used to model selection processes in successive generations only, when the relative faithful reproduction of a unit (e. g., a particular allele combination) in successive generation can be taken for granted, and is not without further qualification applicable to cases, where this is not true, e. g., due to sexual recombination. Hence, models based on the Price equation tend to overemphasize contextinsensitivity of fitness (high r_s) over reliability of variant reproduction (high r_q) as condition for a unit of selection. The same problem limits the applicability of other approaches to compare the relative importance of different nonorthogonal units of selection (e. g., Lande and Arnold 1983; Heisler and Damuth 1987; Goodnight et al. 1992).

tire organisms) or low level units defining very focal partitionings (e. g., nucleotides, single genes), because variants of the former will tend to be easily disrupted by recombination (r_q will be close to zero), whereas variants of the latter will tend to exhibit highly fluctuating fitness values depending on the context (r_s will be close to zero).

Frequencydependence

To complicate matters, there may not only be frequency dependent selection (with frequency dependent selection coefficients s), but the reliability parameters r_s and r_q themselves will also depend on the frequency of certain constituents in the population. For variant types of more comprehensive partitionings, it is particularly r_q, which is frequency dependent, (the frequency of characterizing constituents determines the frequency of disruption of a variant by recombination) whereas r_s will be close to 1. In contrast, for variants of more focal partitionings it is particularly r_s, which is frequency dependent (the frequency of constituents of the context determines the reliability of fitness reproduction of a variant), whereas r_q will be close to 1. As long as a particular variant ($\mathbf{a_2}$) of a constituent (**A**) occurs at very low frequency in the population, variant types of a focal partitioning ($\mathbf{a_2..}$) will be much more relevant for selection than variant types of a more comprehensive partitioning ($\mathbf{a_2b_2.}$) including it as a characterizing constituent, because there is a very high probability that variants of the more comprehensive partitioning will be disrupted by recombination as long as they are rare, i. e., r_q for rare variants of more comprehensive partitionings will be low, but will increase with frequency. In contrast, for variants of more focal partitionings ($\mathbf{a_2..}$) r_s and hence the relevance of these variants for selection will be relatively high, if variants of constituents of the context (e. g., $\mathbf{b_1}$, $\mathbf{b_2}$) occur at either very high or very low frequency, but will be minimal when the frequencies of constituents of the context are more evenly distributed, because in the latter case the probability is maximal that the characterizing constituents ($\mathbf{a_2}$) find themselves in different contexts after reproduction.

This frequency dependence has two consequences. First, for comparing relevance or effectivity for selection between different units, it is necessary to compare them for a defined value of constituent frequencies, for instance for equal frequencies of all variants of constituents. Second, during a selection process, the frequencies of constituents will change and with them will the relevance and effectivity of selection for different variants. In general, it might be expected, given the different types of frequency dependence described above, that lower level units of selection (more focal partitionings) play a more important role for the invasion of new variants into a population as well as for the final steps to fixation, whereas higher level units of selection (more comprehensive partitionings) will be more effective during the phase of equal distribution of variants.

The fact that the level of highest relevance or effectivity of selection can change during a selection process, is important for the invasion of rare variant types into a population. Two cases need to be distinguished. First, the case where the fitness of variant types of comprehensive and focal partitionings are not conflicting: While there is fitness epistasis between the characterizing constituents of the comprehensive partitioning, it does not reverse the sign of fitness advantages (e. g., when the average fitness in the population for $\mathbf{a_1..}$ is higher than for $\mathbf{a_2..}$, the average fitness for $\mathbf{a_1b_1.}$ and $\mathbf{a_1b_2.}$ will not be lower than for $\mathbf{a_2b_1.}$ and $\mathbf{a_2b_2.}$, respectively). In this case, rare advantageous variants of comprehensive partitionings ($\mathbf{a_2b_2.}$) can relatively easily invade the population either by initial selection for fitness advantages of variants of a more focal partitioning ($\mathbf{a_2..}$) or – if there are no fitness differences at the level of the more focal partitioning – by neutral drift. Second, the case where the fitness of variant types of comprehensive and focal partitionings are conflicting: There will be fitness epistasis between the characterizing constituents of the comprehensive partitioning, so that for at least one variant type of the comprehensive partitioning the sign of fitness advantages will be reversed (e. g., when the average fitness in the population for $\mathbf{a_1..}$ is higher than for $\mathbf{a_2..}$, whereas the average fitness for $\mathbf{a_2b_2.}$ is higher than for $\mathbf{a_1b_2.}$)[25]. When there is conflict, invasion of rare advantageous

[25] Conflicts between units of selection can also occur when constituents are not tightly numerically coupled in reproduction and there is overlap of reproducers and multiplicators as discussed in section 3.1. Meiotic drive by segregation distorter alleles (such as the t-allele in mice or the *Sd*-locus in *Drosophila*), intragenomic conflicts involving selfish DNA, conflicts between unicellular or multicellular units of reproduction or conflicts between individual and group selection provide famous examples for this. These have been discussed extensively elsewhere (see e. g., Lewontin 1962, 1970; Buss 1987; Lloyd 1988; Maynard Smith and Szathmáry 1995; Sober and Wilson 1998; Maynard Smith 1998; Michod 1999), so I will here only briefly indicate how the notation that I introduced can be extended to accomodate these cases. Let us assume that there are several constituents **A**, **B** and **C** of a reproducer, and that only **B** and **C** are tightly numerically coupled in reproduction, whereas **A** is not. In this case, separate fitness values can be determined

(1) for each numerically uncoupled group of constituents such as variants of **A** in a particular context of **B** and **C** ([**A/BC**]), **A** in any context of **B** and **C** ([**A/..**]), **B** and **C** in a particular context of **A** ([**BC/A**]) etc., and

(2) for variants of reproducing units encompassing all constituents such as [**ABC**] or [**A..**]. Because **A** can reproduce independently, its number may vary relative to the number of **B** and **C**, so a more appropriate notation in this case may be [$\mathbf{(A)_nBC}$] or [$\mathbf{(A)_n..}$], respectively.

How can there be conflict between units of selection such as [**A/..**] and [**ABC**]? For illustration, assume a transposable genetic element ("selfish DNA"; Doolittle and Sapienza 1980; Orgel and Crick 1980) **A**, which may exist in different variants $\mathbf{a_1}$ and $\mathbf{a_2}$ and [**A/..**] may act as a unit of selection. For instance, variant $\mathbf{a_2}$ may reproduce at a higher rate than the genomes **BC** in which it resides, because it produces additional copies of itself within the same genome, whereas $\mathbf{a_1}$ does not (Fig. 9 C). Hence, the fitnesses for [**A/..**] and for [**../A**] differ for variant $\mathbf{a_2}$: The fitness for $\mathbf{a_2/..}$ (= fitness of $\mathbf{a_2}$ in the context of any variant of **BC**) will be higher than for $\mathbf{../a_2}$ (= fitness of any variant of **BC** in the presence of $\mathbf{a_2}$), while the fitness of $\mathbf{a_1/..}$ and $\mathbf{../a_1}$ will be identical. In addition, the organism including **A** itself may act as a unit of selection [**A..**] or [**ABC**] and reproduce with a definite rate. Conflict between the units [**A/..**] and [**A..**] will arise, if selection for one unit counteracts selection on the other. This

variants of comprehensive partitionings $\mathbf{a_2b_2.}$ is difficult, because there will be selection against them at low frequencies (e. g., Crow et al. 1991)[26].

3.3 Units of evolution and coevolution probabilities

In the last section I have considered an actual selection process among a particular given constellation of variants of a reproducer and have defined a unit of selection with respect to these actual variants. I now want to broaden the perspective and turn attention from a particular combination of variants to an entire set of various possible constellations of variants of a reproducer (e. g., the set of all possible constellations of all one mutant neighbors of the constituents). This will allow us to define a new kind of unit, that will be referred to as *unit of evolution*. It will be a unit of those constituents, which tend to coevolve by recurrently constraining each others evolutionary modification.

Units of reciprocal fitness dependence define units of evolution

In any selection process among a given constellation of variants, there may be two kinds of ambiguities, that preclude to generally determine, which constituents are necessary to codetermine a particular fitness value and which constituents make independent fitness contributions. First, it is impossible to assess the fitness contribution of constituents, that are not actually present in different variants. And second, it is impossible to decide

---

will be the case, if the fitness for $\mathbf{a_2..}$ is smaller than for $\mathbf{a_1..}$ – for instance because individuals are less fertile the more copies of the **A** locus are present – whereas the fitness of $\mathbf{a_2/..}$ is higher than for $\mathbf{a_1/..}$ Similar conflicts might arise, when altruistic behavior $\mathbf{a_1..}$ is favored over egoistic behavior $\mathbf{a_2..}$ by selection between reproducing trait groups (unit of selection [**A.. A.. A..**]: fitness of $\mathbf{a_2}$-dominated groups $\mathbf{a_2.. a_2.. a_1..}$ is smaller than for $\mathbf{a_1}$-dominated groups $\mathbf{a_1.. a_1.. a_2..}$), whereas $\mathbf{a_2..}$ is favored by selection of organisms within groups (unit of selection [**A../A..**]: fitness for $\mathbf{a_2../a_1..}$ is bigger than for $\mathbf{a_1../a_2..}$).

Combinations of constituents that are not tightly numerically coupled can act as a unit of selection only, when there is relatively effective selection for those units. In the case of trait groups this requires in particular that r_q ("group heritability") is high (see Wimsatt 1980; Maynard Smith 1987; Sober 1987; Brandon 1990; Sober and Wilson 1998). Such group heritability is favored by certain conditions of nonrandom mating among progeny, e. g., group formation due to kin recognition or assortative interactions between like trait-bearers (see e. g., Sober and Wilson 1998). It is still hotly debated, how often the conditions for effective group selection are met in nature and which of the mechanisms of group reproduction is most prevalent (e. g., Sober and Wilson 1998; Maynard Smith and Szathmáry 1995; Maynard Smith 1998).

[26] Wright's shifting balance theory (see e. g., Wright 1931, 1988; Crow et al. 1990; Ridley 1993; Coyne et al. 1997) suggests a process, by which rare advantageous variants can nonetheless get a foothold in a population in such cases. It is still controversial whether the rather special demands on a variety of parameters in Wright's model are frequently met in real-world selection processes (e. g., Ridley 1993; Coyne et al. 1997). In addition, even though we can reasonably extrapolate from current knowledge in physiology and genetics that pervasive fitness epistasis will be the rule rather than the exception, it is a completely open question, how frequently such epistasis will in fact lead to a conflict between units of selection at different levels (Ridley 1993; Whitlock et al. 1995; Coyne et al. 1997).

whether two constituents have nonepistatic effects on fitness, when not all possible combinations of their variants are instantiated (e. g., when $\mathbf{a_1b_1.}$ and $\mathbf{a_2b_2.}$ are instantiated, but not $\mathbf{a_1b_2.}$ and $\mathbf{a_2b_2.}$). I will consider these two ambiguities in turn.

First, in any selection process, selection can take place only among the elements which actually exist in several variants. Nonetheless, the fitness of each variant may be contextsensitive in that it depends strongly epistatically on some of the constituents that do not vary. For instance, the relative fitness of $\mathbf{a_1b_1.}$ vs. $\mathbf{a_2b_1.}$ may be very different from the relative fitness of $\mathbf{a_1b_2.}$ vs. $\mathbf{a_2b_2.}$ (e. g., when $\mathbf{a_1}$ and $\mathbf{a_2}$ are two ligands that bind better to receptors $\mathbf{b_1}$ and $\mathbf{b_2}$, respectively). Consequently, the relative fitness of $\mathbf{a_1..}$ vs. $\mathbf{a_2..}$ will be very different for a selection process $\mathbf{P_1}$ among the two variants $\mathbf{a_1b_1.}$ and $\mathbf{a_2b_1.}$ than for a selection process $\mathbf{P_2}$ among the two variants $\mathbf{a_1b_2.}$ and $\mathbf{a_1b_2.}$. However, constituents, which do not exist in different variants such as **B** in $\mathbf{P_1}$ and $\mathbf{P_2}$ are not considered part of a unit of selection in an actual selection process despite their importance for fitness determination; accordingly, [**A..**] but not [**AB.**] will be a unit of selection for $\mathbf{P_1}$ and $\mathbf{P_2}$[27].

Usually, such nonvarying constituents important for fitness determination are considered part of the selective environment, which exert "selection pressures" on the varying constituent. However, thereby, the role of co-reproduced constituents **B** for selection among variants of **A** is treated analogously to the role of contingent environmental factors **E** (such as the humidity of the air, the brightness of the sun etc.), ignoring that the former role is special in that variants of **B** themselves may depend on **A** for their reproduction in a series of iterated selection processes. I want to suggest instead that constituents, e. g, **A** and **B**, should be considered part of the same *unit of evolution*, when they form a nondecomposable unit of *reciprocal* fitness dependence (this excludes **E**), even though they may not act as an effective unit of selection in an actual selection process either due to the absence of variants of one constituent or due to the low reliability of variant reproduction (low r_q) because of high degrees of polymorphism, low degrees of linkage etc.

Second, in case that not all constellations of variants of two constituents are observed in a certain selection process, it is impossible to decide, whether only one or both of them belong to the same unit of selection, i. e., whether [**AB.**] or rather [**A..**] and [**B..**] act as unit of selection. Consider, for example, the following fitness table:

---

[27] For instance, when units of selection are defined by levels of additive variance (Wimsatt 1980, 1981; Lloyd 1988; see above), nonvarying constituents are by definition not part of it. This "obsession with the actual" (Sober 1984, p. 272) and its neglect of the causes of selection processes has been severely criticized by Sober (1984; see also Sober 1981; Sober and Lewontin 1982; Sober and Wilson 1994).

	$\mathbf{b_1}$	$\mathbf{b_2}$
$\mathbf{a_1}$	4	not instantiated
$\mathbf{a_2}$	not instantiated	1

Two different situations may underly this situation. First, **A** and **B** may be independently variable. In that case, there are two possibilities: Either the fitness values of **A** and **B** depend epistatically on each other or **A** and **B** make independent fitness contributions. In order to decide between these alternatives, the fitness values of $\mathbf{a_2b_1.}$ and $\mathbf{a_1b_2.}$ would need to be determined. Second, **A** and **B** may not be independently variable and combinations $\mathbf{a_2b_1.}$ and $\mathbf{a_1b_2.}$ cannot be realized. In that case, the fitness values of **A** and **B** are also not independent of each other, although not due to epistasis but rather due to the lack of independent variability.
Again, the newly introduced notion of a unit of evolution is useful to accomodate such cases. Constituents **A** and **B**, should be considered part of the same *unit of evolution*, when they form a nondecomposable unit of reciprocal fitness dependence, be it due to fitness epistasis or due to the lack of independent variability, even though they may not unambiguously be identified as an effective unit of selection in an actual selection process due to the absence of particular constellations of variants.
But how can we determine, which constituents have interdependent fitness values and hence should be considered part of the same unit of evolution, when they do not vary in actual selection processes or when not all constellations of varying constituents are realized? For instance, how can we decide, whether [**A..**] or [**AB.**] is a unit of evolution, when only **A** exists in several variants but not **B** or when particular variants of **A** are always associated with particular variants of **B**? There are two procedures for deciding empirically, if **B** is important for the determination of fitness differences between $\mathbf{a_1..}$ and $\mathbf{a_2..}$ in selection processes. First, comparison of different selection processes involving different variants of **B** or different constellations of variants of **A** and **B**. And second, introduction of new variants of **B** or new constellations of variants of **A** and **B** , e. g., by experimental manipulation (see e. g., Cheverud and Routman 1995; Wagner et al. 1998). When it can be shown by any of these two procedures that either particular variants of **A** and **B** cannot be generated independent from each other, or that variants of **B** strongly and epistatically affect the fitness of variants of **A**, **A** and **B** may be considered part of the same unit of evolution[28].

[28] Epistatic fitness interactions can be determined either by direct comparison of the fitness values of four variants ($\mathbf{a_1b_1./a_2b_1./a_1b_2./a_2b_2.}$) or by four pairwise comparisons of fitness values between the pairs ($\mathbf{a_1b_1./a_2b_1.}$), ($\mathbf{a_1b_2./a_2b_2.}$), ($\mathbf{a_1b_1./a_1b_2.}$) and ($\mathbf{a_2b_1./a_2b_2.}$). More comprehensive partitionings (e. g., [**ABC**]) may qualify as units of evolution, when there is at least pairwise (or twodimensional) epistasis between its characterizing constituents (e. g., reciprocal fitness interactions between **A** and **B**, **B** and **C**), but no epistasis between characteriz-

Coevolution probabilities and units of evolution

Straightforward as this may seem, the answer to the question whether **A** and **B** belong to the same or to different units of evolution may depend on which variants of **B** are tested as the following table of fitness values illustrates:

	$\mathbf{b_1}$	$\mathbf{b_2}$	$\mathbf{b_3}$
$\mathbf{a_1}$	5	1	10
$\mathbf{a_2}$	1	5	2

In this example, the fitness of $\mathbf{a_1}$ and $\mathbf{a_2}$ differs strongly depending on whether $\mathbf{b_1}$ or $\mathbf{b_2}$ (e. g., receptors with higher binding affinities to ligands $\mathbf{a_1}$ or $\mathbf{a_2}$, respectively) are present, but there are no changes in relative fitness when $\mathbf{b_1}$ is replaced by $\mathbf{b_3}$ (e. g., a receptor with same ligand selectivity as $\mathbf{b_1}$, but higher general binding affinity). As a consequence, the answer is relative to the frequency or probability, with which selection processes among certain constellations of variants (such as C1: ($\mathbf{a_1b_1./a_2b_1./a_1b_2./a_2b_2.}$), C2: ($\mathbf{a_1b_1./a_2b_1./a_1b_3./a_2b_3.}$), or C3: ($\mathbf{a_1b_2./a_2b_2./a_1b_3./a_2b_3.}$)), out of a given set of variants of constituents (e. g., the set with the members $\mathbf{a_1b_1.}$, $\mathbf{a_1b_2.}$, $\mathbf{a_2b_1.}$, $\mathbf{a_2b_2.}$, $\mathbf{a_1b_3.}$, $\mathbf{a_2b_3.}$) occur. While [**AB.**] will be a unit of evolution for some combinations (such as C1, C3), [**A..**] and [**B..**] will be units of evolution for others (such as C2). A similar argument applies to lack of independent variability: For instance, variants $\mathbf{a_1}$ and $\mathbf{a_2}$ may be independently variable of variants $\mathbf{b_1}$ and $\mathbf{b_2}$, whereas $\mathbf{a_4}$ and $\mathbf{b_4}$ may not be realizable independently. Again [**AB.**] may be a unit of evolution for some constellation of variants, [**A..**] and [**B..**] for others.

For each given set **V** of variants of constituents and with defined probability of occurrence for each variant, there will be a certain probability $P^e_{\mathbf{AB}}$ that constituents **A** and **B** will make interdependent fitness contributions (due to pairwise epistasis for fitness or lack of independent variability). For the set of variants listed in the table above, this probability $P^e_{\mathbf{AB}}$ is 2/3 assuming that C1, C2 and C3 are equally likely. Because small epistatic effects are likely to be found for almost every combination of variants, it will be useful for all practical purposes to calculate $P^e_{\mathbf{AB}}$ as the probability that constituents **A** and **B** will lack independent variability or exhibit epistasis for fitness with each other *exceeding a certain reference threshold*[29].

---

ing constituents and the remaining constituents. Higherdimensional epistasis (reciprocal fitness interactions between **A**, **B** and **C**) may occur, but is not necessary. Dimensionality of epistasis refers to the number of constituents which are involved in reciprocal epistatic fitness interactions. In case of pairwise epistasis between **AB** and **BC**, interactions between **A** and **B** can be selected independently from interactions between **B** and **C**, while this is not the case when epistasis between **A**, **B** and **C** is threedimensional.

[29] This requires introduction of some measure of the degree of epistasis in order to allow quantification (see Lewontin and Kojima 1960 for an example).

Furthermore, it should be possible to calculate for each partitioning (e. g., [**AB .**] or [**ABC**]) of a reproducing system the probability P^c that it will be a nondecomposable unit of reciprocal fitness dependence relative to a given set of variants. I will call $P^c_{[\mathbf{AB.}]}$ and $P^c_{[\mathbf{ABC}]}$ the *coevolution probabilities* of [**AB .**] and [**ABC**], respectively. Under the simplifying assumption that all fitness dependences can be resolved into pairwise fitness interactions between constituents, the coevolution probability of a partitioning (e. g., [**AB .**]) relative to a set of variants **V** might be defined as the probability that there will be (suprathreshold) pairwise interdependence of fitness of each of its characterizing constituents (**A**, **B**) with at least one other characterizing constituent (**B**, **A**), but that there will be no (suprathreshold) pairwise interdependence of fitness of any of its characterizing constituents with any other constituent (**C**) of the reproducing system. In our example above, $P^c_{[\mathbf{AB.}]}$ will be identical with $P^e_{\mathbf{AB}} = 2/3$, as long as there is no fitness dependence of **A** and **B** with any variants of another constituent **C** ($P^e_{\mathbf{AC}} = P^e_{\mathbf{BC}} = 0$; consequently $P^c_{[\mathbf{ABC}]} = 0$).

However, when coevolution probabilities are calculated from the probabilities of pairwise fitness dependence of *any variants* of a given set **V**, these probabilities do not adequately represent the transition probabilities for evolutionary processes, where selection takes place between an established type (e. g., $\mathbf{a_1b_1c_1}$) and a newly introduced variant (e. g., $\mathbf{a_1b_1c_2}$). To represent evolutionary processes it is therefore more useful to calculate the coevolution probabilities for different units of evolution from the probabilities of pairwise fitness dependence of all pairs of constituents, with the additional assumption *that all constellations of variants contain one reference type of variant* $\boldsymbol{a_1b_1c_1}$, relative to a set of variants $\mathbf{V(a_1b_1c_1)}$, which, for instance may be defined to include the reference type and all its one mutant neighbors (e. g., $\mathbf{a_1b_1c_2}$, $\mathbf{a_1b_2c_1}$) as well as all recombinants (e. g., $\mathbf{a_1b_2c_2}$) among them.

Analogously, it will be possible for every partitioning (e. g., [**AB .**]) to calculate not only a coevolution probability but also a *disruption probability* relative to a set of variants **V**. The latter can be defined as the probability that any units encompassing some but not all of its characterizing constituents (**A** or **B**) plus at least one remaining constituent (e. g., **C**) of the reproducing system exhibit (suprathreshold) pairwise interdependence of fitness exclusively among themselves.

In general, a partitioning, qualifies as a unit of evolution, when it is likely that all and only its characterizing constituents will make interdependent fitness contributions relative to a certain set of variants **V**, i. e., when its coevolution probability relative to **V** exceeds its disruption probability. Because only hereditary constituents are open to the repeated introduction of variants (see above), a unit of evolution should be typically definable exclusively in terms of the coevolution probabilities among its hereditary constituents.

Implications of coevolution probabilities: hierarchies and constraints

Coevolution probabilities $P^c_{[\mathbf{AB}.]}$ serve both as a measure of the importance of a partitioning as a potential unit of selection and as a measure of the reciprocal constraints that constituents of a reproducing system exert on each other, because they make several predictions. For example, $P^c_{[\mathbf{AB}.]}$ determines the probability that [**AB.**] will be an actual nondecomposable fitness unit (high r_s) in selection processes among different variants of **A** as well as **B**, that may be effective as a unit of selection when the reliability of variant reproduction r_q is high enough, i. e., whenever there is low degree of polymorphism or tight linkage among characterizing constituents[30]. On the other hand, for iterated sequences of selection processes, in each of which either **A** or **B** vary but not both simultaneously, $P^c_{[\mathbf{AB}.]}$ determines the probability that fitness differences between variants of each constituent in a more recent selection process depend on the outcome of selection on the other constituent in a previous selection process. Importantly, **A** and **B** can be assigned a coevolution probability even when they never vary together in actual selection processes and, consequently, relatively comprehensive partitionings (e. g., a gene complex **ABC**) can act as unit of evolution even in large populations, although selection in any actual selection process takes place only among those of its constituents (e. g., the gene **A**) which exist in several variants simultaneously (e. g., the alleles $\mathbf{a_1}$, $\mathbf{a_2}$).

Because all constituents of a reproducing system are reciprocally necessary for their reproduction, constituents will tend to have a certain coevolution probability with every other constituent of the reproducing system. Therefore, multiple units of evolution (**AB**, **ABC**, **BCD**, **ABCD**, etc.) can be typically recognized for any set of variants of a reproducing system (**ABCD**). However, this does not imply that all such units are equally important, because their coevolution probability may differ widely. As will be illustrated in more detail below (section 4; Fig. 12), strong epistatic fitness interactions or lack of independent variability are much more probable for variants of constituents that are directly and strongly connected resulting in high coevolution probabilities. Therefore, **AB** and **CD** may each have high coevolution probability, whereas the coevolution probability for **ABCD** may be very small (i. e., **AB** and **CD** may be a unit of evolution for many types of variants of **A**, **B**, **C** or **D**, whereas **ABCD** may be a unit of evolution for much fewer such types). These different coevolution probabilities should be reflected in different frequencies of *dissociated co-*

[30] Although it has been concluded from several studies (see e. g., Lewontin 1974) that the average degree of polymorphism in natural populations is relatively high, this conclusion is based mainly on polymorphisms for enzymes in a few species (mainly of *Drosophila*). The degree of polymorphism for regulatory (e. g., developmental) genes, may turn out to be much lower (see e. g., Purugganan 1998). Theoretically, the probability that a partitioning [**AB.**] will act as an actual unit of selection with a particular effectivity should be calculable from its coevolution probability, the average degree of polymorphism of its constituents and the linkage between constituents, but lack of sufficient empirical data will usually preclude this.

evolution of constituents (i. e., the frequencies with which certain constituents evolve in a coordinated fashion, but independent from other constituents) in a certain evolving lineage (Schlosser, 2001, in press a, b).
For example, the reactions of basal metabolism (see e. g., Stryer 1981) such as the citric acid cycle involving enzymes like aconitase and isocitrate dehydrogenase (**A** and **B**) followed by oxidative phosphorylation of NADH in the respiratory chain supply ATP molecules that are necessary for a plethora of other biochemical processes like the movement of myosin heads along actin filaments during muscle contraction (**C**). Therefore, the fitness of actin depends strongly on the fitness of variants of enzymes of the citrate cycle. However, under the assumption that the majority of variants of actin affect its interaction with myosin molecules (**D**) without interfering with the capacity of the latter to bind ATP, this dependence will be typically nonepistatic and not result in coevolution, because any of these variants will be equally affected by changes in ATP level. In contrast, there may be many variants of actin and myosin that will affect the interaction of these molecules and these are likely to have epistatic fitness effects, due to the key-lock type interaction of these molecules.
Concluding this section, I should emphasize that the concept of coevolution probabilities for different units of evolution, as I have introduced it here, represents a measure for a phenomenon that is often discussed under the heading of developmental or functional "constraints" (see e. g., Gould and Lewontin 1979; Alberch 1980; Gould 1982; Cheverud 1984; Maynard Smith et al. 1985; Roth and Wake 1989; Resnik 1995; Raff 1996; Schwenk 1994; Wagner and Schwenk 2000) on selection processes. The term "developmental constraints" is usually applied to cases, where variants of different constituents cannot be generated independent from each other, whereas "functional constraints" refers to those cases, where the fitness of a constituent of a reproducing system depends epistatically on other constituents. Coevolution probabilities indicate the degree to which different constituents of a reproducing system will tend to coevolve or, putting it differently, the degree to which they will "constrain" their possibilities to change, either by reciprocally restricting variability or by exerting the most dominant and persistent reciprocal selection pressures on each other. In addition, of course, the fitness of a constituent will often depend on the presence or absence of certain contingent and nonhereditary environmental conditions (such as a certain temperature etc.), which cannot coevolve by coselection (see e. g., Schlosser 1993; Arthur 1997). However, epistatic fitness interactions among the characterizing constituents of a unit of evolution may hold for a broad range of environmental conditions; in these cases reciprocal interactions among the constituents may form the most prevalent selection pressures on their evolution[31].

[31] The role of other constituents for epistatic fitness determination of a particular constituent has sometimes been called "internal selection" (e. g., Whyte 1965; Riedl 1975; Arthur 1997; Wagner and Schwenk 2000), but this term is misleading, because coevolution probabilities can

Coevolution probabilities also embody what Darwin (1859) referred to as "laws of variation": Because constituents with high coevolution probabilities will be the most important factors that reciprocally codetermine their fitness values, they will also be the most important coevolving factors determining which variants are viable at all (i. e., have a non-zero fitness) and therefore can form the raw-material for evolution, be it by selection or by drift (see Cheverud 1984, Wagner and Altenberg 1996 for similar arguments)[32].

Although coevolution probabilities represent reciprocal fitness dependences that constrain the direction of evolution of a reproducing system, it deserves to be pointed out that they are themselves subject to evolutionary change (see e. g., Wagner and Mezey 2000). For instance, when **ABC** has a much higher coevolution probability than **AB** for constellations of variants of the set $\mathbf{V(a_1b_1c_1)}$ including all one mutant neighbors and their recombinants of a specific variant $\mathbf{a_1b_1c_1}$, there will often be also a relatively high coevolution probability for **ABC** for combinations of variants of the set $\mathbf{V(x)}$, where **x** is a member of $\mathbf{V(a_1b_1c_1)}$. Nonetheless, this may not be true for all **x**. For some particular set, e. g., $\mathbf{V(a_1b_1c_6)}$, the probability for pairwise fitness dependence of **B** and **C** may be much lower, thereby increasing the coevolution probability of **AB** over that of **ABC**. Such shifts in coevolution probabilities accompany evolutionary changes in the degree of modularity as will be discusssed below.

4. Modules as units of evolution

After having introduced the concepts of modularity and unit of evolution, I will argue in the following paragraphs that in reproducing systems with a modular organization, modules are in fact promising candidates for units of evolution, because the (hereditary) constituents of a single module will often form a unit of high coevolution probability, when certain additional conditions are met. As a consequence, constituents of a single module will

---

be determined for all hereditary constituents of a reproducing system that define fitness, "internal" autoconstituents (e. g., other genes of the same organism) as well as "external" alloconstituents (e. g., genes of other coevolving organisms; see above).

[32] Although some "structuralists" or "internalists" regard the role of "internal factors" as antithetical to the role of selection in evolution (e. g., Webster and Goodwin 1996), structuralist and selectionist perspectives can be reconciled (e. g., Wake and Larson 1987). In order for selection to occur, all that matters is that there are reproducible fitness differences between reproducible variants. It is irrelevant if these fitness differences are due to differences in interactions with a certain kind of "external" and contingent environmental factor or are rather predominantly due to differences in "internal" interactions with other hereditary constituents (reflected in high coevolution probabilities). Although it may be true that the fitness of variants will more frequently and strongly differ due to the latter type of effects, because of the tight and multiple interconnections among hereditary constituents, variants nonetheless might spread in populations via selection.

tend to be part of a single unit of selection in actual selection processes when the degree of polymorphism is not too high. Modules are more likely to delimit actually important units of selection than either entire organisms or single genes because they are less easily disrupted by recombination (r_q will be higher) than organisms, while having less contextsensitive fitness values (higher r_s) than genes. Hence, I suggest in agreement with Wagner's "building block hypothesis" (Wagner 1995; Wagner and Altenberg 1996)[33] that under certain conditions (see below) modules will not only be building blocks of development and/or function of a reproducing system, they will also tend to be units of its change during evolution.

4.1 Modularity and coevolution probabilities

Coevolution probabilities are likely to reflect patterns of couplings among constituents of a reproducing system

Modules are good candidates for units of evolution, because there is likely to be a close tie between the pattern of couplings of the constituents of a reproducing system and their independent variability on the one hand, their epistatic effects on each others fitness values on the other hand (Fig. 11): Any perturbation of constituents is likely to affect mainly those other constituents with which they are strongly connected. Moreover, some perturbations of a constituent with pleiotropic roles may have pleiotropic effects on many of the other constituents to which it is connected (for instance, if the latter are all dependent on the same output variable). It is important to note, however, that not all perturbations of a constituent with pleiotropic roles need to have pleiotropic effects, because different connections may be independently perturbable from each other.

However, heritable variations comprise only a special class of perturbations. Therefore the question arises, whether the probabilities for integrated and contextinsensitive behavior of modules relative to a class of developmental perturbations will be reflected in the coevolution probabilities among their hereditary constituents relative to a class of heritable variations. In order to answer this question, it first needs to be analyzed, how heritable variations will affect the fitness of constituents.

In general, the variation of a hereditary constituent should most likely affect only the fitness of such other constituents, with which it is functionally and/or developmentally connected. All constituents which are (directly or indirectly) functionally coupled in the reproduction of any constituent **A** collectively determine its fitness value; they also contribute to the determination of the fitness values of constituents developmentally coupled to each of them. It is important to point out that fitness depends on all links in an uninterrupted reproductive cycle (see Fig. 11), therefore

[33] This hypothesis was initially born in computer science to understand the evolution of genetic algorithms (Holland 1975; Goldberg 1989; Forrest and Mitchell 1993).

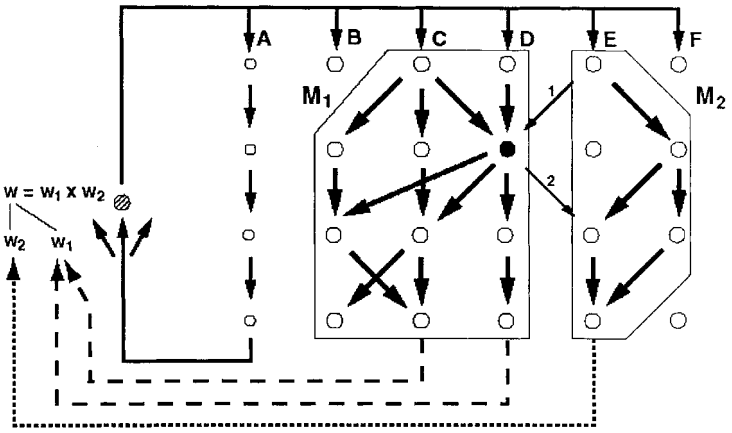


Fig. 11. Fitness interactions of constituents of a reproductive process reflect their pattern of connectivity. For general notation see Fig. 2. Arrows between circles indicate patterns and strengths of dependences between constituents. The diagram can be interpreted, for example, as a highly schematic and simplified representation of an organism, that develops from a zygote (hatched) and establishes different cell lines (columns of circles), including a germ cell line (column of small circles) and several somatic cell lines (columns of large circles), which express different genes and interact in a modular pattern (in 2 modules $\mathbf{M_1}$ and $\mathbf{M_2}$). The zygote arises from the germ line, but its fitness w, i. e, the expected number of zygotes produced depends on the interactions of somatic cell lines with each other and the environment. Several constituents co-operatively affect a component w_1 of the probability of reproduction of the zygote (dashed lines). Other constituents have separate and largely independent effects w_2 on the probability of zygote reproduction (dotted lines). Fitness cannot only be determined for the zygote, but for all constituents. The fitness of a certain constituent **D** (black circle) depends on all constituents, which are functionally coupled in its reproduction. This includes constituents "upstream" of **D** and directly functionally coupled in its realization (**C**, **D**) as well as those constituents "downstream" of **D** and developmentally coupled by it (**B**, **C**, **D**), which are involved in the same reproductive cycle. Under the assumption that the interactions (1 and 2) between modules are negligible, constituents of module $\mathbf{M_1}$ such as **D** would have fitness w_1, while constituents of $\mathbf{M_2}$ would have fitness w_2 in the example depicted. If, however, interaction 1 or 2 was important (i. e, in case $\mathbf{M_1}$ was not insensitive to $\mathbf{M_2}$ or not dispensable for $\mathbf{M_2}$, respectively) the fitness of **D** or other constituents of $\mathbf{M_1}$ would depend on the fitness of constituents of $\mathbf{M_2}$, i. e., there would be no independent fitness contributions of both modules. See text for further explanation.

any variations of a constituent **A** not only affect **A**'s fitness and the fitness of constituents dependent on its output variables but also the fitness of constituents, which affect the *input* variables of **A**. For the same reason, the fitness of **A** may be affected by changes of constituents directly affecting its input variables as well as by changes of constituents affected by its output variables (because the latter may in fact be functionally coupled in the reproduction of **A**; Fig. 11).

The strength of the effect of perturbation on fitness will tend to be correlated with the strength and number of the connections perturbed by the alteration of a constituent variable. Moreover, the fitness effects of variants of two connected constituents **A** and **B** (see e. g., Fig. 2) may be epistatic or non-epistatic. They are most likely to be epistatic for those variants of

each constituent which affect the same connection between **A** and **B** (e. g., x_2(**A**) in Fig. 2) or reciprocal connections between **A** and **B**, whereas they may well be non-epistatic, when the variants of at least one of the constituents affects another of its connections (such as y_3(**A**) in Fig. 2). If **B**, for instance, represents a ligand and **A** its receptor, epistatic effects will be observed between those variants of **A** and **B**, which both affect ligand binding: two variants of the ligand $\mathbf{b_1}$ and $\mathbf{b_2}$ may bind with different affinity to two variants of the receptor in the extracellular ligand binding domain $\mathbf{a_1}$ and $\mathbf{a_2}$, resulting in fitness epistasis between these four variants. In contrast, there may be independent fitness effects of **A** and **B** in case that variants $\mathbf{b_1}$ and $\mathbf{b_2}$, which affect ligand binding, encounter variants $\mathbf{a_1}$ and $\mathbf{a_3}$, which differ in the affinity of *intra*cellular binding sites of the receptor to second messengers (Fig. 12).

Modules act as units of evolution under certain conditions

Because constituents participating in modules are strongly, multiply and possibly reciprocally connected predominantly with other constituents of the same module, variants of their constituents will more likely exhibit strong epistatic fitness effects with variants of other constituents of the same module than with variants of most constituents not belonging to the module. However, the fact that several constituents behave as an integrated and contextinsensitive module, alone does not guarantee that they also will act as a unit of elements that reciprocally constrain each others evolution. Several additional conditions have to be met, because heritable variations are a special class of perturbation and the fitness effects of heritable variations of a constituent on fitness are determined by an entire reproductive cycle and hence spread to input constituents as well as to output constituents. First, the constituents of a module should not only act in an integrated and contextinsensitive manner, they should also be relatively *dispensable* for the context. Second, the effects of heritable variations of constituents should be more or less similar or *congruent* with the effects of those kinds of perturbations of a process, relative to which its modularity is defined, such as environmental or stochastic perturbations of organismal development. And third, modules must exhibit a certain *perseverance* even after iterated heritable variation. These three requirements shall now briefly be discussed in turn.

First, in order to act as a unit of evolution with high coevolution probability, a module must not only act in a contextinsensitive manner but must also be largely dispensable for the context itself (e. g., when the latter is also organized in a modular fashion). Only then will a module not only act as an integrated and contextinsensitive unit relative to some arbitrary input-output transformation, but relative to its own self-reproduction, thus forming a unit of contextinsensitive fitness contribution (Fig. 11). The contextinsensitive behavior of a module relative to some arbitrary input-output transformation alone does not rule out that the constituents **B**, **C**

and **D** of a module have strong and multiple effects on constituents of the context such as **E** that may affect the fitness of **B**, **C** and **D** by affecting the fitness of the reproducer in which they reside. In that case a higher order unit comprising the module plus these constituents of its context (**BCDE**) may be more likely to act as a unit of evolution (having a higher coevolution probability) than the module (**BCD**) itself. Under the simplifying assumptions that all connections of constituents can vary independently and that fitness epistasis will be most likely for those variants of two constituents that affect their interactions with each other (see above), this coevolution probability of constituents of a module with constituents of their context should be the smaller the smaller the proportion of functionally relevant interactions of these constituents with each other is among the total number of interactions of each constituent. Because modules are typically neither absolutely contextinsensitive, nor absolutely dispensable for their context, but rather are spatiotemporally embedded into more comprehensive modules in a hierarchical fashion (see section 2.3), this should be reflected in a hierarchy of coevolution probabilities.

Second, in order to act as a unit of evolution with high coevolution probability, modules should also act as integrated and contextinsensitive units in the face of heritable variation. In other words: modules will act as unit of evolution only, if the effects of heritable variations of constituents relatively faithfully reflects the effects of those kinds of perturbations relative to which modularity was defined. Such congruence of heritable variability and general (e. g., developmental) perturbability (akin to the "plastogenetic congruence" of Ancel and Fontana 2000) does not necessarily exist, because heritable variation may have pleiotropic effects on multiple connections of constituents even when these are independently perturbable by other kinds of perturbation.

Non-congruent pleiotropic effects of heritable variation are particularly likely to occur in hereditary constituents with pleiotropic roles (Fig. 12). As discussed below (4.2) this may be important in tying together different domains of a module to a single unit of evolution. Pleiotropic roles of constituents in different modules (overlap of modules; see Fig. 6), however, tend to diminish the importance of modules as units of evolution, because the fitness of variants in pleiotropic constituents (e. g., **B**, **C** and **D**) will depend on all of the modules (e. g., **ABCD** and **XBCD**) in which they (or their descendants) participate (see e. g., Duboule and Wilkins 1998; Niehrs and Pollet 1999). Consequently, some variants with pleiotropic effects will be subject to different epistatic fitness interactions (e. g., between **B** and **A**, and **B** and **X**, respectively) in each module. With increasing probability for non-congruent pleiotropic effects of heritable variation there will be an increasing coevolution probability for a unit (**AXBCD**), that is not a module itself and is more inclusive than each of the overlapping modules (**ABCD** and **XBCD**). Given that the diffusible protein sonic hedgehog has pleio-

tropic roles in vertebrate development – its floor plate expression is instrumental for motor neuron induction in the spinal cord, whereas its expression in the limbs is important for determining their anteroposterior polarity (Hammerschmidt et al. 1997) – the fitness of mutations in the coding region of this gene, for example affecting its diffusion properties, will probably depend epistatically on the genes it helps to regulate in the spinal cord as well as in the limb.

Importantly, however, not all heritable variations of a pleiotropic constituent are likely to have pleiotropic effects: Mutations in the regulatory regions of sonic hedgehog may specifically affect its mode of interactions in one but not the other expression domain. Therefore, the modular nature of gene regulation itself contributes to the independent variability of developmental interactions, thereby promoting the congruence of heritable variability and developmental perturbability in organisms.

Third, in order to act as a unit of evolution not only transitorily but for longer periods of evolution, the integrated and contextinsensitive nature of modules as well as their dispensability for their context and the congruence of heritable variation and other kinds of perturbation needs to persist even after iterated heritable variation (see e. g., Wagner and Mezey 2000). Only then can we expect the perseverance of coevolution probabilities in the chain of generations, that is required for a evolutionarily robust unit of reciprocal constraints.

Modules that fulfill these three conditions – they may be referred to as *reproductive modules* for the sake of brevity – will exhibit a modular and evolutionarily robust "genotype-phenotype map" (Riedl 1975; Cheverud 1984, 1996; Wagner 1996; Wagner and Altenberg 1996; Mezey et al. 2000) and will be units of high coevolution probability that will often act as units of evolution: The fitness of a newly introduced variant of a constituent will mainly depend on the other constituents of the same module. At the same time, such modules limit the spread of variation (see also Conrad 1990; Gerhart and Kirschner 1997). The few, weak and ususally nonreciprocal connections between such modules imply a relatively low (although typically non-zero) coevolution probability of constituents across module boundaries. In other words, the boundaries of these modules will be boundaries of units of relatively high coevolution probability. As a consequence, the modules will tend to have high reliability of fitness reproduction (r_s) for most constellations of variants of their constituents and will tend to act as actual and effective units of selection, whenever the degree of polymorphism is low enough to allow for sufficiently reliable variant reproduction (r_q).

Modules and units of evolution in coevolving reproducing sytems

Modules are often parts of a single organism: Developing organ primordia, regulatory networks of genes maintaining a certain state of cell differentia-

tion (muscle, neuron etc.) or signaling networks as in hedgehog signaling and Delta-Notch mediated lateral inhibition are paradigmatic examples (see e. g., Weintraub 1993; Gerhart and Kirschner 1997; Hammerschmidt et al. 1997; Artavanis-Tsakonas et al. 1999; Guillemot 1999; Gilbert 2000). Each of these paradigmatic cases of modules may act as unit of evolution under the conditions specified in the previous section. However, this view of modules is too narrow. As discussed above (see sections 3.1 and 3.2, footnote 25), reproducers can be overlapping and constituents, which are involved in the reproduction of both reproducers, may be tightly integrated into modules although not numerically coupled in reproduction. Examples for modules in such coevolving systems are not difficult to find. "Trait groups" (Wilson 1983; Sober and Wilson 1998), for instance, which figure prominently in recent models of group selection, are modules of certain interindividual interactions. They are modules because social behavior is not strongly connected to several other traits of the same individual (such a s skull or heart development), while being strongly connected to the social behavior of other individuals. Coevolution probabilities in this case depend on the interaction of constituents for social behavior not only in individual neural circuits but also across the population. Effective selection for trait groups is, however, only possible if particular trait group variants (e. g., altruist dominated groups) can be reestablished with sufficient reliability r_q (see section 3.2, footnote 25), for instance due to kin recognition or other assortative interactions (Sober and Wilson 1998). But modules of interindividual interactions need not be confined to the same species (e. g., Dawkins 1982; Futuyma 1986; Goodnight 1990 a, b; Ridley 1993). The mouthparts of pollinating insects and the architecture of flowers ensure lock and key type of interactions and illustrate beautifully that there can be interspecific modules with high coevolution probability. A plethora of other examples can be found in many other mutualistic interactions as well as in the "arms races" of coevolving species of predators and prey or hosts and parasites.

4.2 Modularity and evolvability

As units of high coevolution probability that confine the spread of variation, reproductive modules contribute in two important ways to the "evolvability" of reproducing systems (e. g., Wagner and Altenberg 1996; Dawkins 1996; Gerhart and Kirschner 1997; Kirschner and Gerhart 1998; Brandon 1999; Schank and Wimsatt, 2001). The stability of intramodular connections and the flexibility of intermodular connections (termed "robustness and flexibility" by Gerhart and Kirschner 1997; Kirschner and Gerhart 1998) on the one hand allows the *mosaic evolution* (e. g., Futuyma 1986) of different traits, because selection for variations in one module can proceed relatively independently from selection for variations in another

module. On the other hand it allows the generation of *combinatorial complexity* by the redeployment of modules in new contexts[34].

Mosaic evolution: Quasi-independence and continuity of evolutionary change

Modularity guarantees "quasi-independence" and "continuity" of selection on different traits, which Lewontin (1978) identified as the two conditions of their separate adaptability (see also Brandon 1999; Schank and Wimsatt, 2001)[35]. The quasi-independence of modules is apparent from their being units of high coevolution probability. The latter also guarantees continuity: Because a module will tend to be a unit of relatively contextindependent fitness changes, the total changes in fitness of a complex organism will be composed of many relatively independent fitness changes of each of its modules (see Schank and Wimsatt, 2001). Consequently there tends to be "strong causality" regarding variants of constituents **A** and **C** belonging to different modules or, expressed differently, **A** and **C** will tend to coevolve on smooth fitness landscapes (Wagner and Altenberg 1996), i. e., small changes in **A** will typically have small and predictable (typically nonepistatic) fitness effects on **C** ([**A . .**] and [**. . C**] will be independent units of selection for many constellations of their variants). In contrast, strong causality will not hold for variants of constituents **A** and **B** of the same module: **A** and **B** will tend to be either canalized (see e. g., Waddington 1957; Hall 1992; Wagner et al. 1997; Gibson and Wagner 2000; Rutherford 2000) due to strong regulatory capacities of the module. In that case small changes in **A** will be buffered and have negligible effects on **B**. Or **A** and **B** will coevolve on rugged fitness landscapes, so that small changes in **A** may have large and unpredictable (epistatic) fitness effects on **B** (for many constellations of variants, [**AB .**] rather than [**A . .**] and [**. B .**] will act as units of selection, assuming that r_q is sufficiently high).

Under the assumption that large effects on fitness are typically detrimental, the coevolution probability of a unit of evolution also predicts patterns

---

[34] The same two properties of modules, which favor evolvability – robustness and flexibility – also allow the diversification and increase in complexity of processes in development thereby promoting what might be called "developability" by being a precondition for the evolution of development in multicellular organisms (see Gerhart and Kirschner 1997 for related arguments).

[35] It is important to point out that being merely a unit of function does not suffice for separate adaptability. Functional units will only act as units of evolution (and hence be adaptable independently), if they are indeed modules, i. e., in case they are not only functionally but also developmentally relatively independent from each other. Strong developmental coupling between functional units, however, strongly constrains their separate adaptability. The basic organization (e. g., number and arrangements of bones) of fore- and hindlimbs (in particular their proximal parts), for instance, tends to be more or less identical in each tetrapod species due to shared developmental genetic networks (e. g., Shubin et al. 1997), despite the fact that there can be strong functional divergence in the elaboration of this basic pattern (e. g., lengths and shapes of bones) in fore- and hindlimbs).

of robustness and flexibility during evolution. The higher the coevolution probability of a unit, the more will it tend to be conserved. Reduced coevolution probabilities across module boundaries therefore imply that the connections between modules will be the preferred sites of evolutionary flexibility. Changes of "extramodular connections" of the constituents of a module (i. e., connections to constituents which are not part of the module) are less likely to have large epistatic and therefore potentially detrimental effects on fitness than changes of the "intramodular connections" between different constituents of the same module. Therefore, it should be expected that modules will tend to be relatively conserved, while their interrelations may be not.

Combinatorial complexity: Redeployment and multiple instantiation of modules

Modularity also allows the generation of combinatorial complexity during evolution because it facilitates the redeployment of modules in new contexts. While typically increasing pleiotropy during evolution inadvertently will lead to additional constraints on the pleiotropic constituents (see above; Waxman and Peck 1998; Duboule and Wilkins 1998), this will not be necessarily true, when entire modules acquire new roles and become instantiated repeatedly in different domains. In those cases, where there will be pleiotropy of a number of constituents **B**, **C**, and **D** that interact with each other as a module **BCD** themselves (Fig. 12), **BCD** may continue to act as a unit of evolution with high coevolution probability despite its embedding into several different contexts. The lateral inhibition mechanism involving the cells surface proteins Notch and Delta and the intracellular signaling molecule Suppressor of hairless is a case in point (Simpson 1997; Kimble and Simpson 1997; Beatus and Lendahl 1998; Artavanis-Tsakonas et al. 1999). While these molecules operate in a multitude of different contexts (e. g., in the neural plate, sensory epithelia of the ear, somites of vertebrates), interactions between these and several other constituents have been preserved in each domain. In each context they ensure that adjacent cells adopt different fates, while the particular cell fates involved vary from context to context.

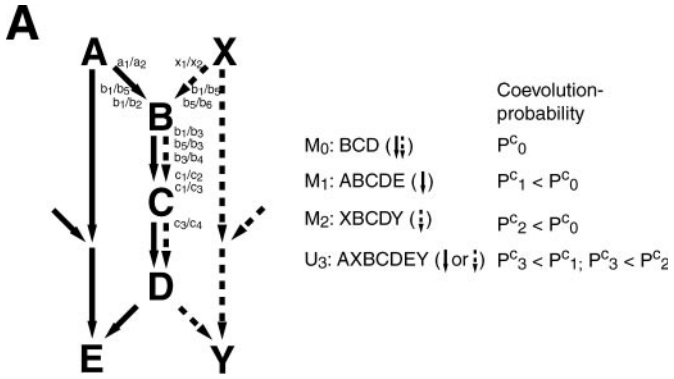
Constituents of a module that are pleiotropically employed in different domains of that module, as in the case of the Notch-pathway, may have a high coevolution probability and act as a largely conserved unit of evolution for most variants of **B**, **C** and **D** because as constituents of a module, **B**, **C** and **D** will have the majority of strong connections between themselves. It is, therefore more likely that a new variant will affect one of those many "intramodular connections" rather than one of the few "extramodular connections" and its fitness is more likely to be epistatically dependent on other constituents of the module rather than on the constituents of the context, in case it is only relatively sparsely and weakly con-

nected with the latter. In other words, a module will tend to act as unit of evolution even if it is instantiated in multiple domains. However, because **BCD** is only *relatively* independent from the context, there will inevitably be some variants of its constituents (e. g., variant $\mathbf{b_2}$ vs. $\mathbf{b_1}$ of **B**), that will differ in their extramodular connections, such as the connection between **B** and **A** (Fig. 12). For such variants, epistatic fitness interactions between **B** and the context, in which it is embedded, are to be expected. As a consequence, there will be a certain coevolution probability for the unit **ABCD** (as there will be for the unit **XBCD**), but it will be smaller than the coevolution probability for **BCD**. Therefore, a hierarchy of coevolution probabilities will tend to reflect the hierarchy of modules embedded into more inclusive modules, although there may, of course, still be some variants that have epistatic fitness effects in more than one context resulting in small coevolution probabilities for units such as **ABC** or **AXBCD**, which are not modules (Fig. 12).

Thus, contrary to the suggestion that the degree of pleiotropy between constituents will generally tend to decrease with increasing modularity (Cheverud 1984; Wagner 1995; Wagner and Altenberg 1996), modularity can, in fact, be expected to *favor* the evolution of this particular kind of pleiotropy, viz. cases, where entire modules are repeatedly employed in different contexts thereby allowing a rapid increase of complexity of reproducing systems in evolution. This expectation rests first on the reduced coevolution probability and hence increased evolutionary flexibility across module boundaries, which facilitates the dissociation of modules from a certain context and their integration into a new one, and second on the assumption that more reliable or effective modules are more likely to be retained in case of redundancy. Before I elaborate the latter point below, let me discuss the role of flexibility.

Redeploying and recombining modules

The redeployment of a (reproductive) module and its embedding into a new context during evolution may require only few changes of extramodular connections, which are unlikely to have large epistatic effects on fitness. An entire network of interactions, for instance, may be reactivated in a new context, even when only one or a few of its elements becomes responsive to a new inducer (e. g., Niehrs and Pollet 1999). Importantly, this reactivation of a module – which I will refer to as "domain duplication" of the module – does, therefore, not necessarily involve the duplication of its hereditary constituents such as genes. However, the latter is additionally deconstraining because it reduces the probability that variants of a constituent may have epistatic fitness effects affecting more than one context, in particular, when gene duplication is followed by the complementary loss of regulatory regions from the two duplicates (Force et al. 1999).



Redeployment (domain duplication) and multiple use in many different contexts seems to be the rule rather than an exception for many important networks among regulatory genes (e. g., Zuckerkandl 1994; Raff 1996; Gerhart and Kirschner 1997; Duboule and Wilkins 1998; Gilbert 2000). Numerous examples could be cited, but let me just point here briefly to some previously mentioned examples, e. g., hedgehog- and Notch-networks as well as *Hox* genes (see also Hammerschmidt et al. 1997; Keys et al. 1999; Simpson 1997; Kimble and Simpson 1997; Beatus and Lendahl 1998; Artavanis-Tsakonas et al. 1999; Warren et al. 1994; Maconochie et al. 1996; Gellon and McGinnis 1998).

Domain duplication of a module may be due to the fact that the regulatory regions of some of the pleiotropically expressed genes became responsive to new transcription factors, by acquiring new response elements or enhancers during evolution; these now ensure their activation in different contexts, while the regulatory regions mediating reciprocal intramodular interactions were conserved[36]. This is not an unlikely scenario. It is becoming increasingly clear that gene regulation is itself modular. This is reflected in the composite nature of regulatory regions of genes, where different clusters of response elements (enhancers) interact with different combinations of transcription factors thus directing the expression of genes in different contexts (e. g., Štanojevic et al. 1989, 1991; Kirchhamer et al. 1996; Arnone and Davidson 1997; Yuh et al. 1998; Dover 2000). Also, the exons of the coding regions may be translated into different pro-

[36] Because different clusters of response elements may direct the pleiotropic expression of genes in different contexts, one could argue that while there may be pleiotropic roles of the proteins generated by the coding region $\mathbf{B_C}$ of gene **B** in the two contexts **ABCD** and **XBCD**, there is no pleiotropy for transcription factor binding to response elements $\mathbf{B_A}$ and $\mathbf{B_X}$, which direct expression in these respective contexts. As a consequence, the combination of constituents $\mathbf{AXB_CCD}$ may act as a unit of evolution with a certain coevolution probability, although the coevolution probability for $\mathbf{AXB_ACD}$ and $\mathbf{AXB_XCD}$ is zero, while the coevolution probability for $\mathbf{AB_ACD}$ and $\mathbf{XB_XCD}$ is correspondingly higher than for $\mathbf{AB_CCD}$ and $\mathbf{XB_CCD}$, respectively.

B

Pairwise fitness epistasis between different combination of variants of A, X, B and C								
A		**X**		**B**		**C**		**epistasis**
a_1	a_2			b_1	b_2			+
a_1	a_2			b_1	b_5			+
a_1	a_2			b_1	b_3			-
a_1	a_2			b_5	b_3			-
a_1	a_2			b_3	b_4			-
a_1	a_2			b_5	b_6			-
		x_1	x_2	b_5	b_6			+
		x_1	x_2	b_1	b_5			+
		x_1	x_2	b_1	b_3			-
		x_1	x_2	b_5	b_3			-
		x_1	x_2	b_3	b_4			-
		x_1	x_2	b_1	b_2			-
a_1	a_2					c_1	c_2	-
a_1	a_2					c_1	c_3	-
a_1	a_2					c_3	c_4	-
				b_1	b_3	c_1	c_2	+
				b_5	b_3	c_1	c_2	+
				b_3	b_4	c_1	c_2	+
				b_1	b_3	c_1	c_3	+
				b_5	b_3	c_1	c_3	+
				b_3	b_4	c_1	c_3	+
				b_1	b_3	c_3	c_4	-
				b_5	b_3	c_3	c_4	-
				b_3	b_4	c_3	c_4	-

Fig. 12. Modules may be instantiated in multiple domains. A) The depicted pattern of connections among constituents may, for example, be interpreted as follows: **B** may be a cell surface receptor, that is either activated by a ligand **A** or by a ligand **X** and in turn activates an intracellular protein **C**; **C** then turns on expression of a gene **D**, which activates either downstream gene **E** or **Y**, depending on which other transcription factors it is coupled with. A module $\mathbf{M_0}$ consisiting of constituents **BCD**, may be repeatedly instantiated (exist in several domains) during a certain process such as the life cycle of an organism. For example, the activation of **B** by **A** (solid arrows) in one context and by **X** in another context (dashed arrows), will be automatically followed by the activation of **C** and **D** in each of the two contexts. Consequently, module $\mathbf{M_0}$ is instantiated twice, being part of a higher order module $\mathbf{M_1}$ (**ABCDE**) in one context, while being part of another higher order module $\mathbf{M_2}$ (**XBCDY**) in another context. Therefore, $\mathbf{M_1}$ and $\mathbf{M_2}$ are overlapping, although they may be spatiotemporally distinct (being realized during different developmental phases and in different parts of the organism). Pairs of variants, which differ with respect to a certain connection of a constituent are indicated next to this connection. For instance, variant $\mathbf{b_2}$ of constituent **B** may differ from $\mathbf{b_1}$, with respect to its binding affinity to extracellular ligand **A**, while $\mathbf{b_3}$ differs from $\mathbf{b_1}$ with respect to its binding affinity to its intracellular ligand **C**. There is likely to be pairwise fitness epistasis for those constellations of variants of two constituents, which affect the same connection, e. g., between variants $\mathbf{a_1 . b_1 \ldots / a_2 . b_1 \ldots / a_1 . b_2 \ldots / a_2 . b_2 \ldots}$ but not between $\mathbf{a_1 . b_1 \ldots / a_2 . b_1 \ldots / a_1 . b_3 \ldots / a_2 . b_3 \ldots}$. The table in B) gives an example for how the presence (+) or absence (–) of fitness epistasis may be distributed among several possible constellations of a given set of variants. From such information it is possible to calculate the probabilities P^e for the pairwise fitness dependence (which is assumed here only to be due to epistasis) between any two constituents (e. g., $P^e_{\mathbf{AB}}$) as well as the coevolution probabilities P^c for module $\mathbf{M_0}$ as well as the higher order modules $\mathbf{M_1}$ and $\mathbf{M_2}$. As indicated in A) the hierarchy of modules is reflected in a hierarchy of coevolution probabilities. However, there is also a small coevolution probability (e. g., because there are epistatic fitness interactions of $\mathbf{b_1/b_5}$ with $\mathbf{a_1/a_2}$ as well as with $\mathbf{x_1/x_2}$) for a unit $\mathbf{U_3}$ (**AXBCDEY**), which is not a module.

teins after alternative splicing depending on the context (reviewed in Gilbert 2000; Lewin 2000). The modularity of gene regulation itself facilitates the "tinkering" (Jacob 1977) by recombination, domain multiplication and embedding of modules into new contexts during evolution, because constituents can acquire new connections (become responsive to other inputs or acquire other outputs) by the simple mechanism of genetic recombination ("shuffling") between existing response elements in the regulatory region or exons (see e. g., Gilbert 1978; García-Bellido 1996; Gerhart and Kirschner 1997; Patthy 1999; Eickbush 1999; Boeke and Pickeral 1999; Dover 2000)[37].

While the domain duplication of a module in a reproducing system may be achievable by a single change of its extramodular connections during evolution, it will typically take at least two independent such changes for the evolution of real multifunctionality, where the duplicate acquires a new and distinct role for the reproduction of the system (has a distinct function), rather than being just a neutral addendum (Sidow 1996; Schlosser, in press a). While a module such as the Delta-Notch pathway may be activated in a new context after one or some of its elements become responsive to a new inducer (e. g., by acquiring a new response element), none of the elements of the new context may be initially responsive to any constituent of the duplicate domain. In such cases, activity of the duplicate domain of the module will be initially spurious and it will not affect the fitness of the constituents of its new context. The initial domain duplication of a module is then selectively neutral and is more likely to become established by drift rather than by selection. However, the duplicate domain will alter the coevolution probabilities of its constituents with the constituents of its new context, because it is now more likely that there will be epistatic fitness interactions with a subset of variants of context constituents, viz., those variants which acquire responsiveness to the dupli-

[37] I have argued that modules can be relatively easily reshuffled during evolution, because variants of their constituents are less likely to affect their external connections and variants affecting external connections of constituents are less likely to have strong epistatic effects on their fitness. This reasoning is applicable to input connections as well as to output connections and it is therefore not to be expected that reproducing systems with a modular organization will generally develop according to a "von Baerian" pattern, i. e., in a way that earlier processes tend to be more evolutionarily conserved than later ones, as is frequently suggested (e. g., Arthur 1997). It has been reasoned that a von Baerian pattern can be expected in systems, whose complexity increases in time, because events at early stages will tend to be more highly "generatively entrenched", i. e., affect a higher number of adaptive or fitness relevant events, than events at later stages (Wimsatt 1986; Arthur 1997). However, in modular reproducing systems, this may not generally be true: Here, the constituents **B**, **C** and **D** of the same module will exhibit high coevolution probabilities (i. e., show a high degree of generative entrenchment with respect to each others fitness), while a constituent such as **A**, which acts earlier and may indeed contribute to the establishment of the module may be much less likely to exist in variants with strong and epistatic fitness effects on **B**, **C** or **D** (i. e., may be less generatively entrenched with respect to their fitness), for instance because **A** is only one out of many possible inducers of **BCD**.

cate module (e. g., by acquiring a new response element for Suppressor of hairless or other downstream components of the Notch pathway)[38].

Redundancy and replacement of modules

Redeployed modules may acquire a novel function in their new context or they may replace another module. Replacement may occur, when the module $\mathbf{M_1}$ evolves so that the duplicate domain in its new context mimicks the external connections of another module $\mathbf{M_0}$. This is particularly likely, when the module $\mathbf{M_1}$ has the same logical properties than $\mathbf{M_0}$ (i. e., embodies the same function mapping input on output variables), while its internal connectivity may be completely different. If the logical properties of $\mathbf{M_1}$ and $\mathbf{M_0}$ are identical (or similar), they may replace each other functionally, if $\mathbf{M_1}$ acquires the same input and output variables as $\mathbf{M_0}$ as discussed in the last paragraph (e. g., when a constituent of $\mathbf{M_1}$ acquires a new response element for the inducer of $\mathbf{M_0}$, whereas the constituents causally dependent on $\mathbf{M_0}$ acquire response elements for constituents of $\mathbf{M_1}$). In such cases one of the two redundant modules or domains (and their hereditary constituents) will often disappear during evolution, because there will be no effective selection against disruptive mutations (Ohta 1987; Nowak et al. 1997; Wagner 1998; Krakauer and Nowak 1999). Under certain conditions, e. g., when functionally deficient variants of each module will occur with equal frequency, but when variants of one module are more likely to have higher relative fitness advantages over alternatives, the probability for retainment may be higher for that module. By this mechanism, a more efficient or reliable module may spread through the organism and replace a less efficient or reliable one with the same logical properties[39].

Such replacements may underly cases where clearly homologous structures in different organisms develop by different mechanisms (see e. g., Striedter and Northcutt 1991; Dickinson 1995; Bolker and Raff 1996; Abouheif 1997; von Dassow and Munro 1999). Segmentation in insects illustrates this: While segmentation in all insects depends on the iterated expression of so-called segment-polarity genes, this expression pattern is set up in very dif-

[38] This scenario of the evolution of multiple domains for modules conforms to what Katz (1987) has labeled "phylogenetic ratcheting": There will be a trend for increasing complexity during evolution, because new features can be added relatively easily without adverse effects on fitness, and with the accumulation of neutral additions the probability increases that one of them may become selectively advantageous later, i.e., will form an "exaptation" (Gould and Vrba 1982). This scenario predicts that evolution should follow a pattern of "punctuated equilibria" (Gould and Eldredge 1977, 1993), where protracted periods of neutral evolution are punctuated by sudden increases in fitness. Such punctuated equilibria were indeed observed in experiments and models on RNA evolution (Huynen et al. 1996; Fontana and Schuster 1998).

[39] Multiple use of the same module may also serve as a basis for several subsequent evolutionary modifications, because it allows the coordinated optimization of different structures, such as the fore- and hindlimbs in vertebrates (as argued by Niehrs and Pollet 1999).

ferent ways in *Drosophila* and in other insects (e. g., Patel et al. 1992; Patel 1994), suggesting that gene networks upstream of segment polarity genes have been replaced by different gene networks during insect evolution.
Replacement of modules, of course, may also take place for modules, which act as submodules of higher level modules. However, this is not a counterargument (as suggested by von Dassow and Munro 1999) to the idea that modules generally will tend to be relatively conserved units of evolution/adaptation. When $\mathbf{M_0}$ is replaced by $\mathbf{M_1}$ as submodule of some larger module **M**, the pattern of fitness epistasis and coevolution probability for the constituents of $\mathbf{M_1}$ may be largely conserved although it is now acting in a new context. Despite this replacement of one of its submodules (and possibly several of its hereditary constituents), the logical structure of connectivities between **M**s submodules as well as the pattern of fitness epistasis among them and their coevolution probability may also be largely preserved for the higher order module **M**. Therefore, a module **M** may (1) retain its relational structure across generations in a lineage of organisms and therefore may be considered homologous in all these organisms, and (2) retain its coevolution probability across generations even when some of its constituents have been replaced (see also Roth, 1991).

Other factors contributing to evolvability

It is important to bear in mind that modularity is not the only mechanism but rather synergizes with other factors in promoting evolvability and developability. I will only briefly list a few examples, as it would be beyond the scope of this paper to discuss their role in detail. First, *regulatory capacities* involving disjunctive pleiotropy and feedback mechanisms allow for plasticity or canalization of processes and thereby buffer environmental and/or genetic perturbations (e. g., Davidson et al. 1995; Gerhart and Kirschner 1997). Plasticity (involving several alternative trajectories of interactions among constituents depending on the environment) allows the moulding of modules to varying external conditions (e. g., size regulation; "exploratory mechanisms" such as in the formation of microtubules, blood vessels, muscles, synapses, immune cells etc.; see Gerhart and Kirschner 1997). Canalization (involving the convergence of several alternative trajectories of interactions on common end states) guarantees relative invariant behavior of the module under varying conditions, e. g., in metabolic homeostasis as well as in developmental "homeorhesis" (Waddington 1957; Gibson and Wagner 2000; Rutherford 2000)[40]. Second, mechanisms of *replication* allow for the duplication (multiplication) of structures and thereby open up on the one hand new possibilities for

[40] The evolution of development itself can be viewed as the evolution of mechanisms for the plastic exploitation of stochastic or environmental asymmetries (which allow diversification of genetically identical cells during development) paired with the canalized reproduction of the totipotent founder entity (e. g., germ line cells).

recombination among constituents (e. g., by duplication and transposition of exons or regulatory elements like response elements or enhancers; see e. g., Gilbert 1978; Eickbush 1999; Patthy 1999; Boeke and Pickeral 1999). On the other hand they permit the generation of redundancy of constituents (e. g., duplication of regulatory genes), which may increase stability and facilitates increases in complexity even in cases where there is no modularity, because redundant elements are unconstrained and free to diverge gradually (see e. g., Ohno 1970; Jacob 1977; Ohta 1987; Zuckerkandl 1994; Raff 1996, Wagner 1998; Krakauer and Nowak 1999; Shimeld 1999; Force et al. 1999). Third, *sex* allows recombination to take place not only between constituents of a single reproducing system, but between different reproducing systems with comparable organizations, again raising the flexibility. While it remains controversial, how sex itself evolved (e. g., Ridley 1993), many theories assume that counteracting the perpetuation of linkage between favorable variants of one constituent and unfavorable variants of another played a crucial role (e. g., Barton and Charlesworth 1998). Fourth, and most importantly, only the capacity for *multiplicative reproduction* makes modifications of a modular structure evolvable at all. I have taken this for granted in this section so far, because I have only discussed the role of modularity for reproducing systems. It needs to be emphasized, however, that not all systems with a modular organization are necessarily reproducing systems, but that only reproducing systems are evolvable in the proper sense of the term, because only such systems have heritability due to variant and fitness reproduction (see section 3.1).

4.3 The evolution of modularity

I have argued so far that (reproductive) modularity defines relatively conserved units of evolution as well as the flexible connections between them. But how does modularity itself evolve? Many suggestions have been made, but there seem to be three major classes of models. First, modularity may indeed have been selected for its ability to promote evolvability itself. This may happen by different mechanisms such as selection for adaptation rate (due to their reduced intermodular coevolution probabilities, modular systems are also able to respond more quickly to selective pressures, see e. g., Riedl 1975; Wagner 1981) or clade selection (e. g., Gerhart and Kirschner 1997; Kirschner and Gerhart 1998). However, selection for adaptation rate is only effective in the absence of recombination (Wagner and Bürger 1985) and the importance and prevalence of clade selection in evolution still remains controversial (see e. g., Gould and Eldredge 1993; Ridley 1993).
Second, modularity may be the inevitable result of simultaneous selection for several uncorrelated interactions of a reproducing system with its environment. Wagner (1996, 42) expresses this very clearly: "In this scenario the pattern of modularity … would reflect the statistical pattern of selec-

tion episodes, such that characters that tend to be under simultaneous directional selection get integrated into a module of phenotypic change, while the characters that rarely adapt to environmental changes at the same time will be represented by genes that have no or only limited pleiotropic effects among them". Generally, interactions of a system with its environment are the more reliable (and hence possibly fitter), the more the pattern of connections among constituents matches the pattern of correlation among factors of the environment (Clarke and Mittenthal 1992). This will result in the evolution of coevolution probabilities itself, because fitness increases, the more the coevolution probabilities of constituents (more precisely: autoconstituents) matches the coincidence probability of the environmental conditions (alloconstituents) on which they depend for reproduction. If each of two strongly and/or multiply connected constituents **A** and **B** is dependent on different and independent contextual (e. g., environmental) factors, then simultaneous optimization of their interactions with the contextual factors may only be possible when ties between the 2 constituents are broken. Hence, uncorrelated selection pressures on **A** and **B** will favor their parcellation into different modules. Conversely, integration of constituents **A** and **C** into a module are only likely to be favored, if selection pressures on **A** and **C** tend to be correlated. In other words, there will be selection for increasing pleiotropy between elements of the same functional unit and decreasing pleiotropy between different functional units (Riedl 1975; Cheverud 1984, 1996). A combination of directional selection (on intramodular connections becoming intermodular connections or vice versa) and stabilizing selection (on remaining intramodular connections) may be required to explain why some connections are differentially suppressed or enhanced during this process whereas others are maintained (Wagner 1996; Wagner and Altenberg 1996). One prediction of this model is that functional units will typically be congruent with developmental units because uncorrelated environmental constraints on different functional units in the adult will come to be reflected in reduced pleiotropy among their developmental precursors. Therefore, the suggestion that evolvability may be due to the direct selection for *developmental* robustness and flexibility (Conrad 1990; Gerhart and Kirschner 1997; Kirschner and Gerhart 1998) is in accordance with this model and emphasizes one of its implications.

A third type of model for the evolution of modularity is suggested by my arguments presented in section 4.2. It is intended as a supplement rather than an alternative to the other two models. The core idea is that once some degree of modularity is established, modularity is basically a self-organizing process, in which modularity generates more modularity. In modular systems, variants are more likely to neutrally or even positively affect fitness while at the same time having significant phenotypic effects when they alter intermodular connections. In contrast, changes of intramodular

connections will tend to be either disruptive or canalized. Hence, in modular systems there is an intrinsic bias for evolution due to intermodular changes. The latter permit what Jacob (1977) has called "tinkering": the multiple and combinatorial deployment of entire modules in new contexts. This will be particularly easy to achieve in systems where mechanisms for the duplication and recombination of hereditary constituents, such as genes or their components (e. g., response elements, exons or clusters of those) exist (although the latter is only one among several possible mechanisms for the multiplication of domains)[41]. Therefore, modular reproducing systems facilitate the increase in complexity by a kind of "modular ratcheting" akin to the "phylogenetic ratcheting" proposed by Katz (1987): There may be a trend for increasing modularity during evolution, because new modules can be added relatively easily without adverse effects on fitness, and with the accumulation of new modules the probability increases that one of them may become selectively advantageous later (see also Zuckerkandl 1997).

5. Conclusions

In summary, modularity plays an important role for the evolution of reproducing systems, in particular if some additional conditions are fulfilled and modules act as reproductive modules. Because such modules are relatively tightly integrated among themselves, but are only sparsely connected to their neighbors, they on the one hand tend to be relatively conserved units of evolution, i. e., units of high coevolution probability that tend to frequently coevolve in a coordinated fashion thereby constraining each others evolution. On the other hand, such modules limit the spread of perturbations/variations, thereby facilitating flexible changes between them, which form the basis for evolutionary innovation, diversification and increase in complexity. Consequently, modules can be optimized relatively independently from other modules, thereby forming the quasi-independent atoms of adaptation (Lewontin 1978). However, the embedding of modules into higher order units implicates that most constituents will not merely be part of a single unit of evolution, but rather participate in a hier-

[41] Importantly, however, in this model the duplication of genes or regulatory elements is only one out of several possible mechanisms for the multiplication of domains of modules. Another model for the self-organization of modularity, which depends more heavily on gene duplication, has been suggested by Altenberg (1995; Wagner and Altenberg 1996). According to his model of "constructional selection", new genes with few pleiotropic effects (more specifically: with few conflicting effects on the fitness of different characters), are more likely to be preserved in evolution. Under the assumption that there will typically be a correlation between the pleiotropic effects during a gene's first addition and the pleiotropic effects of subsequent allelic variants or duplicates of that gene, it can be expected that variants and duplicates of genes with little pleiotropic effects are also more likely to be preserved leading to the preferential spread of such genes in the genome.

archy of units of evolution with different coevolution probabilities. Modularity also is an important motor of increasing complexity and modularity in evolution, because modules can easily be redeployed in new contexts and recombined in a combinatorial fashion. The argument developed here is a general one. It is applicable to all reproducing systems with a modular organization, be it genetic algorithms or organisms, although the self-maintaining organization of living systems, of course, places special demands on the process of self-reproduction.

The claim that modules tend to be units of evolution is not merely vacuous speculation. There are at least three possibilities, how it can be tested after experimental perturbations in a few model organisms have unravelled the boundaries of modules, by identifying networks of interacting genes, proteins etc. that act as integrated and contexinsensitive units. First, comparative studies in an explicit phylogenetic framework and based on robust phylogenetic trees can test the prediction that intramodular connections tend to be more conserved resulting in repeated dissociated coevolution of the constituents of a module from their context (e. g., Schlosser, 2001, in press a, b). Second, this prediction may also be directly tested in selection experiments, which are however only feasible in simple systems with rapid reproductive cycles such as bacteria. Finally, the role of modules as units of evolution may be explored in computer models. This approach, however, is only practicable when many simplifying assumptions are made and it needs to be carefully assessed to what extent insights from computer models are relevant to the evolution of living systems.

Such empirical tests of the role of modularity are as urgently needed as further theoretical efforts to elucidate the role of modules in evolution in more detail. It is very likely that the insights gained in such studies will have an enormous impact on evolutionary theory. They will allow us to make the vague notion of "constraints" more precise and thereby form the key for understanding trends of evolution that are due to biases in variation or to selection pressures inherent in the organization of a reproducing system. In addition, there may not only be theoretical fruits to be harvested. Medical sciences, for example, will profit from elucidating the modularity of development and function of living systems, which may allow to identify the common basis for complex medical syndromes. Finally, understanding modularity will also greatly benefit evolutionary technologies (e. g., Rechenberg 1973; Goldberg 1989; Kauffman 1993, 1995; Wagner and Altenberg 1996), that are gaining firm ground in engineering, drug design and computer programing.

Acknowledgments

I thank Jim Cheverud, Richard Michod, Denis Thieffry, William Wimsatt and Günter Wagner for critical comments on the manuscript. This work was supported by a fellowship of the Hanse Institute for Advanced Study, Delmenhorst.

References

Abeles, M., Prut, Y., Bergman H., Vaardia, E. (1994) Synchronization in neuronal transmission and its importance for information processing. Progress Brain Res. **102**: 395–404.

Abouheif, E. (1997) Developmental genetics and homology: a hierarchical approach. Trend. Ecol. Evol. **12:** 405–408.

Alberch, P. (1980) Ontogenesis and morphological diversification. Am. Zool. **20:** 653–667.

Altenberg, L. (1995) Genome growth and the evolution of the genotype-phenotype map. In: Banzhaf, W., Eeckman, F. H. (eds) Evolution and biocomputation. Springer, Berlin, pp. 205–259.

Ancel, L. W., Fontana, W. (2000) Plasticity, evolvability and modularity in RNA. J. Exp. Zool. (Mol.Dev.Evol.) **288:** 242–283.

Andrews, J. H. (1998) Bacteria as modular organisms. Annu. Rev. Microbiol. **52:** 105–126.

Arnone, M. I., Davidson, E. H. (1997) The hardwiring of development: organization and function of genomic regulatory systems. Development **124:** 1851–1864.

Artavanis-Tsakonas, S., Rand, M. D., Lake, R. J. (1999) Notch signaling: Cell fate control and signal integration in development. Science **284:** 770–776.

Arthur, W. (1997) The origin of animal body plans. Cambridge University Press, Cambridge.

Barton, N. H., Charlesworth, B. (1998) Why sex and recombination? Science **281:** 1986–1990.

Beatus, P., Lendahl, U. (1998) Notch and neurogenesis. J. Neurosci. Res. **54:** 125–136.

Bernstein, H., Byerly, H. C., Hopf, F. A., Michod, R. E., Vemulapalli, G. K. (1983) The Darwinian dynamic. Quart. Rev. Biol. **58:** 185–207.

Bhalla, U. S., Iyengar, R. (1999) Emergent properties of networks of biological signaling pathways. Science **283:** 381–387.

Boeke, J. D., Pickeral, O. K. (1999) Retroshuffling the genomic deck. Nature **398:** 108–111.

Bolker, J. A., Raff, R. A. (1996) Developmental genetics and traditional homology. Bioessays **18:** 489–494.

Bonner, J. T. (1988) The evolution of complexity. Princeton Univ. Press, Princeton.

Brandon, R. N. (1990) Adaptation and environment. Princeton Univ. Press, Princeton.

Brandon, R. N. (1999) The units of selection revisited: the modules of selection. Biology and Philosophy **14:** 167–180.

Bray, D. (1995) Protein molecules as computational elements in living cells. Nature **376:** 307–312.

Bryant, E. H., McCommas, S. A., Combs, L. M. (1986) The effect of an experimental bottleneck upon quantitative genetic variation in the housefly. Genetics **114:** 1191–1211.

Burstein, Z. (1996). A network model of developmental gene hierarchy. J. Theor. Biol. **174:** 1–11.

Buss, L. W. (1987) The evolution of individuality. Princeton Univ. Press, Princeton.

Cheverud, J. M. (1984) Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. **110:** 155–171.

Cheverud, J. M. (1996) Developmental integration and the evolution of pleiotropy. Am. Zool. **36:** 44–50.

Cheverud, J. M., Routman, E. J. (1995) Epistasis and its contribution to genetic variance components. Genetics **139:** 1455–1461.

Cheverud, J. M., Vaughn, T. T., Pletscher, L. S., King-Ellison, K., Bailiff, J., Adams, E., Erickson, C., Bonislawski, A. (1999) Epistasis and the evolution of additive genetic variance in populations that pass through a bottleneck. Evolution **53:** 1009–1018.

Clarke, B. S., Mittenthal, J. E. (1992) Modularity and reliability in the organization of organisms. Bull. Math. Biol. **54:** 1–20.
Conrad, M. (1990) The geometry of evolution. Biosystems **24:** 61–81.
Coveney, P., Highfield, R. (1995) Frontiers of complexity. Fawcett Columbina, New York.
Coyne, J. A., Barton, N. H., Turelli, M. (1997) A critique of Sewall Wright's shifting balance theory of evolution. Evolution **51:** 643–671.
Crow, J. F., Engels, W. R., Denniston, C. (1990) Phase three of Wright's shifting balance theory. Evolution **44:** 233–247.
Darwin, C. (1859) On the origin of species. Murray, London.
Davidson, E. H., Peterson, K. J., Cameron, R. A. (1995) Origin of bilaterian body plans: Evolution of developmental regulatory mechanisms. Science **270:** 1319–1325.
Dawkins, R. (1976) The selfish gene. Oxford Univ. Press, Oxford.
Dawkins, R. (1978) Replicator selection and the extended phenotype. Zeitschr. Tierpsychol. **47:** 61–76.
Dawkins, R. (1982) The extended phenotype. Oxford Univ. Press, Oxford.
Dawkins, R. (1996) Climbing Mount Improbable. Norton, New York.
Dickinson, W. J. (1995) Molecules and morphology: Where's the homology. Trends Genet. **11:** 119–121.
Doolittle, W. F. (1999) Lateral genomics. Trends Genet. **15:** M 5–M 8.
Doolittle, W. F., Sapienza, C. (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature **284:** 601–603.
Dover, G. (2000) How genomic and developmental dynamics affect evolutionary processes. Bioessays **22:** 1153–1159.
Duboule, D., Wilkins, A. S. (1998) The evolution of bricolage. Trends Genet. **14:** 54–59.
Eickbush, T. (1999) Exon shuffling in retrospect. Science **283:** 1465–1466.
Eigen, M., Gardiner, W., Schuster, P., Winkler-Oswatitsch, R. (1981) The origin of genetic information. Sci. Am. **244** (4): 88–118.
Eldredge, N. (1985) Unfinished synthesis. Oxford Univ. Press, New York.
Falconer, D. S. (1960) Introduction to quantitative genetics. Oliver and Boyd, London.
Fenster, C. B., Galloway, L. F., Chao, L. (1997) Epistasis and its consequences for the evolution of natural populations. Trends Ecol. Evolut. **12:** 282–286.
Fontana, W., Buss, L. (1994 a) "The arrival of the fittest": toward a theory of biological organization. Bull. Math. Biol. **56:** 1–64.
Fontana, W., Buss, L. W. (1994 b) What would be conserved if "the tape were played twice"? Proc. Natl. Acad. Sci. USA **91:** 757–761.
Fontana, W., Schuster, P. (1998) Continuity in evolution: On the nature of transitions. Science **280:** 1451–1455.
Fontana, W., Wagner, G., Buss, L. W. (1995) Beyond digital naturalism. In: Langton, C. G. (ed) Artificial life. MIT Press, Cambridge.
Force, A., Lynch, M., Pickett, F. B., Amores, A., Yan, Y.-L., Postlethwait, J. (1999) Preservation of duplicate genes by complementary degenerative mutations. Genetics **151:** 1531–1545.
Forrest, S., Mitchell, M. (1993) Towards a stronger building-block hypothesis: effects of relative building-block fitness on GA performance. In: Whitley, L. D. (ed) Foundations of Genetic Algorithms. Morgon Kaufman, Palo Alto, pp. 109–126.
Freeman, M. (2000) Feedback control of intercellular signalling in development. Nature **408:** 313–319.
Futuyma, D. J. (1986) Evolutionary biology. Sinauer, Sunderland.
García-Bellido, A. (1996) Symmetries throughout organic evolution. Proc. Natl. Acad. Sci. USA **93:** 14229–14232.
Gellon, G., McGinnis, W. (1998) Shaping animal body plans in development and evolution by modulation of Hox expression patterns. Bioessays **20:** 116–125.
Gerhart, J., Kirschner, J. (1997) Cells, embryos, and evolution. Blackwell Science, Malden.
Gibson, G., Wagner, G. (2000) Canalization in evolutionary genetics: a stabilizing theory? Bioessays **22:** 372–380.
Gilbert, S. F. (1998) Conceptual breakthroughs in developmental biology. J. Biosci. **23:** 169–176.
Gilbert, S. F. (2000) Developmental biology. Sinauer, Sunderland.

Gilbert, S. F., Opitz, J. M., Raff, R. A. (1996) Resynthesizing evolutionary and developmental biology. Dev. Biol. **173:** 357–372.
Gilbert, W. (1978) Why genes in pieces? Nature **271:** 501.
Goldberg, D. E. (1989) Genetic algorithms in search, optimization, and machine learning. Addison-Wesley, Reading.
Goodnight, C. J. (1988) Epistasis and the effect of founder events on the additive genetic variance. Evolution **42:** 441–454.
Goodnight, C. J. (1990 a) Experimental studies of community evolution. I. The response to selection at the community level. Evolution **44:** 1614–1624.
Goodnight, C. J. (1990 b) Experimental studies of community evolution. II. The ecological basis of the response to community selection. Evolution **44:** 1614–1624.
Goodnight, C. J., Schwartz, J. M., Stevens, L. (1992) Contextual analysis of group selection, soft selection, hard selection, and the evolution of altruism. Am. Nat. **140:** 743–761.
Gould, S. J. (1982) Darwinism and the expansion of the evolutionary theory. Science **216:** 380–387.
Gould, S. J., Eldredge, N. (1977) Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology **3:** 115–151.
Gould, S. J., Eldredge, N. (1993) Punctuated equilibrium comes of age. Nature **366:** 223–227.
Gould, S. J., Lewontin, R. C. (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lond. B **205:** 581–598.
Gould, S. J., Vrba, E. S. (1982) Exaptation – a missing term in the science of form. Paleobiology **8:** 4–15.
Gray, R. (1992) Death of the gene: developmental systems strike back. In: Griffiths, P. (ed) Trees of life. Kluwer, Dordrecht, pp. 165–209.
Griffiths, P. E., Gray, R. D. (1994) Developmental systems and evolutionary explanation. J. Philos. **91:** 277–304.
Guillemot, F. (1999) Vertebrate bHLH genes and the determination of neuronal fates. Exp. Cell Res. **253:** 357–364.
Hall, B. K. (1992) Evolutionary developmental biology. Chapman and Hall, London.
Hammerschmidt, M., Brook, A., Mc Mahon, A. P. (1997) The world according to hedgehog. Trends Genet. **13:** 14–21.
Harper, J. L., Rosen, B. R., White, J. (1986) The growth and form of modular organisms – Preface. Phil. Trans. R. Soc. Lond. B **313:** 3–5.
Hartwell, L. H., Hopfield, J. J., Leibler, S., Murray, A. W. (1999) From molecular to modular cell biology. Nature **402** Suppl.: C 47–C 52.
Hedrick, P., Jan, S., Holden, L. (1978) Multilocus systems in evolution. Evol. Biol. **11:** 101–184.
Heisler, I. L., Damuth, J. D. (1987) A method for analyzing selection in hierarchically structured populations. Am. Nat. **130:** 582–602.
Hertz, J., Krogh, A., Palmer, R. G. (1991) Introduction to the theory of neural computation. Addison-Wesley, Redwood City.
Holland, J. H. (1975) Adaptation in natural and artificial systems. University of Michigan Press, Ann Arbor.
Hull, D. L. (1980) Individuality and selection. Annu. Rev. Ecol. Syst. **11:** 311–332.
Hull, D. L. (1981) Units of evolution: a metaphysical essay. In: Jensen, U. J., Harré, R. (eds) The philosophy of evolution. The Harvester press, Brighton, pp. 23–44.
Hurst, L. D. (1999) The evolution of genomic anatomy. Trends Ecol. Evol. **14:** 108–112.
Huynen, M. A., Stadler, P. F., Fontana, W. (1996) Smoothness within ruggedness: the role of neutrality in adaptation. Proc. Natl. Acad. Sci. USA **93:** 397–401.
Jacob, F. (1977) Evolution and tinkering. Science **196:** 1161–1166.
Katz, M. J. (1987) Is evolution random? In: Raff, R. A., Raff, E. C. (eds) Development as an evolutionary process. Liss, New York, pp. 285–315.
Kauffman, S. A. (1993) The origins of order. Oxford Univ. Press, New York.
Kauffman, S. A. (1995) At home in the universe. Oxford University Press, New York.
Keys, D. N., Lewis, D. L., Selegue, J. E., Pearson, B. J., Goodrich, L. V., Johnson, R. L., Gates, J., Scott, M. P., Carroll, S. B. (1999) Recruitment of a hedgehog regulatory circuit in butterfly eyespot evolution. Science **283:** 532–534.
Kimble, J., Simpson, P. (1997) The lin-12/notch signaling pathway and its regulation. Annu. Rev. Cell Dev. Biol. **13:** 333–361.

Kirchhamer, C. V., Yuh, C.-H., Davidson, E. H. (1996) Modular cis-regulatory organization of developmentally expressed genes: two genes transcribed territorially in the sea urchin embryo, and additional examples. Proc. Natl. Acad. Sci. USA **93:** 9322–9328.
Kirschner, M., Gerhart, J. (1998) Evolvability. Proc. Natl. Acad. Sci. USA **95:** 8420–8427.
Krakauer, D. C., Nowak, M. A. (1999) Evolutionary preservation of redundant duplicated genes. Semin. Cell Dev. Biol. **10:** 555–559.
Lande, R., Arnold, S. J. (1983) The measurement of selection on correlated characters. Evolution **37:** 1210–1226.
Lewin, B. (2000) Genes VII. Oxford Univ. Press, New York.
Lewontin, R. C. (1962) Interdeme selection controlling a polymorphism in the house mouse. Am. Nat. **96:** 65–78.
Lewontin, R. C. (1970) The units of selection. Annu. Rev. Ecol. Syst. **1:** 1–18.
Lewontin, R. C. (1974) The genetic basis of evolutionary change. Columbia Univ. Press, New York.
Lewontin, R. C. (1978) Adaptation. Sci. Am. **239** (3): 156–169.
Lewontin, R. C. (1983) The organism as the subject and object of evolution. Scientia **118:** 65–82.
Lewontin, R. C., Kojima, K.-I. (1960) The evolutionary dynamics of complex polymorphisms. Evolution **14:** 458–472.
Lloyd, E. (1988) The structure and confirmation of evolutionary theory. Princeton Univ. Press, Princeton.
Maconochie, M., Nonchev, S., Morrison, A., Krumlauf, R. (1996) Paralogous hox genes: function and regulation. Annu. Rev. Genet. **30:** 529–556.
Maturana, H. R., Varela, F. J. (1975) Autopoietic systems: A characterization of the living organization. Report 9.4 Biological Computer laboratory, University of Illinois, Urbana.
Maynard Smith, J. (1987) How to model evolution. In: Dupré, J. (ed) The latest on the best. MIT Press, Cambridge, pp. 119–131.
Maynard Smith, J. S. (1989) Evolutionary genetics. Oxford Univ. Press, New York.
Maynard Smith, J. (1998) The units of selection. Novartis Foundation Symp. **213:** 203–217.
Maynard Smith, J., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., Wolpert, L. (1985) Developmental constraints and evolution. Quart. Rev. Biol. **60:** 265–287.
Maynard Smith, J., Szathmáry, E. (1995) The major transitions in evolution. Freeman, Oxford.
Mayr, E. (1963) Animal species and evolution. Belknap Press, Cambridge.
McAdams, H. H., Shapiro, L. (1995) Circuit simulation of genetic networks. Science **269:** 650–656.
Mendoza, L., Thieffry, D., Alvarez-Buylla, E. R. (1999) Genetic control pf flower morphogenesis in Arabidopsis thaliana: a logical analysis. Bioinformatics **15:** 593–606.
Mezey, J. G., Cheverud, J. M., Wagner, G. P. (2000). Is the genotype-phenotype map modular?: A statistical approach using mouse quantitative trait loci data. Genetics **156:** 305–311.
Michod, R. E. (1997) Evolution of the individual. Amer. Nat. **150:** S5–S21.
Michod, R. E. (1999) Darwinian dynamics. Princeton Univ. Press, Princeton.
Mjolsness, E., Sharp, D. H., Reinitz, J. (1991) A Connectionist Model of Development. J. Theor. Biol **152:** 429–453.
Niehrs, C., Pollet, N. (1999) Synexpression groups in eukaryotes. Nature **402:** 483–487.
Nowak, M. A., Boerlijst, M. C., Cooke, J., Smith, J. M. (1997) Evolution of genetic redundancy. Nature **388:** 167–171.
Ohno, S. (1970) Evolution by gene duplication. Springer, New York.
Ohta, T. (1987) Simulating evolution by gene duplication. Genetics **115:** 207–213.
Orgel, L. E., Crick, F. H. C. (1980) Selfish DNA: the ultimate parasite. Nature **284:** 604–607.
Oyama, S. (1985) The ontogeny of information. Cambridge Univ. Press, Cambridge.
Patel, N. H. (1994) Developmental evolution: Insights from studies of insect segmentation. Science **266:** 581–590.
Patel, N. H., Ball, E. E., Goodman, C. S. (1992) Changing role of even-skipped during the evolution of insect pattern formation. Nature **357:** 339–342.
Patthy, L. (1999) Genome evolution and the evolution of exon-shuffling – a review. Gene **238:** 103–114.

Price, G. R. (1972) Extension of covariance selection mathematics. Ann. Human Genetics **35:** 485–490.
Purugganan, M. D. (1998) The molecular evolution of development. Bioessays **20:** 700–711.
Raff, R. A. (1996) The shape of life. Univ. of Chicago Press, Chicago.
Rechenberg, I. (1973) Evolutionsstrategie. Frommann-Holzboog, Stuttgart.
Reggia, J. A., Armentrout, S. L., Chou, H.-H., Peng, Y. (1993) Simple systems that exhibit self-directed replication. Science **259:** 1282–1287.
Reichardt, L. F., Fariñas, I. (1997) Neurotrophic factors and their receptors. In: Cowan, W. M., Jessell, T. M., Zipursky, S. L. (eds) Molecular and cellular approaches to neural development. Oxford Univ. Press, New York, pp. 220–263.
Resnik, D. (1996) Developmental constraints and patterns: Some pertinent distinctions. J. Theor. Biol. **173:** 231–240.
Ridley, M. (1993) Evolution. Blackwell, Cambridge.
Riedl, R. (1975) Die Ordnung des Lebendigen. Parey, Hamburg.
Roth, G., Wake, D. B. (1989) Conservatism and innovation in the evolution of feeding in vertebrates. In: Wake, D. B., Roth, G. (eds) Complex organismal functions: Integration and evolution in vertebrates. Wiley, Chichester, pp. 7–21.
Roth, L. (1991) Homology and hierarchies: problems solved and unresolved. J. Evol. Biol. **4:** 167–194.
Rutherford, S. L. (2000) From genotype to phenotype: buffering mechanisms and the storage of genetic information. Bioessays **22:** 1095–1105.
Sasai, Y., De Robertis, E. M. (1997) Ectodermal patterning in vertebrate embryos. Dev. Biol. **182:** 5–20.
Schank, J. C., Wimsatt, W. C. (1986) Generative entrenchment and evolution. PSA 1986 **2:** 33–60.
Schank, J. C., Wimsatt, W. C. (2001) Evolvability: adaptation and modularity. In: Singh, R. S., Krimbas, C. B., Paul, D., Beatty, J. (eds) Thinking about evolution. Cambridge Univ. Press, Cambridge, pp 322–335.
Schlosser, G. (1993) Einheit der Welt und Einheitswissenschaft. Grundlegung einer Allgemeinen Systemtheorie. Vieweg, Braunschweig.
Schlosser, G. (1996) Der Organismus – eine Fiktion? In: Rheinberger, H. J., Weingarten, M. (eds) Jahrbuch für Geschichte und Theorie der Biologie III. Verlag für Wissenschaft und Bildung, Berlin, pp. 75–92.
Schlosser, G. (1998) Self-re-production and functionality. A systems-theoretical approach to teleological explanation. Synthese **116:** 303–354.
Schlosser, G. (in press a) Modules – Developmental units as units of evolution? In: Schlosser, G., Wagner, G. P. (eds): Modularity in development and evolution. University of Chicago Press, Chicago.
Schlosser, G. (in press b) Amphibian variations – the role of modules in mosaic evolution. In: Rasskin-Gutman, D., Callebaut, W. (eds) Modularity: Understanding the development and evolution of complex natural systems. MIT Press, Cambridge.
Schlosser, G. (2001) Using heterochrony plots to detect the dissociated coevolution of characters. J. exp. Zool. (Mol. Dev. Evol.) **291:** 282–304.
Schlosser, G., Thieffry, D. (2000) Modularity in development and evolution. Bioessays **22:** 1043–1045.
Schwenk, K. (1994) A utilitarian approach to evolutionary constraint. Zoology **98:** 251–262.
Sharp, D. H., Reinitz, J. (1998) Prediction of mutant expression patterns using gene circuits. Biosystems **47:** 79–90.
Shimeld, S. M. (1999) Gene function, gene networks and the fate of duplicated genes. Semin. Cell Dev. Biol. **10:** 549–553.
Shubin, N., Tabin, C., Carroll, S. (1997) Fossils, genes and the evolution of animal limbs. Nature **388:** 639–648.
Sidow, A. (1996) Gen(om)e duplications in the evolution of early vertebrates. Curr. Opin. Genet. Develop. **6:** 715–722.
Simon, H. A. (1962) The architecture of complexity. Proc. Am. Phil. Soc. **106:** 467–482.
Simpson, P. (1997) Notch signaling in development. Perspect. Dev. Neurobiol. **4:** 297–304.
Smith, N. G. C., Knight, R., Hurst, L. D. (1999) Vertebrate genome evolution: a slow shuffle or a big bang? Bioessays **21:** 697–703.

Sober, E. (1981) Holism, individualism and the units of selection. PSA 1981: 93–121.
Sober, E. (1984) The nature of selection. Univ. of Chicago Press, Chicago.
Sober, E. (1987) Comments on Maynard Smith's "How to model evolution". In: Dupré, J. (ed) The latest on the best. MIT Press, Cambridge, pp. 133–149.
Sober, E., Lewontin, R. C. (1982) Artifact, cause and genic selection. Philos. Science **49:** 157–180.
Sober, E., Wilson, D. S. (1994) A critical review of philosophical work on the unit of selection problem. Philos. Science **61:** 534–555.
Sober, E., Wilson, D. S. (1998) Unto others. Harvard Univ. Press, Cambridge.
Somogyi, R., Sniegoski, C. A. (1996) Modeling the complexity of genetic networks: understanding multigenic and pleiotropic regulation. Complexity **1:** 45–63.
Štanojević, D., Hoey, T., Levine, M. (1989) Sequence-specific DNA-binding activities of the gap proteins encoded by hunchback and Krüppel in *Drosophila*. Nature **341:** 331–335.
Štanojević, D., Small, S., Levine, M. (1991) Regulation of a segmentation stripe by overlapping activators and repressors in the *Drosophila* embryo. Science **254:** 1385–1387.
Sterelny, K., Kitcher, P. (1988) The return of the gene. J. Philos. **85:** 339–361.
Striedter, G. F., Northcutt, R. G. (1991) Biological hierarchies and the concept of homology. Brain Behav Evol **38:** 177–189.
Stryer, L. (1981) Biochemistry. Freeman, San Francisco.
Szathmáry, E. (1995) A classification of replicators and lambda-calculus models of biological organization. Proc. R. Soc. Lond. B **260:** 279–286.
Szathmáry, E., Maynard Smith, J. (1995) The major evolutionary transitions. Nature **374:** 227–232.
Szathmáry, E., Maynard Smith, J. (1997) From replicators to reproducers: the first major transitions leading to life. J. Theor. Biol. **187:** 555–571.
Thieffry, D., Huerta, A. M., Pérez-Rueda, E., Collado-Vides, J. (1998) From specific gene regulation to genomic networks: a global analysis of transcriptional regulation in *Escherichia coli*. Bioessays **20:** 433–440.
Thieffry, D., Romero, D. (1999) The modularity of biological regulatory networks. Biosystems **50:** 49–59.
Thieffry, D., Thomas, R. (1995) Dynamical behaviour of biological regulatory networks – II. Immunity control in bacteriophage lambda. Bull. Math. Biol. **57:** 277–297.
Thomas, R. (1978) Logical analysis of systems comprising feedback loops. J. Theor. Biol. **73:** 631–656.
Thomas, R. (1991) Regulatory networks seen as asynchronous automata: a logical description. J. Theor. Biol. **153:** 1–23.
Thomas, R., Thieffry, D., Kaufman, M. (1995) Dynamical behaviour of biological regulatory networks – I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. Bull. Math. Biol. **57:** 247–276.
Tsai, M.-J., O'Malley, B. W. (1994) Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. 63: 451–486.
Uexküll, J. v. (1928) Theoretische Biologie. 2nd ed. Springer, Berlin.
Varela, F., Maturana, H. R., Uribe, R. B. (1974) Autopoiesis: The organization of living systems, its characterization and a model. Biosystems **5:** 187–196.
Von Dassow, G., Munro, E. (1999) Modularity in animal development and evolution: Elements of a conceptual framework for EvoDevo. J. Exp. Zool. (Mol. Dev. Evol.) **285:** 307–325.
Von Dassow, G., Meir, E., Munro, E. M., Odell, G. M. (2000) The segment polarity network is a robust development module. Nature **406:** 188–192.
Waddington, C. H. (1957) The strategy of the genes. George Allen and Unwin, London.
Wade, M. J. (1985) Soft selection, hard selection, kin selection, and group selection. Am. Nat. **125:** 61–73.
Wade, M. J. (1992) Epistasis. In: Fox Keller, E., Lloyd, E. A. (eds) Keywords in evolutionary biology. Harvard University Press, Cambridge, pp. 87–91.
Wagner, A. (1998) The fate of duplicated genes: loss or new function? Bioessays **20:** 785–788.
Wagner, G. P. (1981) Feedback selection and the evolution of modifiers. Acta Biotheor. **30:** 79–102.
Wagner, G. P. (1995) The biological role of homologues: a building block hypothesis. N. Jb. Geol. Palont. Abh. **19:** 36–43.

Wagner, G. P. (1996) Homologues, natural kinds and the evolution of modularity. Am. Zool. **36:** 36–43.
Wagner, G. P., Altenberg, L. (1996) Complex adaptations and the evolution of evolvability. Evolution **50:** 967–976.
Wagner, G. P., Bürger, R. (1985) On the evolution of dominance modifiers. II. A non-equilibrium approach to the evolution of genetic systems. J. Theor. Biol. **113:** 475–500.
Wagner, G. P., Booth, G., Bagheri-Chaichian, H. (1997) A population genetic theory of canalization. Evolution **51:** 329–347.
Wagner, G. P., Laubichler, M. D. (2000) Character identification in evolutionary biology: the role of the organism. Theory Biosci. **119:** 20–40.
Wagner, G. P., Laubichler, M. D., Bagheri-Chaichian, H. (1998) Genetic measurement theory of epistatic effects. Genetica **102/103:** 569–580.
Wagner, G. P., Mezey, A. (2000) Modeling the evolution of genetic architecture: A continuum of alleles model with pairwise A × A epistasis. J. Theor. Biol. **203:** 163–175.
Wagner, G. P., Schwenk, K. (2000) Evolutionarily stable configurations: functional integration and the evolution of phenotype stability. Evol. Biol. **31:** 155–217.
Wake, D. B., Larson, A. (1997) Multidimensional analysis of an evolving lineage. Science **238:** 42–48.
Warren, R. W., Nagy, L., Selegue, J., Gates, J., Carroll, S. (1996) Evolution of homeotic gene regulation and function in flies and butterflies. Nature **372:** 458–461.
Waters, K. (1991) Tempered realism about the force of selection. Philos. Science **58:** 553–573.
Waxman, D., Peck, J. R. (1998) Pleiotropy and the preservation of perfection. Science **279:** 1210–1213.
Webster, G., Goodwin, B. (1996) Form and transformation. Cambridge University Press, Cambridge.
Weintraub, H. (1993) The MyoD family and myogenesis: Redundancy, networks and thresholds. Cell **75:** 1241–1244.
Whitlock, M. C., Phillips, P. C., Moore, F. B.-G., Tonsor, S. J. (1995) Multiple fitness peaks and epistasis. Annu. Rev. Ecol. Syst. **26:** 601–629.
Whyte, L. L. (1965) Internal factors in evolution. Tavistock Publications, London.
Wilkins, A. S. (1997) Canalization: a molecular genetic perspective. Bioessays **19:** 257–262.
Wilkins, A. S. (1998) Evolutionary developmental biology: where is it going? Bioessays **20:** 783–784.
Williams, G. C. (1966) Adaptation and natural selection. Princeton Univ. Press, Princeton.
Wilson, D. S. (1983) The group selection controversy: history and current status. Annu. Rev. Ecol. Syst. **14:** 159–187.
Wimsatt, W. C. (1980) Reductionistic research strategies and their biases in the unit of selection controversy. In: Nickles, T. (ed) Scientific discovery: case studies. Reidel, Dordrecht, pp. 213–259.
Wimsatt, W. C. (1981) Units of selection and the structure of the multilevel genome. PSA 1980 **2:** 122–183.
Wimsatt, W. C. (1986) Developmental constraints, generative entrenchment, and the innate-acquired distinction. In: Bechtel, W. (ed). Integrating scientific disciplines. Nijhoff Publ. Dordrecht, pp. 185–208.
Wray, G. A. (1994) Developmental evolution – new paradigms and paradoxes. Dev. Genet. **15:** 1–6.
Wright, S. (1931) Evolution in Mendelian populations. Genetics **16:** 97–159.
Wright, S. (1988) Surface of selective value revisited. Am. Nat. **131:** 115–123.
Xu, X. L., Weinstein, M., Li, C. L., Deng, C. X. (1999) Fibroblast growth factor receptors (FGFRs) and their roles in limb development. Cell Tissue Res. **296:** 33–43.
Yen, P. M., Chin, W. W. (1994) New advances in understanding the molecular mechanisms of thyroid hormone action. Trends Endocrinol. Metab. **5:** 65–72.
Yuh, C.-H., Bolouri, H., Davidson, E. H. (1998) Genomic cis-regulatory logic: axperimental and computaional analysis of a sea urchin gene. Science **279:** 1896–1902.
Zuckerkandl, E. (1994) Molecular pathways to parallel evolution. 1. Gene nexuses and their morphological correlates. J. Mol. Evol. **39:** 661–678.
Zuckerkandl, E. (1997) Neutral and nonneutral mutations: the creative mix – evolution of complexity in gene interaction systems. J. Mol. Evol. **44**, Suppl. 1: S2–S8.