

# Four decades of teaching developmental biology in Germany

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**ABSTRACT** I have taught developmental biology in Essen for 30 years. Since my department is named Zoophysiologie (Zoophysiology), besides Developmental Biology, I also have to teach General Animal Physiology. This explains why the time for teaching developmental biology is restricted to a lecture course, a laboratory course and several seminar courses. However, I also try to demonstrate in the lecture courses on General Physiology the close relationship between developmental biology, physiology, morphology, anatomy, teratology, carcinogenesis, evolution and ecology (importance of environmental factors on embryogenesis). Students are informed that developmental biology is a core discipline of biology. In the last decade, knowledge about molecular mechanisms in different organisms has exponentially increased. The students are trained to understand the close relationship between conserved gene structure, gene function and signaling pathways, in addition to or as an extension of, classical concepts. Public reports about the human genome project and stem cell research (especially therapeutic and reproductive cloning) have shown that developmental biology, both in traditional view and at the molecular level, is essential for the understanding of these complex topics and for serious and non-emotional debate.

**KEY WORDS:** *Evo Devo, Eco Devo, human, genome project, signaling, mesoderm, neural induction, stem cell, cloning*

## Background Information

### Scholarly Interests of Author

The amphibian embryo is one of the best studied organism in developmental biology both in respect to classical embryology and molecular genetics (see reviews Grunz, 1996b, 1997, 1999b, 2001). Therefore, I use the amphibian embryo as a model organism to explain many basic mechanisms of developmental biology, including molecular principles. Since I have used both urodeles (*Triturus*, Axolotl) and *Xenopus* embryos in this research, the theoretical aspects as shown in textbooks can be supplemented by personal experimental experiences. Special research interests include especially gene activation and regulation during mesodermal and neural induction. I received my first training in traditional developmental biology in Cologne. As a postdoctoral fellow in the lab of Heinz Tiedemann at the Institute of Biochemistry and Molecular Biology, Free University of Berlin, and in the lab of Tuneo Yamada, Oak Ridge National Laboratory, Tennessee, I, step by step, added biochemical approaches to my studies, especially regarding the function of the vegetalizing (activin-like) factor. After sabbaticals, in Igor Dawid's lab in 1987 and recently in Eddy De Robertis's lab in 1997/1998, I introduced molecular research and teaching into the laboratory and the classroom.

### Representative Publications

The list of citations below includes representative publications and a videotape, prepared by myself in cooperation with the media center of the university, which can be used as background information and as the basis for laboratory and seminar courses:

**Videotape on Amphibian Embryogenesis and the Main Classical Experiments.** The tape (PAL, NTSC or SECAM format) contains time-lapse sequences of normogenesis of *Triturus alpestris* and *in vivo* and *in vitro* experiments. Short sequences are shown on my home page ([www.uni-essen.de/zoophysiologie](http://www.uni-essen.de/zoophysiologie)).

The topics are listed here.

1. Breeding activities of the four German (European) newts (*Triturus vulgaris* [taeniatus], *T. alpestris*, *T. palmatus* [helveticus] and *T. cristatus*).
2. Normogenesis of *T. alpestris* (cleavage, gastrulation, neurulation, larval development).
3. Blood circulation in gills and tail.
4. Spemann-Mangold Einsteck experiment.
5. Sandwich experiment after Holtfreter (animal cap assay: test of the first highly purified inducing factor, now known as activin - shown are time-lapse sequences of the elongation of mesodermalized ectoderm).

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6. Disaggregation and reaggregation of ectoderm (basic experiment for the detection of the importance of BMP-4 (Grunz and Tacke, 1989, 1990; Wilson and Hemmati-Brivanlou, 1995).
7. Time-lapse sequences of reaggregating cells (mutual cell affinity).

#### Papers

- GRUNZ, H. (1996a). The long road to chemical and molecular embryology. What amphibians can teach us on differentiation. An interview with Professor Heinz Tiedemann. *Int. J. Dev. Biol.* 40: 113-122 (special issue: Developmental Biology in Germany).
- GRUNZ, H. (1996b). Factors responsible for the establishment of the body plan in amphibian embryos. *Int. J. Dev. Biol.* 40: 279-289 (special issue: Developmental Biology in Germany).
- GRUNZ, H. (1997). Neural Induction in amphibians. *Curr. Top. Dev. Biol.* (Eds. Roger A. Pederson and Gerald P. Schatten), 35: 191-228 Academic Press. San Diego.
- GRUNZ, H. (1999a). Amphibian embryos as a model system for organ engineering: in vitro induction and rescue of the heart anlage. *Int. J. Dev. Biol.* 43: 361-364.
- GRUNZ, H. (1999b). Gene expression and pattern formation during early embryonic development in amphibians. *J. Biosci.* 24: 515-528.
- GRUNZ, H. (2000). *Entwicklungsbiologie*. Spiral-bound booklet. 140 pages.
- GRUNZ, H. (2001). Developmental Biology of amphibians after Hans Spemann in Germany. *Int. J. Dev. Biol.* 45: 39-50 (special issue: The Spemann-Mangold Organizer)
- TIEDEMANN, H., ASASHIMA, M., GRUNZ, H., KNÖCHEL, W. (2001). Pluripotent cells (stem cells) and their determination and differentiation in early vertebrate embryogenesis. *Dev. Growth Differ.* 43: 469-502

### Developmental Biology Lecture Course

This undergraduate lecture course reviews representative organisms to demonstrate principles of early embryonic development. Fertilization, cleavage, gastrulation and organogenesis in *Drosophila*, sea urchins, amphibians, zebrafish, birds and mammals, including humans, are covered. Special attention is focused on new molecular genetic aspects of early embryogenesis, especially gene structure and function in normogenesis, and including the close relationship of developmental biology to evolution, teratogenesis, carcinogenesis, and the influence of environmental factors on ontogenesis. Since the human genome project and stem-cell research received much attention, a substantial part of the lecture focuses on the advantages and risks of therapeutic and reproductive cloning. Students are advised to include in their study a spiral-bound brochure prepared by myself, which contains most of the topics of the lecture. In addition, textbooks of developmental biology and cell biology are recommended by the instructor. A videotape with the title "Amphibian embryogenesis and the main classical experiments" is shown during the lecture course. I prepared this videotape, including personal preparations and time-lapse sequences, myself. Questions during the lecture are welcome to encourage feedback from the students to the instructor.

#### Author's Goals in teaching Undergraduates

Since in our university many students focus their interest on fields of biology other than developmental biology, it is important to inform the students about the central and connecting role of developmental biology in the field of biology. Molecular genetic techniques have improved our knowledge about the exact mechanism of embryonic development. Sometimes, unexpected improvements to or extensions of traditional biological concepts can be observed, especially correlations between developmental biology and evolution (for example, the genes *hox* and *pax 6* are

involved in axis formation, eye development or the bilateralia-hypothesis in nearly all phyla of the animal kingdom). Furthermore, the students are taught about how environmental factors affect early embryonic development (Ecological Developmental Biology). I always try to show the close relationship between basic and applied research in developmental biology. The student should understand the need for basic research in model organisms, which form the basis for understanding complex developmental biological processes, especially in humans. It is easy to explain, for example, why knock-out experiments will never be performed in humans because of ethical considerations. On the other hand, such experiments in model organisms are excellent tools to understand mechanisms that are evolutionarily conserved in humans.

#### General Features of the Lecture

About 50-60 students of junior (3rd-year) level participate in this lecture. A prerequisite for this lecture is previous enrollment in a general zoology lecture course and a zoological microscope course. They should also have participated in lectures of general and organic chemistry and general genetics. The lecture runs for a total of 12 weeks in the summer semester for 90 minutes each week. Participants are future schoolteachers of the so-called *Sekundarstufe II* (high school level up until the final examination). The students are encouraged to participate in a separate laboratory-based developmental biology course (see description below). This course mainly deals with developmental biology in amphibians and hydra.

#### Abbreviated Lecture Content

##### A. General Principles in Developmental Biology

- I. Historical background of developmental biology
  - a. Descriptive Embryology (Aristotle, Wolff, Cuvier, DeHillaire, von Baer, Haeckel, Boveri, etc.)
  - b. Mechanistic Embryology (Driesch, Spemann etc.)
  - c. Biochemical Embryology (Brachet, Yamada, Tiedemann)
  - d. Molecular (genetic) Embryology and Developmental Biology
- II. Early embryonic development
  - a. Oocytes ("Eggs") and sperm of different species (yolk content and cleavage type of different species)
  - b. Fertilization
  - c. Cleavage and prelocalization of factors important for animal/vegetal and dorsal/ventral polarity
  - d. Gastrulation (formation of germ layers) and neurulation, organogenesis
  - e. Metamorphosis and regeneration (neoteny, direct development, metaplasia)
- III. Principles of Embryonic Development
  - a. Embryonic induction, including new concepts on the molecular level
  - b. Classical experiments
  - b. Evolutionarily conserved genes
  - c. Axis formation and eye development
  - d. Stem cells, totipotent cells, pluripotent cells, advantages and limitations of reproductive and therapeutic cloning
  - e. Carcinogenesis

##### B. Special Features of Embryogenesis in Different Selected Model Organisms (Description of Normogenesis)

- I. *Drosophila*

- II. Sea urchins
- III. Amphibians
- IV. Birds
- V. Mammals, including humans

### Textbooks for Assigned Readings

GRUNZ, H.: *Developmental Biology*. Spiral-bound booklet  
 WOLPERT, LEWIS: *Developmental Biology*, German version: *Entwicklungsbiologie*  
 GILBERT, S.R. (2000). *Developmental Biology*. Sinauer, Sunderland, MA

### Visual Aids

Videotape: GRUNZ, H.: Early Development of Amphibians and the Main Classical Experiments. 1997. (Short sequences for preliminary information only are also on my home page: <http://www.uni-essen.de/zoophysiologye>)

### Examination

The lecture is, together with information in the textbooks mentioned above, the basis for the first examination (the so-called *Zwischenprüfung*). The format of the questions is multiple choice.

### The Developmental Biology Laboratory Course

This course, which uses live material, for future school teachers and students of ecology (up to 15 students) contains two main parts:

- A. *Hydra* – Mechanism of regeneration (5 half days)
- B. *Xenopus laevis* – early development and selected classical experiments (5 half days)

The instructor of the hydra part (5 days) is Dr. Lothar Tacke, a senior lecturer in my department. I teach the *Xenopus* part with the assistance of two Ph.D. candidates and a graduate student. More details about the *Xenopus* part are described below:

Since the time is limited (5 days), I concentrate on 5 central topics (*all experiments, including glass-needle preparation, are shown in my videotape*):

**Part 1.** Introduction, viewing of the videotape (mentioned above), and general discussion, followed by training in the preparation of Spemann glass needles

**Part 2.** Disaggregation and reaggregation-experiments  
 concepts: biological membranes, cell adhesion and mutual cell affinity, importance of BMP-4 as an antagonist to dorsally expressed genes)

**Part 3.** Eye induction and development  
 concepts: (*pax-6*, master control genes, evolutionarily conserved genes, homology, analogy, convergence)

**Part 4.** Sandwich experiment (animal cap assay)  
 concepts: test of inducing factors, dominant negative receptors and ligands etc.

**Part 5.** Einsteck experiment of Hans Spemann and Hilde Mangold  
 concepts: axis formation, embryonic induction, temporal and spatial gene activation and regulation.

Every day starts with a 90-minute lecture. I selected these 5 topics, since they cover a wide spectrum of developmental biology theory. The 5 experimental topics represent well-known techniques and important classical experiments, which form the basis of modern molecular approaches and knowledge.

We start with the technically easiest experiment (disaggregation of animal cap cells; day 1) and end with the most demanding

one (Einsteck Experiment; day 5). I always demonstrate each experiment step by step under a stereomicroscope equipped with a videocamera connected to a large monitor.

### Part 1. Videotape about Early Amphibian Development and Glass-Needle Preparation

First, I show my videotape “Early development of amphibians and the main classical experiments” (40 min). It contains time-lapse material about early cleavage, gastrulation, neurulation and early development up until the swimming larva stage of newts and the main classical experiments to be used in the laboratory course parts 2-5. Furthermore, Spemann glass-needle preparation is shown. The technique is documented in detail also in my videotape “Amphibian development and the main classical experiments,” available in PAL, NTSC, and SECAM standard video/television formats (see also the few sequences on my Web site (<http://www.uni-essen.de/zoophysiologye>)).

During part 1, the students first learn to prepare glass needles, which are used throughout the course. This technique was introduced by Hans Spemann. The very flexible needles with extremely fine tips are superior to other instruments such as a single eyelash fixed with wax to a glass handle or a tungsten needle. In Cologne, I used platinum needles – fine platinum wire fixed within a glass capillary rod. During my postdoc period in Prof. Tiedemann’s lab, his wife, Dr. Hildegard Tiedemann, showed me the preparation of Spemann glass needles. Dr. Hildegard Tiedemann was a research assistant of Otto Mangold in Freiburg, who himself was a colleague of Hans Spemann. That means I myself am a member of the “glass-needles lineage.” Although I did all experiments for my thesis in Cologne with fine platinum needles, I have found that the glass needle is the better tool, since even tiny pieces of the embryo can be isolated without large cell damage. By the way, during my sabbaticals in Igor Dawid’s lab at the U.S. National Institutes of Health (NIH), in Eddy De Robertis’s lab (UCLA), in the Shanghai Institute of Cell Biology, and during a visit to Yosiki Sasai’s lab in Kyoto, Japan, I was encouraged to teach Ph.D. candidates and postdoctoral fellows the preparation of Spemann glass needles and their advantages for isolating tiny pieces of embryonic tissue with high precision.

### Part 2. Disaggregation and Reaggregation Experiments

In the videotape, the disaggregation technique and other classical embryonic techniques are also demonstrated. This technique was the basis for our observation of the neuralization of ectoderm without an inducer, which later led to the detection of the central role of BMP-4 as an antagonist of Chordin, Cerberus, etc.) (Grunz and Tacke, 1989, 1990; Wilson and Hemmati-Brivanlou, 1995). The removal of the vitelline membrane by sharpened watchmakers forceps without severe disruption of the embryo is an important step, which the students must learn for this and subsequent parts of the course.

Pieces of the animal cap are dissected and disaggregated in  $\text{Ca}^{++}$ - and  $\text{Mg}^{++}$ -free Barth culture solution. After placing single cells in Barth solution with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  on microscope slides, the students can observe under the microscope that these vertebrate cells, which through evolution have become highly specialized, still behave like amoebas. Thus, this experiment is an excellent tool to explain the central role of the cytoskeleton, cell migration, cell adhesion and mutual cell affinity. Furthermore, the students learn

about the importance of the structure and function of biological membranes and receptors. Detailed information about receptors, dominant negative receptors and ligands was already included in my lecture during the spring/summer period.

In my 90-minute introduction to the course, the students have already learned about the role of cadherin and catenin and the importance of the BMP-4 for the stabilization of epidermal determination.

The disaggregation and the delayed reaggregation (the re-aggregation is not performed in the course) of animal cap cells results in the neuralization of the ectodermal cells, as we have shown earlier (Grunz and Tacke 1989, 1990). These observations were the basis for the discovery of BMP-4 as an antagonist to genes and their products expressed on the future dorsal side of the embryo. These topics will be further explained in parts 3 and 4 of the course.

### Part 3. Eye Development

The task of this part of the course is the isolation of the presumptive eye anlage. In the early neurula (stage 13), one side of the anterior neural plate will be isolated. Under optimal conditions, the developing larvae will form one eye on the nonoperated side only.

The experiment is an excellent tool for teaching the relationship between historical (traditional) developmental biology and new approaches in molecular biology. In my Development Biology lecture course, as well in the introduction of this course, I discuss the following topics related to eye development:

1. The classical transplantation experiment of the eye-anlagen performed by Hans Spemann in 1912 (Spemann, 1912), including his hypothesis of "double assurance." Today, we know that the development of a free lens formed without an eye cup is possible (Brahma and Grunz, 1988). These newer data can be explained by the fact that already prior to the histotypic cap formation, signals are transmitted from the eye cup anlage (forebrain area) to the presumptive lens field (Hollemann *et al.*, 1998a).

2. Homologous genes (*pax 6*) responsible for eye development are found in all animals that have developed eyes (Halder *et al.*, 1995 a, 1995b). If the gene (a master control gene) is deleted, no eye will be formed (*eyeless* in *Drosophila* or *aniridia* in mammals).

The students will learn how these new molecular data of developmental biology have extended our knowledge about the mechanism of evolution. As an important example of so-called convergence, the textbooks compare the squid eye (Cephalopoda, an invertebrate) with the vertebrate eye, including those of mammalian animals and humans (Tomarev *et al.*, 1997). In the classical view, the squid eye and the vertebrate eye were considered to be examples of analog development. In contrast to the earlier assumption that the about 40 different eye types in the animal kingdom evolved independently of one another, it can now be postulated with high probability on the basis of molecular data that the development of every eye starts with closely related master control genes of the *pax*-family, which are followed by cascades of further genes (in *Drosophila*, about 2000) leading to the final (histotypic) species-specific eye structures (Halder *et al.*, 1995b).

### Part 4. Sandwich Experiment (Animal Cap Assay)

The students learn that the sandwich technique (after Holtfreter; two animal caps of blastula or gastrula stage) and the animal cap

assay are powerful tools for testing inducing factors, dominant negative ligands, receptors, etc. The technique is described in many textbooks and in many original papers. Briefly, during the course, animal caps will be isolated, and a small piece of the organizer zone of early gastrulae will be wrapped by two animal caps. The ectoderm will be neuralized and will form notochord, muscles and brain structures. Alternatively, a single animal cap can be treated with activin, which results in the differentiation of different mesodermal derivatives depending on the concentration of this growth factor. The animal cap assay (single animal cap) is a very powerful test system in conjunction with RT-PCR for the study of the effect of dominant negative receptors, truncated ligands, inhibitors, etc. The sandwich technique is demonstrated in my videotape. The induction effect (elongation of the ectoderm) by the vegetalizing factor (activin) is shown using the time-lapse technique. These time-lapse sequences were already documented in 1970.

### Part 5. Organizer (Einsteck) Experiment, after Hans Spemann and Hilde Mangold

This classical experiment is mentioned in every textbook of developmental biology. As is well known, Spemann earned for this experiment the Nobel Prize for Physiology or Medicine in 1935 (Spemann and Mangold, 1924). During the course, the students are encouraged to implant a small piece of the organizer area from a donor embryo into the blastocoel of a host embryo (early gastrula). Since the experiment is very difficult for the beginner, very seldom are embryos with a complete second axis produced. More frequently, induced protrusions on the ventral side contained fragmentary brain structures and/or somites and notochord. In the theoretical part of the introduction, I inform the students that over the last decade, the molecular nature of the Spemann-Mangold organizer has been studied in detail. Many genes and their products, including secreted inducing factors such as Chordin, noggin, follistatin, cerberus and dickkopf, have been detected and their functions have become fairly well known. Detailed information about this research field can be found in the special issue of this journal, "Spemann and Mangold Organizer," Vol. 45, 2001. The Einsteck experiment is also demonstrated in my videotape.

### Seminar Courses

Three different seminar courses that deal with developmental biology are offered during the winter and summer terms.

A. Evolutionary Developmental Biology (Evo Devo) \*

B. Ecological Developmental Biology (Eco Devo) \*

\*) *These terms with examples have already been used in the latest edition of the textbook by Scott Gilbert. Several examples of these relatively new topics of developmental biology are described there in more detail.*

C. Development, sexuality, reproduction and physiological adaptations of animals under extreme ecological conditions

In these seminar courses (90 min each), two of the students (usually 15–20 students are enrolled) must give a presentation of about 20-30 min on selected review articles and original papers. There are 12-14 such courses per semester. Each student must write an 8- to 15-page paper about the topic of his or her oral presentation.

### A. Evolutionary Developmental Biology

The seminar course may include, for example, a presentation on original papers about eye development (*pax-6* gene), the formation of the dorsal/ventral and anterior/posterior axes (urbilateralia hypothesis; importance of Chordin/Sog – BMP-4/dpp), or on articles about the role of conserved genes encoding transcription factors and homeobox genes in *Drosophila*, nematodes, amphioxus, amphibians, zebrafish and mammals, including humans.

#### Some Typical Examples of Articles used as Topics

- AMORS, A. and 12 others. (1998). Zebrafish Hox clusters and vertebrate genome evolution. *Science* 282: 1711-1714.
- AVEROF, M. and AKAM, M. (1995). Hox genes and the diversification of insect and crustacean body plan. *Nature* 376: 420-423
- CALDWELL, M.W. and LEE, M.S.Y. (1997). A snake with legs from the marine Cretaceous of the Middle East. *Nature* 386: 705-709.
- DEL PINO, E.M. (1989). Marsupial frogs. *Scientific American* 260(5): 110-118.
- CARROLL, S.B., WEATHERBEE, S.D. AND LANGELAND, J.A. (1995). Homeotic genes and the regulation and evolution of insect wing number. *Nature* 375: 58-61.
- GILBERT, S.F., OPITZ, J.M. and RAFF, R.A. (1996). Resynthesizing evolutionary and developmental biology. *Dev. Biol.* 173: 357-372.
- GILBERT, S.F. (1998). Conceptual breakthroughs in developmental biology. *J. Biosci.* 23: 169-176.
- GEHRING, W.J. (1998). *Master Control Genes in Development and Evolution: The Homeobox Story*. Yale University Press, New Haven.
- COHN, M.J. and TICKLE, C. (1999). Developmental basis of limblessness and axial patterning in snakes. *Nature* 399: 474-479.
- ERWIN, D.H. (1999). The origin of bodyplans. *Am. Zool.* 39: 617-629.
- GREER, J.M., PUET, J., THOMAS, K.R. and CAPECCI, M. (2000). Maintenance of functional equivalence during paralogous *Hox* gene evolution. *Nature* 403: 661-664.
- MARQUÉS, G., MUSACCHIO, M., SHIMELL, M.J., WUNNENBURG-STAPLETON, K., CHO, K.W.Y. and O'CONNOR, M.B. (1997). The Dpp activity gradient in the early *Drosophila* embryo is established through the opposing actions of the Sog and Tld proteins. *Cell* 91: 417-425.
- PANGANIBAN, G. and 13 others. 1997. The origin and evolution of animal appendages. *Proc. Natl. Acad. Sci. USA* 94: 5162-5166.
- PENNISI, E. (2000). An integrative science finds a home. *Science* 287: 570-572.
- SHUBIN, N., TABIN, C. and CARROLL, S. (1997). Fossils, genes, and the evolution of animal limbs. *Nature* 388: 639-648.
- PICCOLO, S., AGIUS, E., LU, B., GOODMAN, S., DALE L. and DE ROBERTIS, E.M. (1997). Cleavage of chordin by the Xolloid metalloprotease suggests a role for proteolytic processing in the regulation of Spemann organizer activity. *Cell* 91: 407-416.
- DEROBERTIS, E.M. (1997). Evolutionary biology - The ancestry of segmentation. *Nature* 387: 25-26.D 280

### B. Ecological Developmental Biology (EcoDevo)

In this seminar course, the students will discuss articles about the importance of environmental factors on the genome and on early embryonic development. Examples are the influence of increased amounts of UV on amphibian populations and introduced estrogens in lakes, which may cause a sex shift in crocodiles. The aim of the course is to enable the students to recognize the close relationship between environmental factors (pesticides, hormones, etc.) and *early* embryonic development. This topic is just now (June 2002) a central topic in Germany, because the contamination of "eco" vegetables by pesticides (such as Nitrofen) and, in turn, of chicken eggs and meat (pork) has been detected. Especially the early embryo, in contrast to the adult organism, of all

species is extremely sensitive to nonoptimal or hazardous environmental conditions. So the future biologist must know that even an apparently specific inhibitor of the development of one species might be harmful for others. An excellent example is the selective inhibition of the development of the mosquito by juvenile hormone (the larvae never reach metamorphosis) in some lakes in the United States. The hormone is eventually converted by UV to homologues of retinoic acid, which causes teratogenesis in frogs (reduction or loss of eye and brain area or development of four hind legs). The dramatic effects of retinoic acid on embryogenesis, and, therefore, its environmental impact, are well documented (Durston *et al.*, 1989; Hollemann *et al.*, 1998b).

#### Some Examples of Typical Topics

Temperature and sex determination in reptiles  
Influence of estrogens from different sources on the fertility of animals (alligators, polar bears, etc.)  
Increased UV-B levels - influence on frog populations  
Predator-induced phenotypes  
Pesticides – chemical homology to juvenile hormones and retinoic acid

#### Literature

- 1) GILBERT, S.R. (2000). *Developmental Biology*, 6th Edn, Sinauer, Sunderland, MA
- 2) Search for new articles on the Internet

### C. Development, Sexuality, Reproduction and Physiological Adaptations to Extreme Environmental Conditions

I got the idea for the topic of this seminar course after my two Galapagos journeys, one in the "El Niño" year 1998 and one in the "normal" year 2000. Several species on the Galapagos archipelago are adapted to extreme environmental conditions. Good examples are the marine iguana, the flightless cormorant, the Galapagos penguin, the giant tortoises, and snakes, scorpions and of course the Darwin finches. In the more recent seminar courses (years 2001 and 2002), I extended the topics to other extreme ecological environments outside of the Galapagos. So the students read articles about adaptations to high altitude deserts, the polar region, the deep sea or the dark. An important part of the talk should include reproduction and early development of animals under these extreme environmental conditions. These topics are excellent for the demonstration of the close relationship between developmental biology (for example the rudimentary eye-anlage of the cave-fish in Mexico), special physiological adaptations (salt glands of Galapagos iguanas), and evolution (survival under extreme ecological conditions). Of special interest are special adaptations during embryogenesis, e.g., neoteny (axolotl), shortened development (tropical tree frogs – *Eleutherodactylus coqui*) and frogs with egg pouches (Gastrotheca). Also in this seminar, I try to point out the importance of the new molecular techniques for the study of the close relationship between developmental biology, evolution and the environment.

#### Some Examples of Typical Topics

Adaptation of the avian egg to high altitude  
Adaptation to altitude - hypoxia in vertebrates  
Adaptation to altitude - blood oxygen affinity in high- and low-altitude populations of the deer mouse  
Adaptation to the desert - kangaroo rat

Adaptation to the desert - desert ant  
 Adaptation to the desert - camel  
 Adaptation to the desert - thermal tolerance of desert pupfish  
 Adaptation to the desert - *Oryz gazella*  
 Adaptation to a marine environment without freshwater - marine iguanas, gulls (salt glands)  
 Adaptation to the dark - cave fishes, bats  
 Adaptation to the polar region - polar fish

#### Textbooks

ECKERT, R. (1997). *Animal Physiology*. W. H. Freeman and Company. New York.  
 SCHMIDT-NIELSEN, K. (1997). *Animal Physiology: Adaptation and Environment*. Cambridge University Press German version: Physiologie der Tiere. Spektrum Akademischer Verlag. Heidelberg, Berlin, 1999

### Biographical Information

#### **My Early Years - teaching in Cologne with Amphibian Embryos collected from Local Ponds**

In 1965, I participated as a teaching assistant (Ph.D. candidate) in a course in Developmental Biology for graduate students at the University of Cologne. At that time, developmental biology continued to be a major field at both the Institute of Zoology and the neighboring Institute of Developmental Biology. Since the former director of the Zoological Institute (Prof. Rotmann) was a research assistant of Hans Spemann, a Nobel Prize winner for Physiology/Medicine, a dominant topic was still the early development of the amphibian embryo. My thesis supervisor, Prof. Engländer, and his colleague Prof. Johnen published several important papers about embryonic competence and the role of time as a factor in amphibian embryonic induction. I had the opportunity to assist as junior instructor in their embryology course.

In those years, the lectures and laboratory exercises were focused on the now classical amphibian experiments. Similar to the situation at the institute of Hans Spemann in Freiburg, all contributors to our Developmental Biology course showed a fever-like behavior during spring time. Following our German tradition, all amphibian experiments were performed with newts, mainly with *Triturus vulgaris* (taeniatus) and *T. cristatus*. Recall, the famous organizer experiments (Einstein experiments) of Hans-Spemann and Hilde Mangold were performed with *Triturus cristatus* and *T. vulgaris* (see the special issue of this journal, Vol. 45, "The Spemann-Mangold Organizer"). *Xenopus* embryos were not available at that time. Since the animal half of a *Triturus cristatus* embryo contains nearly no maternal pigment and looks "albino," the donor tissue (derivatives of the dorsal blastopore lip) could be easily distinguished in the host embryo (*Triturus vulgaris*). The cortex of the animal half of *T. vulgaris*, like wild type *Xenopus laevis*, contains significant numbers of maternal pigment granules.

"Newt fever" among the staff began when—during springtime—the first newts could be seen in the shallow ponds in the countryside surrounding Cologne. Then, a small "expedition" of professors and Ph.D. students with special fishing nets and buckets was assembled. It was the ambition of every participant to catch as many newts as possible. Various newt species preferred favorite ponds. *T. vulgaris* and *T. cristatus* like sunny ponds, while *T. alpestris* prefers ponds that are partly shaded and close to or within the forest (Figs. 1 and 2). The different German species are shown in detail on my home page ([www.uni-essen.de/zoophysiologie](http://www.uni-essen.de/zoophysiologie)) and on my videotape.



**Fig. 1. A Ph.D. candidate catching newts in a pond in the countryside in the north of Essen.** Several boys from neighboring villages observed our activities with much interest. Here, in May 1984, we collected *Triturus vulgaris* for our embryological course. This pond, which lacked shadows, was preferred by *T. vulgaris* but not by *T. alpestris*.

For laying eggs, the females and males were placed in a large glass aquarium with bunches of grass. The female lays the eggs very skillfully between the blades of grass, which in nature protects the embryos against predators and intensive sunlight. Every embryologist knows about the negative effect of UV irradiation on early amphibian embryos! I prepared a videotape for teaching Developmental Biology, and it describes, among other things, details of the four German newt species; egg deposition; the staging series up to larvae; the main classical experiments, including the Spemann Einstein experiment; and the animal cap assay. Information about that videotape can be found on my homepage.

In contrast to the clean and sterile laboratory conditions associated with sophisticated experimentation, catching newts is a rather primitive endeavor. Equipped with long fishermen boots, we walk fairly deep into the pond and try to catch the newts between water plants and mud. Meanwhile, the water of the pond becomes muddy and the newts are caught by trial and error.

In our graduate Developmental Biology course, several classical experiments are performed. The newt eggs are especially suitable for explanation and transplantation experiments, since the cells of blastulae, gastrulae and neurulae are not as sticky as those of *Xenopus*. Therefore, portions of the embryonic tissue can easily be extirpated with fine glass needles (Spemann-type glass needles, see details below). Furthermore, *T. vulgaris* eggs are superior to those of all other newt and frog species in that the jelly coat is relatively tough and of oval shape, in contrast to the sticky and round jellycoats of *Xenopus* or other frogs and toads. This oval shape of the jelly coat of *T. vulgaris* was an important factor in the success of the Spemann's constriction experiments. The constriction of newt embryos at the two-cell stage and the experiment of delayed nuclear transfer were both performed by him with *T. vulgaris*. For the partial or complete

separation of the blastomeres at the two-cell stage, a very thin baby's hair was wrapped around the egg in the middle of the elliptical jelly coat with sharpened watchmakers forceps, followed by carefully controlled constriction. In the case of a successful experiment with partial constriction, the resulting embryo has two heads (Spemann, 1936, 1938). It must be pointed out that the preparation of a loop in the baby hair with two forceps under sterile conditions requires a certain skillfulness. Only one or two students out of approximately 20 have been successful in obtaining double-headed larvae.

#### **More of the Same at the Free University of Berlin**

Later, in 1970, as a Postdoctoral fellow, I was a research assistant in a course of Developmental Biology at the Institute of Biochemistry and Molecular Biology at the Free University of Berlin (director: Prof. Tiedemann). For all experiments in this course, we used *T. alpestris*. The animal half of these beautiful eggs is chocolate-colored. Embryos can be easily dissected with fine glass needles. The *T. alpestris* embryos were used in the laboratory to test the activity and mode of action of different fractions of the vegetalizing (mesodermalizing/endodermalizing) factor, since the ectoderm of this species shows no autoneuralization tendencies, in contrast to that of *T. vulgaris* or axolotl. The main topic of the course was the classical test methods employed to demonstrate the activity of proteinaceous inducing factors in the sandwich method. That method is based on the Holtfreter and Spemann-Mangold Einsteck experiment. The vegetalizing factor (now known as activin) was the first highly purified embryonic inducing factor [for details, see Grunz (1996a, in the special issue of this journal "Developmental Biology in Germany") and Grunz (2001, in the special issue "Spemann-Mangold Organizer")]. The macroscopic effect of the vegetalizing factor (these "historical" time-lapse sequences of the elongation of mesodermalized ectoderm were filmed in 1970) can be seen in my videotape "Amphibian development and the main classical experiments" (mentioned above). All experiments in that video and in the Developmental Biology course in Berlin were performed with *T. alpestris*. For the constriction experiment (not shown in the videotape), we used *T. vulgaris* (as we had previously done in the course in Cologne).

The attitude and motivation of students in that course were quite similar to those expressed in Cologne. Many students were highly motivated, but only few were really successful. I remember one of them, a relatively young (and frustrated) participant of the course, who had a difficult time removing the vitelline membrane and broke several glass needles during the isolation of animal caps. He asked me fairly,

*Do you really intend to continue to perform such experiments in your research and teaching your whole life?*

#### **Establishing a Career when Developmental Biology was given a Relatively Low Priority in Germany**

After my *Habilitation* (*venia legendi*) in 1978, I got a permanent position as Professor of Zoophysiology at the University of Essen. Since it was a new university, I had to build the department from the very beginning. In Germany at that time, no professorial positions for new developmental biologists were available. An important reason for this situation was the loss of interest after the gold rush-like activities in the lab of Spemann and his many colleagues in Europe, the United States, the United Kingdom and Japan. The diminished interest was reinforced by various problems, including WWII and the post-war difficulties. In most laboratories, our traditional embryologi-

cal techniques were still available, but many issues were considered worthy of being dealt with only at the biochemical or molecular genetic levels.

It was difficult for me to establish a successful teaching and research career in modern (biochemical/molecular) developmental biology at our university for several reasons. First was the lack of necessary facilities, funds, and manpower. One of the few successful groups in Germany studying biochemical and molecular processes during early embryonic development was at the laboratory of Prof. Tiedemann in Berlin. His group isolated the embryonic vegetalizing factor (activin) (Tiedemann *et al.*, 1992). However, nearly all biologists in Germany were very suspicious as to whether a protein that had been isolated from chicken embryos, using phenol extraction (for a protein!), could be biologically active in another species such as the amphibian embryo. This, of course, reveals that Prof. Tiedemann was quite ahead of his time, for nothing was known then about conserved genes and the closely related signaling pathways that exist in different species. Since biochemical approaches were expensive and time-consuming, that type of research could be performed in only well-equipped medical institutes (Tiedemann's institute was part of the medical faculty) or in one of the Max Planck Institutes. This was still the case until 1995. Indeed, the 1995 Nobel Prize winner—developmental biologist Prof. Christiane Nüsslein-Volhard—performed her studies as a research fellow of the Max Planck Institute of Developmental Biology in Tübingen.

A second reason was the fact that from the 1960s to the 1990s, developmental biology (especially the amphibian model system) was not considered to be a very worthwhile endeavor in many German universities. Interest had shifted to ethology and neurobiology as a consequence of Konrad Lorenz and Nikolaas Tinbergen being awarded the Nobel prize. Furthermore, ecology became very



**Fig. 2.** Here, I myself demonstrate the "strategy" of catching newts hiding between water plants and in the mud of the pond.



**Fig. 3. Participants of the International Conference on Teaching and Research in Developmental Biology in Afro-Asian Countries in 1982.** In the first row (sitting from L to R): G. Eguchi (Nagoya), M.I. Michael (Alexandria), L.S. Ramaswami (Bangalore), R. Bellairs (London), M. Zheng (Shanghai), S.C. Goel (Convenor, Pune), P.N. Srivastava (Acting President, Indian Soc. Dev. Biol, Delhi), T.S. Okada (President Int. Soc. Dev. Biol., Kyoto), G.T. Tonapi (Pune), R.O. Kelly (Albuquerque), H. Grunz (Essen), D.K. Magon (Nairobi), S.P. Modak (Pune), D.E.S. Truman (Edinburgh), B.I. Sunderaral (Delhi).

popular in Germany. Therefore, during that time nearly no chairs for Developmental Biology were offered at most zoological institutes or natural sciences departments.

A third reason concerns the general emphasis of the University of Essen. Our biology students are being trained as future primary and high school teachers, rather than for the Diploma Degree (Master of Science). Until 2001 we had ecology students who could finish their study with a Diploma of Ecology. That program has, however, been cancelled by the ministry. Nevertheless, those ecology students had little interest in developmental biology, despite the fact that there exists a close relationship between ecology and developmental biology (so-called EcoDevo = Ecological Developmental Biology, see below).

Thus, the conditions for teaching developmental biology at the University of Essen were not optimal. As a consequence, I had no graduate students with a Master's degree (Diploma) in my field who could be selected for further Ph.D. studies in my laboratory. In a few cases, graduate students (future high school teachers) with strong interests in research performed a thesis in my laboratory. Fortunately, several outstanding Chinese Ph.D. candidates with a Master's degree from important Chinese universities came to my laboratory. After a 3-month research stay in 1990 at the Shanghai Institute of Cell Biology (at the invitation of the well-known developmental biologist and institute director Prof. Hui Chuang on behalf of the Academia Sinica and the Max Planck Gesellschaft), I kept in close contact with Chinese institutes. Therefore, I followed recommendations for the selection of Ph.D. candidates made by colleagues in Beijing, Shanghai, Lanzhou and Jinan. I was able thereby to perform some research that was internationally quite well recognized despite the difficult situation in my university.

In former times, developmental biology was also called developmental physiology. That is why I was fortunate to obtain a

permanent position—as head and Professor of the Department of Zoophysiology. Nevertheless, my colleagues of the University of Essen did not consider developmental biology to be a central topic of biology. Since no other zoologist was willing or able to teach general animal physiology, I offered in the winter semester such a lecture course. In the early 1980s, I also was encouraged to give morphological/histological courses, simply because none of my small number of colleagues were willing to take on this task. Consequently, I could teach developmental biology only on a very limited scale. That involved a lecture course on “Developmental Biology” for undergraduates, a laboratory course for graduate students (future school teachers and ecologists) and three different seminar courses (see below).

#### ***A Sojourn in India offered an Opportunity to teach more Developmental Biology***

It was therefore a special honor for me to receive in 1982 an invitation to speak at an international meeting which took place in Pune, India, with the topic “Teaching and Research in Developmental Biology in Afro-Asian Countries.” Only a few scientists from Western countries and Japan (including Okada, Eguchi, Bellairs, and Kelly) participated in this meeting (Fig. 3). It was wonderful to learn from various conference speakers that the level of teaching of developmental biology in the so-called “developing countries” was relatively high. Several colleagues in developing countries had spent time in the United States, the United Kingdom, France, or Japan and introduced upon their return modern techniques and ideas to their laboratories and classrooms. For example, Sohan Modak, who, like me, worked in Tuneso Yamada's laboratory in Oak Ridge, Tennessee, in the late sixties, got a full professorship after his return to India. He then introduced modern developmental biology to the University of Pune. I was especially impressed by the strong interest of a large number of participating



Indian students in modern trends in biology, especially in the field of developmental biology.

It is worth mentioning that other fields, such as mathematics, physics and engineering were—around this period—not very popular in Germany. Computer techniques and, later, gene technology could be performed in Germany only under strong restrictions. The number of persons educated in those new techniques was limited. As a result, nowadays, qualified young persons are rare, and the German government has tried to hire Indian computer specialists (especially from Bangalore, India) with, however, limited success. I visited India again in 1998 and also gave a lecture in Bangalore at one of the most prominent Indian universities. In discussions with young postdoctoral fellows and Ph.D. candidates, I learned that they prefer to get a position in India or in another English-speaking country, preferentially the United States, rather than in Germany. On the other hand, many Chinese students are now interested in coming to Germany.

### **Resurgence of Interest in Developmental Biology in Germany**

Developmental biology as a field of study in Germany improved after the Nobel Prize was awarded to Dr. Nüßlein-Volhard—a developmental (genetic) biologist—in 1995. Of central importance was the fact that molecular techniques were introduced in all the leading laboratories in Germany. Recently, the prominence in Germany of developmental biology and developmental genetics has also leaped forward. The cloning of mammals (the sheep Dolly), the human genome project data, and the public discussion of human stem-cell research, have made developmental biology and its role in both biology and society into a central topic of discussion among our faculty and students and the lay public.

### **Optimism for the Future**

Modern developmental biology is now without doubt a central biology topic in Germany. Indeed, the new data from molecular biology have demonstrated the close relationships which exist between the genome, embryonic development, morphology, anatomy, physiology, environment and evolution. Thus, students should learn in both Development Biology/Genetics seminars and lecture courses that there exist mutual relationships among these different fields of biology. In many universities, including those in Germany, developmental biology, evolutionary developmental biology (EvoDevo) and ecological developmental biology (EcoDevo) are being revitalized as central fields in biology. It can be expected, therefore, that the next generation of researchers in those fields will have a much easier time building their careers.

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