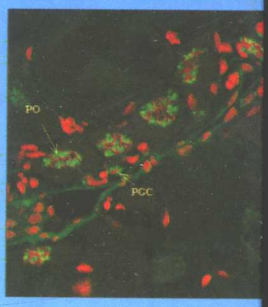
鱼类性别和生殖的遗传基础及其人工控制



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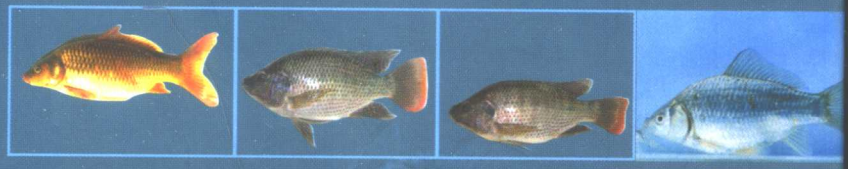

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鱼类性别和生殖的遗传基础及其人工控制

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科学出版社

北京

内 容 简 介

六十多年前，刘建康先生在黄鳝中揭示出雌雄同体和性反转现象，被当时国际权威认为是在低等脊椎动物中的首次发现。六十多年来，鱼类性别和生殖研究不但发展迅速、成果迭出，而且应用价值厚实，已为渔业所用。本书在重印刘建康院士关于黄鳝性别分化研究的两篇论文和英国皇家学会博洛博士针对黄鳝雌雄同体和性反转现象的发现在 *Nature* 上发表的专题评论的基础上，结合作者的研究工作，系统地论述了鱼类性别和生殖的遗传基础及其人工控制，内容包括鱼类生殖策略的多样性与特殊性，鱼类的性别决定与性别决定基因，单性鱼类的生殖方式及其遗传基础，银鲫生殖方式的特殊性及其遗传基础，鲤鱼的性别控制与全雌鲤生产，黄鳝的性反转及其调控机制，罗非鱼性别控制研究及应用，石斑鱼的性反转及其性别分化机制，以及鱼类生长的调控机制及其与生殖的相互关系等。

本书可供从事生命科学研究和教学的人员参考，可用作相关专业研究生的教学用书和大学高年级学生的学习参考书。

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谨以此书献给刘建康院士九十大寿

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序　言

鱼类性别与生殖研究是当前生命科学和生物技术研究的重要领域。六十多年前，刘建康先生在黄鳝中首次揭示出的雌雄同体和性反转现象，被当时国际同仁誉为开创新研究领域的发现。六十多年来，在几代科研人员的努力下，不但在许多鱼类中发现了雌雄同体和性反转现象，而且进一步发现鱼类生殖方式存在多样性和特殊性，且在渔业生产实践中有广泛的应用价值。特别是近十年来斑马鱼和青鳉等鱼类被用作发育生物学等生命学科研究的模式生物之后，鱼类性别与生殖研究的发展更快，酝酿着新的重大突破。鱼类生殖方式的多样性包含有许多生物学研究模型，这些模型或是适用于获得普遍性的脊椎动物生理学和遗传学的新知识，或是适用于发展鱼类养殖体系、开发新的水产生物技术和新品种。

《鱼类性别和生殖的遗传基础及其人工控制》一书是桂建芳研究员等一批相关专家敬献给刘建康先生九十大寿的贺礼。该书重新刊印了刘建康院士关于黄鳝性别分化研究的两篇论文，以及英国皇家学会博洛博士在 *Nature* 上对发现黄鳝雌雄同体和性反转现象的专题评论。在此基础上，作者们结合各自的研究工作，系统地论述了鱼类性别和生殖的遗传基础及其人工控制，内容包括鱼类生殖方式的多样性与特殊性，鱼类的性别决定与性别决定基因，单性鱼类的生殖方式及其遗传基础，银鲫生殖方式的特殊性及其遗传基础，鲤鱼的性别控制与全雌鲤生产，黄鳝性反转的调控机制，罗非鱼的性别决定与全雄鱼育种，石斑鱼的性反转及其性别分化机制，以及鱼类生长的调控机制及其与生殖的相互关系等。可以说该书汇集了我国这一领域的理论和应用研究的最新成果，特别是对一些重要养殖鱼类的个案分析颇具特色，有很强的可读性和启迪性，适合研究生、青年学者和专业人员阅读。

刘建康先生作为我国著名的鱼类学家和淡水生态学家，几十年来在动物解剖学、组织学、胚胎学、生理学、鱼类生态学、动物分类学、湖沼学和淡水鱼类养殖学等领域研究中均有重要建树；作为前所长和名誉所长，为中国科学院水生生物研究所的学科建设和发展做出了重要贡献。我有幸作为刘先生的一名学生，老师的教诲和人格垂范是我毕生的宝贵财富。在此谨祝先生健康长寿，幸福永远。

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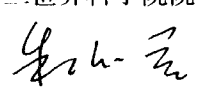


2007 年 5 月 26 日

前　言

六十年前的7月5日，*Nature*杂志发表了英国皇家学会博洛（W. S. Bullough）博士“低等脊椎动物中的雌雄同体”（*Nature*，160：9～11）一文。这是一篇针对我国当时一位青年学者在黄鳝性腺研究中的一个重大发现而写就的一篇专题评论，属有感而发。正如作者开头所说：“从1944年一篇描述黄鳝性腺论文的发表，刘提供了新的和有趣的有关低等脊椎动物性别决定机制的证据，并打开了一个新颖研究领域之门。”这一评价是对时年30岁仅用不到两年时间刚在加拿大麦基尔大学取得博士学位的一位年轻学子的最高祝贺，是对他在中国抗日战争时期那种艰难的环境下所取得的这一创新性发现的褒奖。从那时起，这位青年学子更加坚定了追求科学、心系祖国、一生为鱼所栖居的生态环境而潜心研究的信念，形成了他“重视科学实验，着眼社会实践；不唯上，不唯书，不唯权威；独立思考，敢于创新”的治学格言。正是这一信念和治学精神，造就了令亿万民众受益的一代大家，造就了令几辈学人钦佩敬仰的一代大师。他，就是中国科学院水生生物研究所名誉所长刘建康院士，现已九十高龄的智者。

六十年，按中华民族创立的干支纪时为一甲子，可谓岁月流长；六十年，在人类文化和科学的形成过程中，又可谓弹指一挥间。作为后学，我虽未曾成为先生的嫡传弟子，但作为与先生隔代的所长，又常得亲炙教诲，受益良多。尤其是近年在国家重大基础研究发展计划项目的支持下，有幸跟随先生，开展鱼类生殖调控机制研究，重读先生六十多年前的有关黄鳝始原雌雄同体的论著和博洛先生的评论，钦佩敬仰之情更深。正如博洛先生六十年前所料，鱼类性别和生殖研究不但发展迅速、成果迭出，而且应用价值厚实，已为渔业所用。鉴于此，特联袂现今国内多位同行，以各自之专长，合力编著此书，一是作为刘建康先生九十大寿的贺礼，敬献给我们这一研究领域的先驱，愿先生寿比南山，健康永驻，德高望重，著述等身；二是作为学问的载体，将刘先生六十年前两篇研究黄鳝雌雄同体的论文和博洛先生的评论以及刘先生迄今的著作目录作为引子刊于书前，以便传承创新，延续知识，陶冶身性，启迪后人。

心宽能长寿，德美可延年。食鱼强身体，学问长信念。愿此书对后来者能有所裨益，也期待同行们的批评与斧正。

中国科学院水生生物研究所 所长

2007年5月于武汉东湖之滨

目　录

引　子

刘建康院士关于黄鳝雌雄同体和性反转研究的两篇论文
和博洛博士在 *Nature* 上发表的专题评论

引子 1

RUDIMENTARY HERMAPHRODITISM IN THE SYMBRANCHOID EEL, MONOPTERUS JAVANENSIS

C. K. Liu

INTRODUCTION

Monopterus javanensis, a serpentiform fish of the rice-fields noted for its tastefulness as well as for its tenacity of life, is of wide occurrence in China. It appears to be a gonochorist for the most part of its lifetime, though, owing to the absence of secondary sexual characteristics its sex is difficult to tell from its external appearance. During the course of previous studies of its breeding habits (Wu and Liu, '42) and later of its blood vascular system (Wu and Liu, '43), lot of specimens were dissected and their sexes noticed. Little by little the writer began to be impressed with the idea that there would be something unusual about the sexuality of this fish, since small- and median-sized individuals are overwhelmingly female, while large ones are predominantly male. Spurred by this preliminary finding, he has since then examined, from time to time, hundreds of specimens, recording their sexes in relation to their respective lengths. The data thus accumulated seemed sufficient to warrant an age-sex correlation, which in turn led to the revelation of the rudimentary hermaphroditism of this fish.

Rudimentary hermaphroditism has been known to be a normal condition in the so-called undifferentiated races of frog and in a number of piscine species. The present case, however, has some peculiarity of its own which will become clear when the discussion is taken up.

The writer wishes to acknowledge his indebtedness to Dr. H. W. Wu, under whose guidance this research was pursued.

MATERIAL AND METHOD

With the exception of three individuals of probable significance which were captured from a pond by the members of the institute, all fish over 22 cm. long in this investigation were obtained from the market at various seasons of the year. They were said to have been caught from the rice fields of Pehpei and Hochwan, Szechwan. The fingerlings, possibly stunted in growth, were drawn from a small tank in which they had been reared for one year since hatching.

注：本书 2～38 页为 20 世纪 40～50 年代发表的论文，为保留论文原貌，故影印自原论文。——编辑注

Sex was determined in all instances by direct examination on the gonad. The gonads of the male and the female are precisely similar in form and position, being in either case an unpaired tube like organ lying along the right side of the intestine. The gonoducts are backward prolongations of the gonad; and, having no swellings of any sort, are all the same in both sexes. Whatever its size within the range covered by the present investigation) the female can always be distinguished by the numerous ova which it contains. The male, on the contrary, is characterized by the apparent homogeneity of the spermary. This convenient method of sex identification has been proved compatible with the result obtained by the laborious histological examination. However, the intersexes can only be ascertained by means of microscopic preparations although they might have some macroscopic indications on their gonads.

OBSERVATIONS

A total of 659 specimens ranging from 5.3 to 57.6 cm. in total length were subject to investigation. As indicated in the accompanying table (Table 1), they are grouped into 21 classes with a range of 2 cm. each. In each class, the actual number of either sex is followed by the respective percentage in the bracket. The first three classes represent the fingerlings; the rest as a whole are adult specimens; and between them there is a gap of body length from 11.0 to 21.9 cm. to which we had no access during the entire course of investigation.

TABLE 1

Frequency of male and female individuals in different ranges of body-length

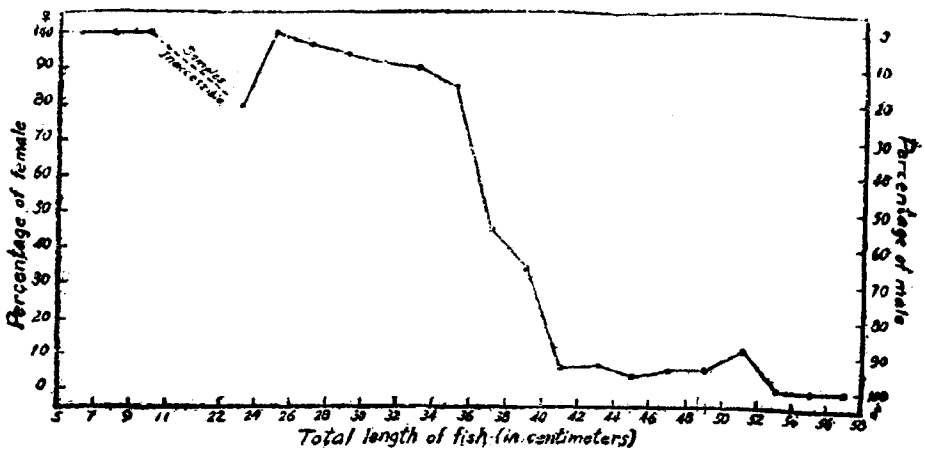
Class range in cm.	Male	Female	Total
5.0-6.9	0 (0)	18 (100)	18
7.0-8.9	0 (0)	58 (100)	58
9.0-10.9	0 (0)	9 (100)	9
22.0-23.9	1 (20)	4 (80)	5
24.0-25.9	0 (0)	8 (100)	8
26.0-27.9	1 (3.27)	30 (96.77)	31
28.0-29.9	3 (6.67)	42 (93.33)	45
30.0-31.9	6 (8.8)	62 (91.2)	68
32.0-33.9	7 (10.3)	61 (89.7)	68
34.0-35.9	12 (16.0)	63 (84.0)	75
36.0-37.9	35 (54.7)	29 (45.3)	64
38.0-39.9	32 (65.3)	17 (34.7)	49
40.0-41.9	32 (94.1)	2 (5.9)	34
42.0-43.9	28 (93.33)	*2 (6.66)	30
44.0-45.9	31 (96.9)	1 (3.1)	32
46.0-47.9	16 (94.1)	1 (5.9)	17
48.0-49.9	17 (94.5)	†1 (5.5)	18
50.0-51.9	8 (88.9)	†1 (11.1)	9
52.0-53.9	11 (100)	0 (0)	11
54.0-55.9	7 (100)	0 (0)	7
56.0-57.9	3 (100)	0 (0)	3

*One of the two samples was collected from a pond.
†Collected from a pond.

It was not until the work of sex identification was half done that intersexes came to be perceived. Since they were still containing eggs in their gonads they were classed as females in identification. The figures given for the females in Table 1, especially in those ranges over 36 cm, therefore possibly include a small number of intersexes.

If the above result is represented graphically, we get a curve as shown in Graph 1, in which the percentages for the male and for the female in the given ranges of body-lengths are indicated on the right and the left respectively.

Graph I. *Correlation between length and sexuality of Monopterus javanensis*



From the table and the graph two points are to be observed. First, all individuals of this fish should pass through a juvenile female phase, as the 85 fingerlings examined showed 100 per cent female. It will be assured that there is no uncertainty concerning the femaleness of the fingerlings, because ova were already well differentiated (diameter up to 75 μ in fixed specimen) even in the shortest individual (5.3 cm. in total length). Second, sex ratio is evidently correlated with the length, and hence with the age, of this fish—smaller specimens are predominantly female while large ones are predominantly male. The normal 1:1 ratio is maintained only in the range of 36.0-37.9 cm, in the 300 specimens below 36 cm. (exclusive of the fingerlings) female predominated to the extent of 90 per cent (270 individuals), whereas in the 274 specimens above 36 cm. male predominated to the extent of 80.3 per cent (220 individuals).

The preponderance of male in the larger specimens and of the female in the smaller ones appears as if it were due to sexual dimorphism, that the male were capable of growing to greater size than the female. That this is not the case can be easily seen from the fact that there were by far too few males in the young adults.

Neither selective capture-rate nor differential viability would account for it, because it would be rather far-fetched to think that female should be more labile to capture at first and male later, or viability should be in favor of one sex at one time and another sex at other time. The peculiar sex-ratio therefore can be explained only on the ground of sex reversal.

If sex reversal is the true explanation to the changes of sex-ratio, transitory ambisexuality should be expected to occur in individuals right in the progress of sex transformation; and this actually has been found. In dealing with females of relatively large size it was noticed that certain of them had fewer ova than normal and, what is more, the yolk massses of the ova were somewhat deformed. Microscopic examination revealed that side by side with the retrogression of ova there was admixture of male element. The proportion of the testicular and ovarian tissue in the ambisexual gonad varied so greatly in different specimens that intersexes grading from prevailingly female to prevailingly male had altogether been observed. Since normal gonad of *Monopterus* has been found to be strictly either male or female, ambisexuality affords an unequivocable proof of sex reversal in this fish.

The histological changes of the gonad on the moment of sex transformation will be described in a future publication dealing with the germ-cell origin of this fish. It would be sufficient to mention here that male element first appears as such in the trabaculæ of the connective tissue capsule of the original (female) gonad, and henceforth spreads out and undergoes hypertrophy so as to convert the ovary into a typical spermary. With the rising of the testicular tissue there is always a corresponding retrogression in the ova, so that when the former approaches its perfection the latter have become entirely out of existence. A "female" is thereby transformed into a "male." Obviously male and female in this fish are not distinct categories of individuals but are merely temporary phases in the life of the same individual.

From the table and the graph it will be apparent that the critical moment or "switch-over" of sex reversal for the great majority of the population should lie within the range of 34-40 cm. in length. But occasionally, precocious or delayed transformation are also met with. Thus a specimen as young as 23 cm. long was found to be already in the male phase perfectly, while others with lengths of 48 and 50 cm. respectively were still in the true female phase. Since large male specimens never show tendency of ovarial reformation it is quite certain that there is no further change of sexuality in the male phase. The exceptional large females just alluded to are therefore to be regarded as cases of delayed transformation rather than of secondary inversion. In this connection the writer wishes to remark that the two exceptional females were captured, along with another of same sex, from a pond wherein there is no drying up during the winter months and the natural food for *Monopterus* is much more abundant as compared with ordinary paddy fields. It seems not impossible that sex reversal in this fish might be in some way influenced by the nutrition of the animal.

That the female phase of *Monopterus* is already functional when the fish is as young as 22 cm. in length can be visualized in the fact that in quite a number of fish of such length the ova were fully mature and ready for shedding. Since 22 cm. is far short of the "'turning-point" length it seems safe to conclude that in general, sex reversal takes place only after the animal has laid its eggs. Regarding the male phase, there is every evidence of its being functional. Sex reversal in *Monopterus* is therefore of the "complete" type, notwithstanding that whether those exceptional, small males had ever produced eggs is open to doubt.

DISCUSSION

In view of the universal femaleness of the fingerlings, the reversal of sex-ratio and the actual occurrence of sex transformation, the writer is led to the conclusion that *Monopterus*, male and female alike, starts its sexual life as female, produces eggs, and afterwards experiences a sex reversal, eventually transforms into a sperm-producing individual. The condition resembles that of a proterogynic hermaphrodite to some extent. However, it should be emphasized that *Monopterus* is merely a hermaphrodite in time, and not a hermaphrodite in space, the latter condition being only found during the brief course of sex transformation. It seems doubtful whether the antecedent cells of the sperm coexist with the ovary before sex reversal; if so, they could hardly be distinguished as such at all. Hence we are dealing here with a case of rudimentary hermaphroditism, which has been referred to the condition in which both sexes exhibit distinct hermaphroditic traits but are still clearly separated for the main part of their lifetime.

In Gnathostomata, rudimentary hermaphroditism as a normal condition has been known in the undifferentiated race of the frog, *Rana temporaria* (Witschi, '29); in the teleosts, *Anguilla* (Grassi, '19), *Salmo irideus* (Mrsic, '23) and notably a group of viviparous cyprinodontids, namely, *Xiphophorus helleri* (Essenburg, '23, '26), *Lebistes reticulatus* (Dildine, '36), *Platypoecilus variatus* (Goldschmidt '37), *Limia caudofasciata*, *L. vittata* and *L. nigrofasciata* (Breider, '36). The details of these cases may differ in even closely related forms, but all agree in that the male passes through an initial female phase. As a rule, the female phase of the male is short, so much so that it may have been terminated prenatally (embryonic hermaphroditism, as in *Lebistes reticulatus*); even in the most lengthy case the initial female phase does not carry further than the end of the first year of the animal's life span (juvenile hermaphroditism, as in *Rana temporaria*). Consequently, the female phase of the genetic male scarcely functions as female. In *Monopterus*, however, the condition is rather phenomenal, because the female phase is unduly protracted so that the animal has reached sexual maturity regularly before the onset of sex reversal. More significant is the fact that every member of the population is alike in being a female at first and male later. Now if it could be shown that among the female-phase individuals there are two

different groups, one of which bears functional ova while the other non-functional, we could properly designate the former as genetic female and the latter genetic male after the manner of the undifferentiated frog race. But such distinction probably does not exist, for save a few females that were right on the way of sex reversal all the female-phase individuals examined showed numerous eggs well-developed and apparently normal, there being no indication whatever of the existence of two heterogenous groups among themselves. Following this the writer is inclined to suggest that in *Monopterus* the zygotic constitution for sex is one and the same throughout the population. There would be no such distinction as genetic male and genetic female, and all individuals are endowed with a factorial make-up in which the quantitative ratio of the male and the female gene complexes, as evidenced from the regular occurrence ot sex reversal, must be nearly in equilibrium. There seems to be no chromosomal mechanism underlying the sex to "tip the balance." The early female and later male differentiation might be caused by the activation of female sex in advance of the male sex through physiological factors which in turn are associated with the process of ageing. That environmental factors, preferably nutrition, might play some role in sex differentiation is reflected in the aforementioned cases of delayed transformation.

Whatever the true explanation may be, *Monopterus* is to be considered as a developmental gonochorist rather than a hereditary gonochorist. The differential sex reaction in time as occurred regularly in this fish is therefore brought in line with the condition found in Cymothoidea (Witschi, '32), barring that the latter is an invertebrate and is essentially a parasitic form. So far as the writer is aware, developmental gonochorism is hitherto unknown in vertebrates.

The writer refrains from refering the present case to consecutive sexuality to which it bears some superficial resemblance. Both involve a functional initial phase of one sex followed by a functional secondary phase of opposite sex. But in consecutive sexuality, typified by the quohog (*Venus mercenaria*), it is indicated by Coe ('43) that "the primary, juvenile gonads are invariably more or less ambisexual" although 98% of all individuals are protandric. The quohog therefore has a truly hermaphroditic gonad to start with. In *Monopterus*, as has been stated, the functional juvenile gonads are strictly dioecious in morphological sense before, and after, the occurrence of sex reversal. Moreover, Coe expresses of *Venus mercenaria:* "Following this initial male phase the sexes are with few exceptions strictly either male or female, with approximately equal numbers of each sex." Thus it is clear that in reality there is only one-half of the population that has a consecutive sexuality whereas the sexuality of another half remains unchanged; in this way the situation appears to the writer quite complicated inasmuch as ambisexuality, which is characteristic of a hermaphrodite, turns up to yield a 1:1 sex-ratio which is characteristic of a hereditary fixed gonochorist. In *Monopterus*, on the contrary, practically all individuals change with regularity