

The Development of Animal Form

Ontogeny, Morphology, and
Evolution

ALESSANDRO MINELLI

University of Padova



CAMBRIDGE
UNIVERSITY PRESS

PUBLISHED BY THE PRESS SYNDICATE OF THE UNIVERSITY OF CAMBRIDGE
The Pitt Building, Trumpington Street, Cambridge, United Kingdom

CAMBRIDGE UNIVERSITY PRESS
The Edinburgh Building, Cambridge CB2 2RU, UK
40 West 20th Street, New York, NY 10011-4211, USA
477 Williamstown Road, Port Melbourne, VIC 3207, Australia
Ruiz de Alarcón 13, 28014 Madrid, Spain
Dock House, The Waterfront, Cape Town 8001, South Africa
<http://www.cambridge.org>

© Alessandro Minelli

This book is in copyright. Subject to statutory exception
and to the provisions of relevant collective licensing agreements,
no reproduction of any part may take place without
the written permission of Cambridge University Press.

First published

Printed in the United States of America

Typefaces Utopia 9.5/13 pt. and Avenir *System* L^AT_EX 2_ε [TB]

A catalogue record for this book is available from the British Library.

Library of Congress Cataloguing-in-Publication Data

Minelli, Alessandro.

The development of animal form : ontogeny, morphology, and evolution /
Alessandro Minelli.

p. cm.

Includes bibliographical references (p.).

ISBN 0-521-80851-0

1. Developmental biology. 2. Ontogeny. 3. Morphology. 4. Evolution
(Biology) I. Title.

QH491 .M559 2003

571.3'1 – dc21

2002073694

ISBN 0 521 80851 0 hardback

Contents

<i>Preface</i>	<i>page</i> xiii
<i>Acknowledgements</i>	xvii
1 The Nature of Development	1
Development for the Sake of Development	1
Developmental Competition between Body Parts	7
The Robustness of Morphogenesis	9
2 Everything Begun to the Service of Development: Cellular Darwinism and the Origin of Animal Form	12
Cilia, Cell Division, and Morphogenesis	12
Epithelia without Cilia	13
Origin of the Ecdysozoan Cuticle	14
Cuticle, Body Size, and Internal Fertilisation	16
Origin of Mineralised Skeletons	18
Organic Matrices	19
Coda	19
3 Development: Generic to Genetic	21
Developmental Genes	21
Master Control Genes?	25
Self-Assembly or Cytotaxis?	27
Default Morphology	30
Generic Forms	32
A Bestiary of Generic Forms	35
The Earthworm and the Ankylosaurus	36
Deceptive Numbers	38
Genetic Assimilation	39
Genes and Phenotype	41
Evolutionary Dissociation between Genes and Phenotypes	42

A Role for the 'Developmental Genes'	44
The <i>Hox</i> Code	44
Organic Codes	50
Universal Genetic Tools	51
Genetic Networks and Morphogenesis	53
4 Periodisation	55
The Primacy of Time	56
Time Schedule: Synchronous Versus Metachronous	57
Units in Time	57
Homology of Developmental Stages or Events	58
Comparing Stages	60
What Is a Larva?	65
Metamorphosis as Metagenesis	67
Postembryonic Development	67
One Life throughout the Metamorphosis	67
Developmental Stages as Units of Competition?	68
The Evolution of Moulting Schedules in the Ecdysozoa	71
Number of Moults, Dyar's Coefficient, and Targeted Growth	73
Lazarus Developmental Features	75
Recapitulation	77
5 Body Regions: Their Boundaries and Complexity	79
Tagmosis	79
Instability of Tagmatic Boundaries	83
Homology of Tagmata	85
Number of Tagmata and Convergence	86
The Complexity of Postembryonic Development	88
Williston's Rule	92
Developmental Time and Body Axes	92
The Time Axis of Development and the Patterning of the Proximo-Distal Axis of the Appendages	97
Topology	99
Morphology and Developmental Topology	99
Topology of Coaptations	102
Topological Breakdown	104
6 Differentiation and Patterning	106
Cells as Units of Differentiation	106
Cell Cycle Length	108

Cell Types	109
Cell Autonomy, Induction, and Repatterning	109
Cell Contacts and Cell Communication	110
Asymmetric Cell Divisions	111
Positional Homology and the Hot Spots of Differentiation	111
Positional Information or Informational Position?	111
Zootype and the Patterning of the Nervous System	112
Cellularity and Positional Information	115
Transpatterning	116
Provisional Scaffolding	116
Segments, Vertebrae, and Scales	119
Guidelines to Follow	121
Phylotypic Stage and Phylotypic Period	123
Morphological Assimilation in Ontogeny and Phylogeny	127
Patterning in Regeneration	130
Embryonic Patterning Versus Patterning in Regeneration	130
Terminal or Apical Control Versus Regeneration	131
7 Size Factors	133
Cell Size Critical for Morphogenesis	133
Size and Cell Number in Embryonic Anlagen	135
Early Because Small or Small Because Early?	135
Critical Number of Cells in Embryonic Anlagen	137
Miniaturisation	139
Miniaturisation and Body Patterning	139
Miniaturisation, Segments, and Cells	140
8 Axes and Symmetries	142
The Animal's Main Body Axis	142
Polyps, Sipunculans, and Squids	143
The Dual Animal	147
Sipunculans and <i>ParaHox</i> Genes	149
Morphological Versus Functional Polarity	150
Cartesian Axes, or Not	152
The Syntax of the Body	155
What Is a Tail?	156
The Time Arrow of Growth and Differentiation	158
The Beginnings of Animal Polarity	159
Tapeworm Polarity	160
Differentiating Back to Front	162
Polarity Reversal	163

Axis Paramorphism and Origin of the Appendages	163
Axis Paramorphism	164
Terminal Control and Axis Paramorphism	173
Gene Co-option	175
Limbs and Genitalia	176
Symmetry and Asymmetry	182
Directional Asymmetry	185
9 Segments	188
What Is a Segment?	188
Virtual Versus Physical Segmental Boundaries	190
How Many Times Did Metazoans Evolve Segmentation?	192
Reconstructing Urbilateria	193
Segments in Annelids, Arthropods, and Vertebrates	195
Limits of a Typological View of Segments	197
Segmentation: One Animal, More than One Mechanism	200
Double Segmentation: Eosegments and Merosegments	200
The Naupliar-Postnaupliar-Meromeric Model of Arthropod Segmentation	204
Reliable Patterning of Eosegments and the Variable Schedule of Merosegmentation	209
Heterogeneous Segments in Vertebrates and Annelids	212
Germ Layers and Segmentation	215
Segmental Mismatches and Resegmentation	216
Dorso-Ventral Mismatches	216
Resegmentation	219
10 Evo-devo Perspectives on Homology	222
Concepts and Interpretations	222
Hierarchies and Beyond	222
Homology: Absolute or Relative?	223
Temporal Serial Homology	227
Genes and Homology	228
Genetic Redundancy, Network Degeneracy, and Homology	231
Evolutionary Novelties	232
Units of Description and Comparison	233
Modules	234
Evolutionary Changes in the Discernibility of Developmental Modules: Fusion Versus Non-disjunction	235
Germ Layers and Homology	236
Lesser Developmental Units	241

Contents

xi

Frames of Reference: Muscles and Nerves	243
Muscles and Homology	244
Nerves and Homology	247
<i>Summary and Conclusions</i>	250
<i>References</i>	255
<i>Index</i>	313

CHAPTER ONE

The Nature of Development

Ontogeny is the unfolding of coupled developmental mechanisms whose parameters are largely specified by the genome. We hardly understand when and whether such mechanisms give rise to a few forms robustly or a plethora of forms each requiring the most delicate genetic balance among the control parameters. If robust flow into one or a few morphologies, governed by parameters easily held in vast volumes of parameter space, is the norm when many mechanisms are coupled, then robust morphogenesis could be the norm as well. Robustness may flow from complexity itself.

B.C. Goodwin, S.A. Kauffman and J.D. Murray 1993: 143

The evolution of the cell can be regarded as the 'big bang' of biological evolution even though it took a very long time. The origin of embryonic development from cells can be regarded as the 'little bang' since the cell was already there.

L. Wolpert 1994: 79

Development for the Sake of Development

The shapes of things are temporarily stable configurations compatible with the underlying dynamics. This is obviously true of a flame, a river or a water drop. But this is also true of life in all its manifestations. The origin of life is the origin of a peculiar set of processes rather than the origin of peculiar things. Development is the sum of the never-ending changes of multicellular organisms, a set of processes that transcends the conventional limits of one generation, from egg to adult.

With many examples often drawn from organisms made of a small number of cells, Bonner (2000) has shown that development is the direct consequence of multicellularity. In other words, development is simply the sum of the changes multicellular systems undergo through time. This might

seem like a trivial rephrasing of the conventional notion of development, but it is not. It is the gateway to abandoning the traditional adultocentric view of development. Development, we are accustomed to saying, is the way an egg (or a seed or a spore) turns into an adult, a 'complete' organism. Residuals of finalism are even present in Striedter's (1998) otherwise attractive definition of development as the trajectory of a complex physical system with multiple stable states. What is at stake is the prospect of moving at last toward a *scientific theory of development* – a target, to be sure, far beyond my most ambitious aims with this book.

Finalism has been largely expunged from evolutionary biology, but it is still widely entrenched in developmental biology. Even to those like myself, who refrain from taking Gould and Lewontin's (1979) paper too literally, the lesson of San Marco's spandrels seems to have put an end to that naive adaptationism which looks after purpose in anything less than the most trivial evolutionary change. Things are very different in developmental biology. Take, for example, Davidson's (1991: 11; 1) statements that "development is the execution of the genetic program for the construction of a given species of organism", or "an embryo is not simply equivalent to a set of differentiating cells, even a spatially organized set. A particular function of embryonic cells is to interact in specific ways, in order to generate morphological structure". It is true that function is not a strong word as is purpose (Amundson and Lauder 1994), but to say that embryonic cells are there "in order to generate morphological structure" smells of finalism nevertheless. This finality may seem more tangible, in respect to the putative finality of evolutionary adaptations, as the ontogenetic game is played in a much shorter time dimension than the evolutionary game. One could say: You have simply to watch a hen's egg turning into a chick, and the latter growing into a cock or a hen, or an oak seed turning into an oak seedling, slowly growing into a mature tree, to convince yourself of the purposefulness of development. Consistent with this viewpoint is the current metaphor of the developmental programme inscribed in an organism's genome. Programme for what? For building an adult, of course.

I admit that life would not continue were it not for the fitness of the adult animal, but the same can be said of *any* developmental stage. Van Valen (1970) rightly remarked that a critical examination of some adult structures would help us find restrictive boundary conditions on developmental processes. But this is only true in terms of an objective analysis of the development of a given species, not as a general prescription of how development must run to build the adult.

It seems more sensible to me to follow Oyama (2000a: 161), who describes a developmental stage as “a kind of temporal slice through the life cycle. It carries the evidence of past gene transcriptions, mechanical influences inside and outside the organism, results of past activities, nutrition or lack of it, and so on, and it has certain prospects for change”.

Many criticisms have been levelled at the metaphor of the genetic programme (e.g., Oyama 1985, Nijhout 1990, Müller and Wagner 1991, Bolker and Raff 1996, Neumann-Held 1999, Laubichler and Wagner 2001). Oyama (2000b: 62–63) dares to say “that whenever a program is invoked, a developmental question is being ignored, or worse, being given a spurious answer”. More explicit is Keller (2000), who suggests that to speak in terms of genetic programme is to commit a basic error in categorisation: genetic is equated to programme at the same time as epigenetic is equated to data. But development depends not only on genetic memory, but also on the machinery of the cellular structures, which in turn are set in place by cellular memory rather than by genetic information (see chapter 3).

Even among those who accept the metaphor of the genetic programme, indeed, there are critics of the widespread notion of development as a single control cascade initiated by a first-moving gene. The genomic regulatory system does not constitute a serial-processing algorithm, because at any time many genes are found to act in parallel (Kauffman 1993).

But the very concept of developmental processes initiated by a single gene expression oversimplifies reality by ignoring the load of the system's past history (Minelli 1971, Oyama 2000a), not to speak of the external influences to which it is steadily exposed. A gene ‘initiates’ a sequence of events only if our investigation starts at that point (Oyama 2000a).

I believe that we can replace this finalistic view with a more sober notion of development as quasi-cyclical process, of which the egg (if any) and the adult (if any) are generally the most conspicuous and well-characterized phases rather than the beginning and the end of a non-return way. There is little scope for objecting that the way an egg (or a juvenile, or a larva) gives rise to an adult is quite different from the way an adult gives rise to the next generation's eggs. This is not necessarily true. Consider the different ways a cnidarian polyp may become a medusa. Cubozoan polyps metamorphose into medusae; that is, the whole polyp is changed into a medusa, much as juveniles (but only a fraction of what we call larvae) change into the corresponding adult. In hydrozoans and scyphozoans, however, the medusa buds off from the polyp, or detaches itself from it, much as gametes are released from the adult animal. These rough comparisons only

invest the hard mechanics of the processes, but this seems enough for embracing the concept of a cyclical, rather than goal-directed, nature of development.

The reader will be ready with the next objection: where is the difference between this cyclical notion of development and the common notion of life cycle? Is it not true that this cyclical notion of development simply makes development synonymous with multicellular life?

To some extent, it does. Adopting Griesemer's (2000) suggestion, we can regard development as the set of processes that must occur before a multicellular biological system is capable of reproduction. To study development is thus to study multicellularity (Bonner 2000).

This means that the basic unit of development is the cell. This may seem another truism, perfectly in line with the current perspectives on animal development, in which each chapter of the story begins with that unique cell, the egg, fertilized or not. But to reduce development to the deployment of an egg's potentialities is, at the same time, to give too much emphasis to the egg and, more important, to underrate a basic fact in development. Every cell starts its own version of life business anew, a version differing from those of the other cells, egg included, only because of the constraints provided by local circumstances, both informational and trophic, that result from a more or less long segment of history of the cell lineage to which this cell belongs. Sooner or later, however, fate and metabolic performance of a given cell cluster or sheet become fixed, the only possible alternative being starvation or death. Some cluster of cells, however, may be saved from this irreversible fate, ready to start new ventures at a later stage. Such are some clusters of set-aside cells (e.g., the imaginal discs of the insects or the adult primordium in a sea urchin larva). Also, such are the stem cells, as well as the cells of the germ line, the only survivors, generally, from the final defeat of the whole multicellular company.

From this perspective, there is nothing like a developmental programme. In a sense, there is nothing special in the mechanisms of development and, in particular, nothing corresponding to final causes.

On the other hand, development is much more than a simple sum of cellular behaviours or mechanisms. This also implies that development is much more than the sum of the expression patterns of an arbitrarily long list of genes. Development, even in its simplest forms – those that give rise to the simple multicellular organism so dear to John Tyler Bonner – is the complex *networking* of cellular behaviours and mechanisms influenced by the expression of all these genes.

In particular, it is impossible to understand development if we do not pay enough attention to all those feedback mechanisms whose existence is one of the main conditions explaining the predictability of course and outcome of developmental processes. The very existence of a feedback, however, does not imply the existence of a programme.

All these behaviours, mechanisms and genes are not there *to ensure* the deployment of the wonderfully complex shapes of living beings. Much more modestly, they are simply there and consequently affect other cellular behaviours, mechanisms, or genes and set in place those forms of self-regulation that are the key to avoid developmental bankruptcy.

From this perspective, development is deprived of the mysterious finalistic overtones which have thus far constrained our ability to understand it. On the other hand, development becomes an even more pervasive dimension of biology than we are accustomed to accept. Everything important in the biology of multicellular organisms belongs to development. In Bonner's (1993) words, organisms are not just adults – they are life cycles and life consists of a succession of life cycles. Development is thus a key aspect of the unending continuity of life. We are accustomed to cutting life's thread into generations, but even this periodisation is debatable (Griesemer 1996), especially when we are dealing with haplodiplobiont or agamic organisms.

If we are ready to abandon a finalistic view of development, as the deployment of a programme inscribed in an egg's nuclear genes, we should be also ready to accept Berrill's (1961) view (see also Goodwin 2000) that the simplest and more direct type of development is to be found in the meristematic development of buds or in units of colonial organisms rather than in the eggs with their highly specialised mechanisms of embryogenesis. The *Hydra*, in this sense, is a sort of permanent embryo (Lohmann and Bosch 2000), because even adult polyps have a striking capacity to regenerate, suggesting that molecular mechanisms underlying pattern formation are permanently active and self-regulatory. In terms of phylogeny, the *Hydra* is not basal within the Hydrozoa, or the Cnidaria generally, but this polyp may well work as a model of a primitive metazoan condition, in which morphogenetic potentials were still diffuse within the multicellular assembly, rather than reduced and restricted, as in modern animals generally. A good indicator of this primitive condition in the *Hydra* is its permanent availability to axis formation.

In so far as its cytoplasm preserves the heavy imprint of maternal gene transcription, the egg is more constrained, in terms of morphogenesis,

than a naive cell could be. But this naivety is not a consequence of being, in terms of gene expression, the equivalent of a *tabularasa*. On the contrary, we should expect the transcriptome of an average hydra cell to be very rich and less biased toward some transcripts than may be an egg, under the belated effect of maternal gene transcription.

An argument in favour of this view of development is the presence of organisms (admittedly, not metazoans) which do not have a 'basic', or 'default' morphology. An example is *Candida albicans*, which can switch among forms so diverse as single budding cells, multicellular threadlike hyphae and strings of yeastlike cells plus long septate filaments, known as pseudohyphae (Braun and Johnson 1997, Ishii et al. 1997, Magee 1997). The pervasive character of plasticity and polymorphism suggested to Newman and Müller (2000) that the correspondence of a genotype to one morphological phenotype, as typically seen in higher animals, should be considered exceptional. In other terms, this tight correspondence is a highly derived condition in which an overdetermining genetic circuitry filters out or buffers the impact of extrinsic or intrinsic variables on the organism's morphology. In Newman and Müller's view, the beginning of multicellular era on our planet was a 'pre-Mendelian' world, in which the connection between genotypes and morphological phenotypes was very loose; that is, any given genotype would have mapped onto many phenotypes. A closer linkage between genetic change and phenotypic change would have emerged later, with the evolution of what may now appear as genetic redundancy (but see page 231) and other mechanisms supporting reliability of developmental outcome.

The non-adultocentric notion of development I am advocating here is perfectly compatible with most current concepts of both developmental and evolutionary biology – for example, with the concept of the developmental module (see page 234), a local cell population with its own developmental dynamics, but also interacting with the other modules in a kind of metapopulation of cells (the biological individual or colony).

Moreover, it gives better sense to phenomena, such as dissogony and paedogenesis. Dissogony is a peculiarity of some comb-jellies (Ctenophora) that reproduce twice in their life, the first time at a very early developmental stage, the second when they have reached the conventional adult stage. Paedogenesis, known from several arthropods and flatworms, means the production of mature eggs when the animal is still in a stage comparable with the larva, or juvenile, of its closest relatives.

A finalistic, adultocentric view of development requires every stage to be compatible with the following ones. The alternative view defended here seems more sober, in that it simply requires every stage to be compatible with the previous one. Natural selection will then select and stabilise developmental sequences compatible with the continuity of life.

Developmental Competition between Body Parts

If development is simply the network of dynamics going on in multicellular systems, there is no reason to regard development as a global property of an organism as such. Cells and multicellular units within it are equally involved in these dynamics and will be expected to compete with other units for access to metabolic or informational resources. Wagner's (1996) concept of the developmental module (see page 234) comes close to this idea, as do Buss's (1987) theory of the evolution of individuality or Edelman's (1987) model of neural Darwinism. The fractal geometry of many biological structures (so widespread among trees, inflorescences, corals and branching systems of vessels and tracheae) also speaks in favour of a multicentric view of development.

Apoptosis, in its many manifestations, is also an expression of this differential success of different cell lineages within a developing organism. During the ontogeny of the hermaphrodite individuals of *Caenorhabditis elegans*, 131 of the 1,090 somatic cells normally die by apoptosis, and more than 80% of the ganglion cells in the cat retina die shortly after they are born. In the latter case, differential cell survival depends on competition for limiting amounts of neurotrophic factors secreted by the target cells these ganglion cells 'try' to innervate (Meier, Finch, and Evan 2000). Martin Raff suggested that cell death is the default fate of all metazoan cells. (This would be the same as saying that the lemming voles of the Arctic are programmed to suicide.) Survival would be obtained through the sustained supply of environmental survival signals, including soluble cytokines and hormones, synaptic connections, and direct physical interactions with heterotypic cell neighbours and extracellular matrix (Raff 1992, Raff et al. 1993, Raff, Durand, and Gao 1998, Meier et al. 2000). I do not underrate the importance of these data, but Raff's interpretation is, in my view, one more expression of an adultocentric view of development. I would describe these in more plain terms of Darwinian competition, as Moreno, Basler, and Morata (2002) also do. Every cell simply does all

it is able to do, given its history, its metabolic state, and the influences it receives from outside. Before choosing as prototype of metazoan cells those that die from apoptosis, one should pay attention to the extraordinary potential of individual blastomeres [e.g., in frogs (Spemann 1938) and sea urchins (Driesch 1892)] that are capable of generating a fully formed embryo if isolated during an early cleavage stage.

Competition between broadly equivalent cells may be instrumental in refining early embryonic patterns, as in the case of invertebrate synapses known to change during development through competition between axons (Lnenicka and Murphey 1989).

Competition at the cell level may translate into visible effects of competition between organs (cf. Rensch 1959). In tetrapod vertebrates, there is a fairly consistent inverse relationship between limb reduction and vertebral elongation or, as in the Palaeozoic lepospondyls, an increased number of vertebrae (Carroll 1999). According to Gluesenkamp (1997), limb reduction in lizards is possibly determined by spatial constraints due to vertebral elongation, causing a decrease in the contribution of somites to the limb anlagen.

In scarab beetles, the production of horns reduces the size of neighbouring body parts: antennae, eyes, or wings, depending on the cephalic or thoracic location of the horns (Emlen 2001). Nijhout and Wheeler (1996) have remarked on the unique conditions under which adult structures grow in holometabolous insects. The metamorphosing insect does not feed during the pupal stage. Therefore, at variance with the large majority of growing systems, the imaginal structures grow within a virtually closed system in which, by consequence, body parts are in direct and strict competition for metabolic resources (Roth and Mercer 2000). As noted by Nijhout and Emlen (1998), this is an old notion, familiar to both Darwin and Geoffroy Saint-Hilaire, but it is difficult to demonstrate by experiments. Smith and French (1991), however, obtained relevant results experimenting with the flesh fly *Sarcophaga*. By destroying selected histoblast nests (groups of cells from which a part of an adult segment forms during metamorphosis), they obtained the corresponding deletion of adult structures accompanied by enlargement of adjacent structures within the same segment and in neighbouring segments (Smith and French 1991). Nijhout and Emlen (1998) studied organ competition in two different insects. The butterfly *Precis coenia* was one of them. Nijhout and Emlen removed one or two hind wing imaginal discs from several larvae of this species at the beginning of the final larval instar. After metamorphosis, the relative size of

the adult fore wings showed a compensatory response proportional to the number of hind wing discs removed. Comparable results were obtained by hormonal manipulation of male scarab beetles of the genus *Onthophagus*, in which a reduction in the size of the cephalic horns was accompanied by an increase in the size of the eyes. A spin-off of these studies is the suggestion (Klingenberg and Nijhout 1998) that fluctuating asymmetry may be controlled by competition among growing organs from a limiting resource.

Genes with specific effects on the control of cell competition are known. In *Drosophila*, the *warts* gene is required for cell proliferation to occur in the correct amount and direction, thus allowing a normal course of morphogenesis. Absence of its normal expression leads to the formation of fragmented and overgrown cell clones with hypertrophy of the epithelial cells in the imaginal discs (Justice et al. 1995).

Developmental biology has traditionally emphasised integration and regulation to such an extent that the 'default' independent activity of multiple local foci of growth and differentiation has been often overlooked. This emphasis on the holistic aspects of development is a characteristic expression of the current adultocentric views. However, even in those animals whose development appears to be more sophisticated and subject to a complex network of regulatory interactions, there is still a large scope for local autonomy, possibly culminating in competition between cells or cell lineages. Local autonomy is even compatible with syncytial organisation, in which one would not expect the slightest degree of compartmentalisation to occur. Brentrup and Wolf (1993) experimented on eggs of different developmental stages of the hymenopteran *Pimpla turionella* fused in parabiocotic tandem. The interactions between the two partners were limited to the exchange of a few nuclei, but each of them followed its own temporal schedule of development, although all their nuclei were still contained in a single syncytium.

The Robustness of Morphogenesis

Goodwin, Kauffman and Murray (1993) asked: is morphogenesis an intrinsically robust process? Robust means that it would not be disrupted by temporary disturbances of reasonably modest intensity. Goodwin et al. suggested that some dynamic principles arising from a coupling of different developmental mechanisms (molecular synthesis, gene activation, spatial patterning of substances, cell interactions, cell sorting, and morphogenetic movements) result in significant reduction in the degrees of

freedom available to the whole developmental system. As a consequence, morphogenesis is intrinsically robust.

The amount of external disturbance a developing system may tolerate is often larger than the development of *Drosophila*, *Caenorhabditis* or *Xenopus* would suggest. Think of what cell sorting may achieve in a reaggregating mass of dissociated cells.

Robustness of development may depend on the number of developmental processes going on concurrently in the same system. Goodwin et al. (1993) imagined a developmental system, in which a cell sorting mechanism based on differential cohesion and surface adhesion forces (cf. Steinberg 1970), is coupled to a patterning process based on a Turing mechanism (cf. Turing 1952). In this system, two different cell types, generated as a consequence of the operating Turing mechanism, would start sorting out according to their surface properties. They would thus change position, and in these displacements they would carry with them the morphogen concentrations on which the Turing process depends. Coupling of the two processes will eventually determine the production of a stable form. Generalizing from this example, Goodwin et al. (1993) stated that the plurality of developmental mechanisms acting concurrently in developmental systems could explain the observed robustness of the latter, despite opposite predictions from a consideration of their structural complexity. This would be true, in particular, for the robustness of the so-called phylotypic stage (cf. page 123), a point also made by Galis (1999).

Azevedo and Leroi (2001) have recently criticized the current deterministic trend prevailing in developmental biology, in which due attention is not paid to the considerable level of stochasticity that has been demonstrated in most cellular properties, including gene expression patterns, mitotic rates, and migration routes. It is important to realize that development is much more flexible, *at the individual level*, than textbook schemes usually suggest. More interestingly, this flexibility is not just a property of advanced or terminal developmental stages, but is also widespread in the earliest ones. It is the sheer morphological simplicity of early developmental stages that limits our chances of spotting this variability. Modern technical tools, however, can provide the support we need. With the aid of a 4D-microscope system (multifocal, time-lapse video recording system), Schnabel et al. (1997) revealed, in the normal embryogenesis of *Caenorhabditis elegans*, variability in cell division timing, cell positioning, and cell-cell contacts not seen previously with more traditional techniques. In their analysis of the distributions of the descendants of the early founder blastomeres at

the premorphogenetic stage, they demonstrated that founder blastomeres establish discrete regions in the embryo through a considerable amount of cell movements, with different patterns in different embryos. Cell fate assignment is nevertheless conserved; This is not due to an autonomous invariant specification of cell fates, but to cell–cell interactions occurring at very early stages when the topology of blastomeres in the embryo is sufficiently precise, thus ensuing reproducible patterns of induction. Apparently, the role of cell lineage, despite its strict reproducibility, is not really responsible, per se, for subsequent cell fate. If so, the embryonic development of *C. elegans* would follow the same basic principles seen in the embryos of other animals, in which body regions are more obviously established by cell–cell interactions (Gurdon 1992, Schnabel et al. 1997). Comparative evidence from other nematodes, on the other hand, demonstrates that there has been exaggeration in the traditional view of a precise cell lineage as a universal attribute of nematode development (Voronov and Panchin 1998).

It has been shown recently that the robustness of a developmental system may have something to do with the peculiar topology of the network of interactions existing between cells or other subsystems within the developing organism. Interestingly, robustness is a characteristic of the so-called scale-free networks (other examples being social networks or the Internet), a class of networks with inhomogeneous distribution of wiring. These networks are very sensitive to selected attacks on a limited number of key nodes, but otherwise robust in front of even high degrees of failure at all remaining nodes in the network (Albert, Jeong, and Barabási 2000), which therefore demonstrate their considerable degree of autonomy from the rest of the network.