Hereditary and Nonheritable Vertebral Anchylosis in the Medaka, *Oryzias latipes*.

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Introduction

Two types of mutants having vertebral abnormality, the fused (*f*) and wavy (*w*), are known to occur among domesticated varieties of the medaka, *Oryzias* (formerly *Aplocheilus*) *latipes* (Aida 1930). The former is characterized by fusion (anchylosis) of vertebrae in any region, while the latter has usually two humps as a result of vertebral column being bent without anchylosis of vertebrae. Instances of such vertebral abnormalities in other fishes are referred to later.

In 1957 some abnormal medaka resembling the fused (*f*) were found by one (H. T.) of the writers in a certain region on the eastern outskirts of the City of Nagoya. They were collected together with normal wild medaka from slow-flowing narrow ditches connecting rice paddy-fields. Although among the collected samples the aberrant form was only a few percent, we were able to collect more than 30 specimens, both male and female.

The region in which they were secured is about 0.7 km south of the Nagoya Botanical Garden and adjoins the southern shore of a muddy bottom lake (Okuno-ike). This area is part of one of the drainage basins of the Ueda river which is a tributary of the Tempaku river. There is a land divide between this basin and that in which our laboratory is situated, the latter belonging to the drainage basin of the Yamazaki river, which would make intermingling impossible. The suspicion that they might have arisen through an accidental hybridization between wild medaka and the fused variety (*f*) being reared in our outdoor breeding pools can be ruled out. Although vigorous searches were carried out in approximately fifteen regions due east of Nagoya, we failed to locate any. The aberrant form is confined to the single region where they were first seen.

The aberrant form on which we are reporting here resembles the fused (*f*) in respect to vertebral anchylosis but somewhat differs from it in other traits such as the frequency of fused vertebrae in respect to regions of vertebral column and the number of rays in the anal fin. Breeding experiments disclosed that the aberrant form found in nature is nonheritable and is supposed to be the production of an extraordinary environmental condition during embryonic life.

Attempts have been made for the purpose of inducing this form of anomaly in

genetically normal (+) strain. It happened to us that phenylthiourea and lathrogenic agents can induce anchylosis of vertebrae when treated in embryonic stages (Tomita and Matsuda, 1961). We were able to rear phenylthiourea-induced anomalies to maturity and to obtain offspring of them.

The present note deals with the characteristics of the hereditary fused (*f*) and the aberrant fish found in nature as well as breeding experiments of both of them and of phenylthiourea-induced anomalies. Conventionally, the aberrant fish found in nature are called the "wild fused".

Characteristics of fused (*f*) and wild fused

Over-all appearance of the genetic fused (*f*) and the wild fused is illustrated in Figs. 1 and 3, respectively. Both of them have a foreshortened vertebral column. The form index, the frequency of anal and dorsal fin-rays of normal, fused (*f*) and wild fused are shown in Table 1. The form index (standard length divided by the greatest body depth) of both fused (*f*) and wild fused is significantly smaller than that of the normal. Significance of deviation of the form index of the wild fused from that of the normal wild population is beyond question ($t = 7.2$,

Fig. 1. Above: Genetic fused (*f*) white female with the genotype $f_i f_i$, b/b , r/r . $\times 2.7$
Below: Normal (+) white female with the genotype $+/+$, b/b , r/r . $\times 2.7$

Fig. 2. A: Ankylosed vertebrae (three) in anterior trunk region of genetic fused (*f*). Top view. $\times 23$
B: Ankylosed vertebrae (five) in posterior trunk region of genetic fused (*f*). Lateral view. $\times 23$

Fig. 3. Above: Wild fused (Brown).
 $\times 2.7$
 Below: Wild normal (Brown).
 $\times 2.7$

TABLE 1. Mean form indices, frequencies of anal and dorsal fin-rays of normal, fused (*f*) and wild fused fishes with standard errors.
 n = Number of fish measured

Fish	Form index ($\frac{\text{Standard length}}{\text{Body depth}}$)	Frequency of anal fin-rays	Frequency of dorsal fin-rays
wild normal	3.8 ± 0.03 (3.0 – 4.5, n = 103)	18.8 ± 0.1 (16 – 21, n = 103)	6.1 ± 0.03 (6 – 7, n = 103)
wild fused	3.2 ± 0.07 (2.5 – 3.8, n = 18)	19.2 ± 0.3 (16 – 20, n = 18)	5.9 ± 0.06 (5 – 6, n = 18)
Domesticated normal	$3.9 \pm 0.15^*$ (n = 200)	19.2 ± 0.04 (17 – 22, n = 600)	5.9 ± 0.02 (5 – 7, n = 600)
Genetic fused (<i>f</i>)	3.0 ± 0.05 (2.1 – 4.0, n = 100)	16.1 ± 0.05 (13 – 18, n = 300)	6.0 ± 0.01 (5 – 7, n = 300)

* Calculated by mean standard length with its standard error/mean body depth with its standard error.

Fig. 4. Ankylosed vertebrae (eleven) in middle trunk region of wild fused. Lateral view. $\times 26$

d.f. = 119, $P < 0.001$). The wild fused has the normal number of the anal fin-rays whereas the fused (*f*) has a significantly reduced number. This difference may be associated with the topographical difference in the frequency of vertebral anchylosis. It is noteworthy that anomalies induced by phenylthiourea have reduced number of the anal fin-rays as in the fused (*f*). The frequency of the dorsal fin-rays in the genetic fused (*f*) and the wild fused is the

same as that of the normal. Examples of skeletal preparations of vertebral anchylosis in the fused (*f*) and the wild fused are illustrated in Figs. 2 and 4, respectively, and numerical data on their characteristics are presented in Tables 2 and 3. While the number of neural spines in both anomalies is the same as that of the normal (Average 31, range 29–33), the number of centra is significantly reduced owing to vertebral anchylosis. This means that the fusion of vertebrae is occurring in centra only.

In other words, complete anchylosis of vertebrae involving neural and hemal spines does not take place. This fact enables us to estimate the number of fused centra by examination of the number of neural spines. Thus, let A be the number of neural spines and B that

TABLE 2. Vertebral anchylosis in fused (*f*) variety

FISH No.	(A) NUMBER OF NEURAL SPINES	(B) NUMBER OF CENTRA	(C) NUMBER OF FUSED CENTRA	(D) NUMBER OF FUSED GROUPS	% OF FUSED VERTEBRAE
1	31	27	6	2	19.4
2	30	27	5	2	16.7
3	31	29	3	1	9.7
4	30	23	13	6	43.3
5	32	26	11	5	34.4
6	32	30	4	2	12.5
7	31	27	6	2	19.4
8	31	28	5	2	16.1
9	29	25	8	4	27.6
10	30	25	8	3	26.7
11	31	27	8	4	25.8
12	32	27	8	3	25.0
13	31	30	2	1	6.5
14	30	20	18	8	60.0
15	33	27	10	4	30.0
16	31	26	8	3	25.8
17	31	28	6	3	19.3
18	31	28	5	2	16.1
AVERAGE AND RANGE	30.9 (29-33)	26.6 (20-30)	7.4 (2-18)	3.2 (1-8)	24.1 (6.5-60.0)

TABLE 3. Vertebral anchylosis in wild fused

FISH No.	(A) NUMBER OF NEURAL SPINES	(B) NUMBER OF CENTRA	(C) NUMBER OF FUSED CENTRA	(D) NUMBER OF FUSED GROUPS	% OF FUSED VERTEBRAE
1	32	22	13	3	40.6
2	33	31	4	2	12.1
3	33	32	2	1	6.1
4	30	16	15	1	50.0
5	31	25	8	2	25.8
6	33	23	14	4	42.4
7	30	17	17	4	56.7
8	31	23	11	3	35.5
9	31	24	11	4	35.5
10	30	20	14	4	46.7
11	31	26	8	3	25.8
AVERAGE AND RANGE	31.3 (30-33)	23.5 (16-32)	10.6 (2-7)	2.8 (1-4)	34.2 (6.1-56.7)

of centra. Needless to say that in the normal fish A equals B . In both aberrant forms B is significantly reduced. Let C be the number of fused centra and D be that of groups of fused vertebrae, then it is obvious that these items are represented by the formula $A = B + C - D$. While C and D in both anomalies are not different (tables 2 and 3), the frequency of anchylosis in respect to topographical region is somewhat different between the fused (f) and the wild fused. This difference is clearly illustrated in histograms of Fig. 5. While anchylosis in the fused (f) is most frequent in the posterior region (21–30th) and lowest in the middle region (11–20th), that in the wild fused is more frequent in the middle region (11–20th) and less frequent in both anterior and posterior regions. The difference of the anal-fin frequency between the two aberrant forms may be associated with this difference.

Fig. 5. Topographical difference between genetic fused (f) and wild fused, in respect to vertebral anchylosis.

Artificial induction of vertebral anchylosis by phenylthiourea

Tomita and Matsuda (1961) in our laboratory found that treatment of embryos with phenylthiourea and lathrogenic agents have a remarkable effect in inducing vertebral anchylosis in genetically normal strain (wild and orange-red) of the medaka. Blastulae were immersed in M/400–M/200 phenylthiourea solution and allowed to develop in the solution until they reach the stage of the initial establishment of blood circulation. Thereafter, they were reared in the Ringer's solution for the medaka. If embryos are remained to develop beyond that stage, they succumb before hatching because of defect in circulation. Embryos treated with phenylthiourea during early embryonic stage usually hatched out. Examination of just hatched fry indicates that they have abnormally twisted notochord and aberrant form of body in various degree. Unevenly developed notochord sometimes assumes spiral lumps (Fig. 6). Most of them failed to reach young. Only some fry, abnormality of which is not so severe, can develop further. We have, however, succeeded in rearing a few

Fig. 6. Newly hatched fry treated with phenylthiourea during early embryonic life. Note twisting of notochord. $\times 20$

of them to reach maturity. In over-all appearance, they resemble the genetic fused (f). The number of anal fin-rays is reduced to the degree similar to that of the fused (f). Preparation of vertebral skeleton clearly disclosed fusion of vertebrae although the mode of anchylosis in this induced fused is somewhat different from that of the genetic fused (f).

Breeding experiments with the fused (f) variety

The fused (f) mutant first appeared in Aida's breeds in Kyoto and was reported briefly by him (Aida, 1930). He stated that this was a character recessive to the normal. He mentioned also that this character is independent of all known color genes and of sex. The stock of the fused (f) in our laboratory was originally received from the late Aida in 1947. It has since been kept in our laboratory by the senior author (T. Y.) performing some breeding experiments concerning to aberrant sex ratios (unpublished), details of which will be reported in future. Although Aida (*loc. cit.*) presented the data indicating that the recessive character is governed by a single autosomal factor, data relevant to independency of the factor from color genes and sex were not presented. It seems, therefore, worth while to present our data of breeding experiments performed by the senior author (T. Y.).

In presenting the result of genetic studies, need for a brief account on color varieties in this fish is taken for granted. Aida (1921) established that color varieties of the medaka can be phenotypically symbolized as follows: brown (wild) B, R ; blue B, r ; orange-red b, R ; white b, r ; variegated orange-red B', R and variegated white B', r . The B, B' and b constitute autosomal triple alleles controlling the formation of the black pigment (melanin) while the R and r , governing the deposition of orange pigment (carotenoid), are resident either in the X or Y , namely, sex-linked. The genetic sex-determining mechanism normal to this fish was established by Aida (1921) as $XX = \text{♀}$ and $XY = \text{♂}$ from evidence of sex-linked inheritance. The validity of this fact was ascertained unequivocally by the senior author through progeny testings of sex-reversals induced by treatment with heterotypic sex-hormones (Yamamoto, 1955, 1958).

In 1954 fused white females f, b, r ($f/f, b/b, X'/X'$) were crossed with a normal orange-red males of our $d-rR$ strain with the genotype $+/+, b/b, X'/Y^R$ to get normal white females ($f/+, b/b, X'/X'$) and normal orange-red males ($f/+, b/b, X'/Y^R$) in equal numbers. The F_1 offspring are denoted by (fd). The observed segregations for the two phenotypes ($+, b, r \text{ ♀}$ and $+, b, R \text{ ♂}$) were 19 and 27, respectively (Table 4). The χ^2 analysis indicates that the departure from the expected 1:1 ratio is insign-

TABLE 4. F₁ offspring (*f*d) of fused white females (3) mated with a normal heterozygous orangered male (*d-rR* strain). *f* = fused, + = normal, *b*, *R* = orange-red, *b*, *r* = white. Genotype of (*f*d) +, *b*, *R* ♂ is presumably *f*/+, *b*/*b*, X'/Y^R

SYMBOL OF OFF- SPRING	PARENTS		F ₁ OFFSPRING				χ ² test for 1 ♀ : 1 ♂
	♀	♂	+, <i>b</i> , <i>r</i> ♀	+, <i>b</i> , <i>r</i> ♂	+, <i>b</i> , <i>R</i> ♀	+, <i>b</i> , <i>R</i> ♂	
(<i>f</i> d)	<i>f</i> / <i>f</i> , <i>b</i> / <i>b</i> , X'/X'	+ /+, <i>b</i> / <i>b</i> , X'/Y ^R	19	0	0	27	χ ² = 1.39 P=0.30-0.20

TABLE 5. Backcross of a doubly heterozygous (*f*d) +, *b*, *R* ♂ to fused white femalee (*f*, *b*, *r* ♀♀). *f* = fused, + = normal, *b*, *R* = orange-red, *b*, *r* = white

SYMBOL OF OFF- SPRING	PARENTS		OFFSPRING				
	♀	♂	+, <i>b</i> , <i>R</i> ♀	+, <i>b</i> , <i>R</i> ♂	+, <i>b</i> , <i>r</i> ♀	+, <i>b</i> , <i>r</i> ♂	<i>f</i> , <i>b</i> , <i>R</i> ♀
(<i>f</i> ² d)	<i>f</i> / <i>f</i> , <i>b</i> / <i>b</i> , X'/X'	<i>f</i> /+, <i>b</i> / <i>b</i> , X'/Y ^R	1	63	54	0	0

			Total
<i>f</i> , <i>b</i> , <i>R</i> ♂	<i>f</i> , <i>b</i> , <i>r</i> ♀	<i>f</i> , <i>b</i> , <i>r</i> ♂	
56	51	0	225

χ² for segregation of the +, *f* = 0.54, P = 0.95 - 0.90
χ² for segregation of the *R*, *r* = 1.00, P = 0.90 - 0.80
χ² for joint segregation of the two factor pairs (+, *f* and *R*, *r*) = 0.11, P = 0.99 (No linkage)

nificant. In 1955 an (*f*d) orange-red male presumably having the genotype *f*/+, *b*/*b*, X'/Y^R was backcrossed to the fused white females *f*/*f*, *b*/*b*, X'/X'. Since both parents have the common *b*/*b*, we are here dealing with a dihybrid backcross. The four equal classes *f*, *b*, *r* ♀; *f*, *b*, *R* ♂; +, *b*, *r* ♀ and +, *b*, *R* ♂ are expected to occur. The result of the offspring (*f*²d) is given in Table 5. The four classes were fairly segregated in a 1:1:1:1 ratio (χ² = 1.39, d.f.= 3, P = 0.80-0.70). A single exceptional +, *b*, *R* ♀ appeared, however, in addition. This exception was mated to *f*, *b*, *r* ♂♂ consecutively to decide her genotype. Unfortunately, however, she spawned only undeveloped eggs.

In 1957, (*f*²d) *f*, *b*, *r* ♀♀ (3) were mated to a normal wild (brown) male (+, *B*, *R*) collected on the outskirts of Nagoya to get triply heterozygous females and males in equal numbers. In the next year, a triply heterozygous male presumably of the genotype *f*/+, *b*/*B*, X'/Y^R was backcrossed to the fused white females *f*/*f*, *b*/*b*, X'/X'. The result is shown in Table 6. As expected from the type of mating, the eight classes are segregated in equal numbers (χ² = 5.7, d.f. = 7, P = 0.70-0.50). The χ² subdivided into its six components, *i. e.*, the χ²s for the segregations of the three single factors and others for linkage among the three single factors also indicate no significant deviation from the expectations. This gives a conclusive evidence for the conclusion arrived at by Aida (1930) that the fused (*f*) is governed by a single gene and that the +, *f* segregates independently from the *b*, *B* and the *r*, *R*. In other words, the +, *f* is neither linked with these color genes. All the fish of the pheno-

TABLE 6. Backcross of a triply heterozygous brown male ($f/+$, b/B , X'/Y^R) to fused white females (f/f , b/b , X'/X'). f = fused, $+$ = normal, B , R = brown, B , r = blue, b , R = orange-red, b , r = white

SYMBOL OF OFF- SPRING	PARENTS		OFFSPRING																Total
	♀	♂	$+B,R$ ♀	$+B,r$ ♂	$+b,R$ ♀	$+b,r$ ♂	fB,R ♀	fB,r ♂	fb,R ♀	fb,r ♂	fB,R ♀	fB,r ♂	fb,R ♀	fb,r ♂	fB,R ♀	fB,r ♂	fb,R ♀	fb,r ♂	
(f^2n)	$f/f, b/b, X'/X'$	$f/+ , b/B, X'/Y^R$	0	32	26	0	0	25	40	0	0	30	28	1	0	30	26	0	238

χ^2 for segregation of the $+$, $f = 0.27$, $P > 0.99$

χ^2 for segregation of the B , $b = 0.067$, $P > 0.99$

χ^2 for segregation of the R , $r = 0.067$, $P > 0.99$

type r except for a single exception are females and all the fish of the phenotype R are males as expected from the type of mating in which r is born in the X and R is resident in the Y . A corollary from these results is that the genetic sex-determining mechanism of the wild medaka from Nagoya is also XX for the female and XY for the male as in domesticated varieties as well as the wild type from Kyoto as demonstrated by Aida (1921).

It is worth while to note herein that the sex-ratio among offspring does not deviate significantly from a 1 ♀ : 1 ♂ ratio, when the fused (f) female is mated to male of either $d-rR$ strain or the wild type. Unpublished data indicate that in the reverse type of mating, namely, females of either $d-rR$ strain or the wild type are mated with males of the fused (f) variety, the offspring frequently show aberrant sex-ratios. Full account on the problem will be given elsewhere.

A single exception f, B, r ♂ may either be a recombinant ($f/f, b/B, X'/Y'$) arising as the result of crossing over between the X' and Y^R chromosomes or a male with the genotype $f/f, b/B, X'/X'$ produced by genic imbalance between heterosomal and autosomal sex-genes. The exception was tested by normal white females ($+/+$, b/b , X'/X') of our $d-rR$ strain. Unfortunately, however, he died before reproducing.

Breeding experiments with wild fused and phenylthiourea-induced fused

A question was asked whether the wild fused was hereditary or not because there was a possibility that a naturally appeared mutant population might be thriving, being isolated in the particular region where they were found. In order to answer the question, the following sorts of matings were carried out: (1) matings of the wild fused to the wild fused, (2) matings of the wild fused to the wild normal, (3) matings of the hereditary fused (f) to the wild fused. Matings of the phenylthiourea-induced fused to the phenylthiourea-induced fused were performed in addition. These breeding experiments were rather difficult because of weakness of these anomalies. The results from successful matings are tabulated in Tables 7 and 8. The F_1 offspring yielded by these crosses are all normal in respect to vertebral condition, *i. e.*, none of the fused being produced. It is obvious that both the wild fused here referred to and the phenylthiourea-induced fused are nonheritable.

TABLE 7. Offspring of wild fused mated with wild fused,
with wild normal and with fused (*f*).
B, R = brown, *b, r* = white

MATINGS		COLOR PHENOTYPES OF PARENTS	F ₁	
♀	♂		NORMAL	FUSED
wild fused ♀ No. 1	wild fused ♂ No. 2	<i>B, R</i> <i>B, R</i>	76 71	0 0
wild fused ♀ No. 2	wild fused ♂ No. 2	<i>B, R</i> <i>B, R</i>	44 47	0 0
wild fused ♀ No. 1	wild normal ♂ No. 1	<i>B, R</i> <i>B, R</i>	19 19	0 0
wild fused ♀♀ Nos. 3, 7	wild normal ♂ No. 2	<i>B, R</i> <i>B, R</i>	13 10	0 0
Fused (<i>f</i>) ♀♀ Nos. 1-3	wild fused ♂ No. 1	<i>b, r</i> <i>B, R</i>	4 5	0 0
Fused (<i>f</i>) ♀♀ Nos. 4-6	wild fused ♂ No. 2	<i>b, r</i> <i>B, R</i>	75 78	0 0

TABLE 8. Breeding experiments with fused fish induced by phenylthiourea
during early embryonic stages. The offspring are not sexed.
B, R = Brown, *b, R* = orange-red

MATINGS		COLOR PHENOTYPES OF PARENTS	F ₁	
♀	♂		NORMAL	FUSED
Induced fused ♀ No. 1	Induced fused ♂ No. 2	<i>B, R</i> <i>b, R</i>	69	0
Induced fused ♀ No. 2	Induced fused ♂ No. 3	<i>B, R</i> <i>b, R</i>	95	0

DISCUSSION

The present study is concerned with vertebral anchylosis of the fish. There is another vertebral abnormality in fishes in which vertebral column is curved dorso-ventrally without anchylosis of vertebrae, *viz.*, the hunchback or lordosis. In this type of anomaly, the number of vertebral centra remains unchanged.

Before discussing the present result, it will not be out of place to refer to the latter type (lordosis). Gordon (1934) reported a hunchback in the platyfish, *Platy-poecilus maculatus*, in which the character was inherited in a quite obscure manner. He claimed that the spinal deformity is not a simple recessive character. The anomaly of the lordotic type has been reported also in the guppy, *Lebistes reticulatus* (Kirpichnikov 1935, Goodrich *et al.* 1943, Rosenthal and Rosenthal 1950) and in the swordtail, *Xiphophorus helleri* (Rosenthal *et al.* 1958). Occurrence of lodoscoliosis in the striped panchax, *Aplocheilus (Panchax) lineatus*, was reported by Hisaoka (1957). Not all these anomalies have been successfully submitted to genetic analysis. The lordotic anomalies that have been analyzed indicate that lordosis is a recessive character. Kirpichnikov's case is a noticeable exception. He claimed that the *Curvatus* in the guppy was dominant to the normal.

Aida (1930) described that he found lordotic anomalies in the bream (*Pagurus tumifrons*), the carp (*Cyprinus carpio*), the ayu (*Plecoglossus altivelis*). Since, however,

these fishes have not been subjected to genetic analysis, it is not certain whether they are hereditary or nonheritable. In the medaka, *Oryzias latipes*, Aida (1930) reported that the wavy (*w*) is an autosomal recessive. Takeuchi (1960) in our laboratory performed a detailed study on the wavy (lordosis) of the medaka. He confirmed not only Aida's conclusion but pointed out that there are a number of modifiers aside from the principal gene (*w*).

Returning to our main theme, *viz.*, vertebral anchylosis, a study of the genetic fused (*f*) reported herein gives an additional evidence of the conclusion arrived at by Aida (1930) that this mutant is (1) an autosomal recessive and (2) independent of all known color genes and the sex. The convincing data pertaining to (2) are presented here for the first time. Vertebral anchylosis was also reported to occur in the trout (*Trutta fario*) by Schäferna (1923). According to Wunder (1949), the vertebral shortening and anchylosis characteristic of a race of the carp (*Cyprinus carpio*), the Aischgrund carp, cultivating in certain districts of Germany, is hereditary. He proposed to designate this sort of anomaly as chondrodystrophy and postulated that the anomaly is produced by a defect of ossification in the anteroposterior axis of the vertebral column and a hypertrophied ossification in the dorso-ventral axis.

The present study demonstrates that there are two types of vertebral anchylosis in the medaka, *i. e.*, hereditary and nonheritable. It is, therefore, hazardous to assume *a priori* without breeding experiment that all the abnormal fish having vertebral anchylosis, found either in nature or under domestication, are hereditary. In the cases of the wild fused reporting herein, some unusual environmental situations might have been encountered during embryonic life in the locality where these anomalies were found. The idea naturally suggested itself that the anomaly might have arisen through some chemical substance introduced in the paddy fields where these wild fused were found. Consequently, we asked the farmer whether he had sprayed some organic insecticides over his rice-paddies. The answer was affirmative; Parathione and Folidol were sprayed in the summer months of 1955 and 1956.

Attempts have been made to duplicate such deformities by means of these chemicals by Tomita and Matsuda (1961). These agents were found to produce severe deformities such as reduction in embryonic body, abnormal eyes and defect in circulatory system. Although deformity induced by these insecticides is severe, the notochord is little affected. Embryos with an abnormal notochord are very rare. We have not yet succeeded in rearing treated embryos to reach young or maturity. Although it seems likely that the wild fused might have arisen under the influence of the insecticides during embryonic life, no definite conclusion, therefore, can be drawn as to the etiology of the wild fused herein referred to. Goldschmidt (1935, 1935b, 1938) introduced the term phenocopy. We have an excellent review by Landauer (1948). Whether wild fused and phenylthiourea-induced fused represent phenocopies of the fused (*f*) is dependent on the definition of the term. While both

anomalies are the mimics of the genetic fused (*f*) in respect to the anal fin-ray frequency. Embryologically, however, phenylthiourea induces twisting of the notochord while the genetic fused (*f*) does not show such twisting.

If we define a phenocopy as an environmentally induced phenotype which corresponds exactly to a mutant phenotype in its developmental origin, these anomalies cannot be regarded as true phenocopies in a strict sense. They only represent phenocopy-like anomalies or false phenocopies. According to Stern (1955), however, the terms true and false phenocopies have only limited validity. It is possible that the wild fused and phenylthiourea-induced fused may have their true genetic counterparts in mutants to be appeared in future.

SUMMARY

1. In 1957 a number of the medaka (*Oryzias latipes*) having vertebral anchylosis resembling the genetic fused (*f*) were found in a certain region on the outskirts of Nagoya. Characteristics of the "wild fused" and the genetic fused (*f*) are presented.

2. Breeding experiments performed on the genetic fused (*f*) confirm Aida's (1930) conclusion that it is a Mendelian recessive and independent from known color genes and the sex.

3. Breeding experiments with the wild fused indicated that the vertebral anchylosis is nonheritable. These anomalies might have arisen from some unusual environmental condition during embryonic life.

4. Artificial induction of nonheritable anchylosis is successfully performed by treating early embryos of normal strain with phenylthiourea.

5. It is pointed out that there are two kinds of vertebral anchylosis in this fish, *i. e.*, hereditary and nonheritable.

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