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Growth and Differentiation of Sex in the Pre-Adult Guppy, *Poecilia reticulata* (Peters)

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Abstract

For the purpose of distinguishing the sexes in the living juvenile guppies, the newborn fry were reared during 10 weeks, and their growths of body length and anal fin length, the changes brought about by the growth and the position of the anal fin on the body, and several secondary sex characters were measured every week. The rearing conditions included a constant average temperature at 24.3°C, 12 hours' illumination per day, and sufficient feeding.

The body length of the male was slightly larger than that of the female during the immature period, but no significant difference was observed between their means. The growth of the body length fits von Bertalanffy's growth equation with the asymptotic limits of 20.0 mm in the male and 29.8 mm in the female.

The anal fin is always situated more anteriorly in the male than in the female. From this fact it is more or less possible to distinguish the sexes of guppies through the immature period from the time of birth. The male anal fin length grows abruptly during the 6–8th weeks after birth, on the contrary the female one does with an almost invariable rate.

In the male, the relative growth of the anal fin length to the body length is composed of three periods excluding the first week, where the I period (1–4th weeks) is due to an isauxesis, the II period (5–7th weeks) due to a striking tachyauxesis, which may coincide with the sexual maturation period and accompanied by a larger individual variation, and the III period (8–10th weeks) is due to a bradyauxesis, during which the maturity is almost completed. Whereas, in the female it is composed of two periods, where the I period (0–2nd weeks) is due to a slight tachyauxesis, and the II period (3–10th weeks) is shown by a bradyauxesis, in which the maturation may occur accompanying a larger individual variation and may be completed.

The appearance order of external secondary sex characters in the male is at first the enlargement of the third ray in the anal fin and of the ventral fin, next the appearance of black spots on the tail sides, and at last the coloration on the body sides.

The guppies, *Poecilia reticulata*, show sexual dimorphism when adults, but it is difficult to distinguish any differences between males and females during their immature period from their external appearances. As soon as the guppies come to maturity and their body length attains about 12 mm, the males begin to

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develop secondary sex characters on the body sides[1], but the body length at maturity flucutates considerably due to various factors, especially environmental conditions. Therefore, there are some instances where the sex is not always discernible, although the body length of an individual has already reached 12–14 mm. If one can judge sexes of immature guppies from only external appearances, the life cycle regarding age, growth, pre-adult mortality, sex ratio, population dyanmics, and some other experimental observations will become possible under controled initial sex ratios. From such a point of view, comparing the growth process and the changes of the external form during the immature period between males and females which were discernible after attaining maturity, several results have been obtained as reported in the following chapters.

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Material and Method

The guppies used in this experiment descended from those which had been reared in the Laboratory of Biology of Fish Population, Faculty of Fisheries, Hokkaido University since 1960. During over a 10-year period those guppies were not crossbred with other strains. The guppies treated here may have been identified as a kind of wild type or their hybrids, because their males were multifarious, but not remarkable as to caudal fins.

For this experiment, glass aquaria (24×20×22 cm) filled with about 7 litres of water respectively were placed in the water-bath in the laboratory dark-room. The newborn guppies were separately taken one by one into those aquaria, and every individual was reared and observed throughout 10 weeks. As the partition plates made of opaque plastics were put between the aquaria, each fish was completely isolated keeping itself from visibility. Water temperature was maintained from 24.0°C to 24.6°C, being regulated by a thermostat system inside the water bath. The aquaria were illuminated from above by fluorescent lamps for 12 hours daily with a timing device instead of natural light, consequently the other half of day was dark enough. All the fish were sufficiently fed once a day in the morning with powdered dried *Daphnia*. The water plant (*Anacharis canadensis*) and sand sterilized by boiling were reasonably placed in the aquaria in order to provide them with natural environmental conditions as closely as possible.

The measurements on definite body parts of the fish were carried out under anaesthesia by means of a projector once a week, in addition to the observations of the morphological changes of their ventral and anal fins. After being kept in a 1/5,000 MS–222 solution for about three or four minutes to quiescence, the fish were put on a hole glass for the measurements. The definite points on the projected figure of the fish, to which the ends of the measuring body parts corresponded, were plotted on the tracing paper covering the projective plate of the projector. After plotting, each fish was quickly put back into the aquarium where it was before, in order to shorten the time in narcotized state which might influence its growth and physiological conditions. On the plotted tracing papers, the distances between those points that measured the length of body parts are as follows (Fig. 1):

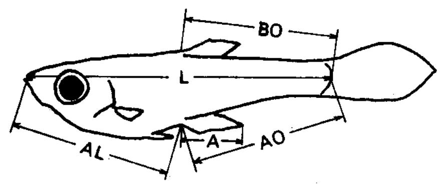

Fig. 1. Diagram to show the measured parts of the body.

L (body length): from the tip of the upper jaw to the base of the caudal fin;
AL: the distance from the tip of the upper jaw to the anterior basal end of the anal fin;
AO: the distance from the anterior basal end of the anal fin to the base of the cadual fin;
BO: the distance from the anterior basal end of the dorsal fin to the base of the caudal fin;
A (anal fin length): the largest length from the anterior basal end to the tip of the anal fin.

Results

Growth of body length

The average body lengths and their 95% confidence intervals every week end from the birth day are shown in Table 1. The female is slightly larger than the male at birth, but this size relation reverses from the first week to the 8th week, and then it reverses again at the 9th and 10th weeks, however no significant difference was observed between the male and the female in the mean body lengths for all ages in weeks. As Walford's finite differences diagram of the body lengths might be accepted as a straight line for the male as well as the female, the regression lines were calculated by the method of the least squares as follows (Fig. 2):

For the male $$L_{t+1} = 0.921\, L_t + 1.58\,,$$

and for the female $$L_{t+1} = 0.960\, L_t + 1.19\,,$$

where L_t and L_{t+1} are the body lengths at the time, t and $t+1$, respectively. Consequently the asymptotic limit of the body length was estimated as 20.0 mm for the male and 29.8 mm for the female. Thus the growth of body length fits von Bertalanffy's growth equation,

$$L_t = L_\infty \{1 - e^{-k(t-t_0)}\}\,,$$

Table 1. *Mean body lengths, L, at each age, compared by the Student's t-test and t′ values in the method of Cochran-Cox between male and female. Variance ratio tested by the F-distribution. The number of individuals observed was 26 (male 12, female 14) every week. * or ** shows respectively a significance level below 5% or 1%.*

Age (weeks)	Averages and their 95% confidence intervals (mm)		Variance		Variance ratio	t	t'
	male	female	male	female			
0	6.93±0.21	6.96±0.20	0.1082	0.1146	1.0591	0.7601	
1	7.99±0.17	7.86±0.27	0.0900	0.2185	2.4275	0.6363	
2	8.83±0.33	8.76±0.41	0.2636	0.5154	1.9552	0.1003	
3	9.79±0.43	9.53±0.41	0.4518	0.4985	1.1034	1.1042	
4	10.50±0.45	10.31±0.56	0.5127	0.9254	1.8050	0.5925	
5	11.33±0.38	11.02±0.63	0.3591	1.1931	3.3224*		0.9145
6	12.13±0.40	11.71±0.63	0.3936	1.1946	3.0351*		1.0825
7	12.89±0.32	12.54±0.68	0.2591	1.4092	5.4388**		1.0029
8	13.55±0.24	13.29±0.61	0.1382	1.1223	8.1208**		0.8581
9	13.93±0.21	14.02±0.57	0.1045	0.9869	9.4440**		0.3203
10	14.26±0.21	14.61±0.47	0.0973	0.6662	6.8469**		1.4831

where L_∞ is the asymptotic limit of growth in the body length, k the growth coefficient, and t_0 the corrective value of t at which $L_t=0$. The growth equations of the body length were practically calculated as follows (Fig. 3):

For the male

$$L_t = 20.0\,\{1-e^{-0.0803(t+4.87)}\}\,,$$

and for the female

$$L_t = 29.8\,\{1-e^{-0.0408(t+6.13)}\}\,.$$

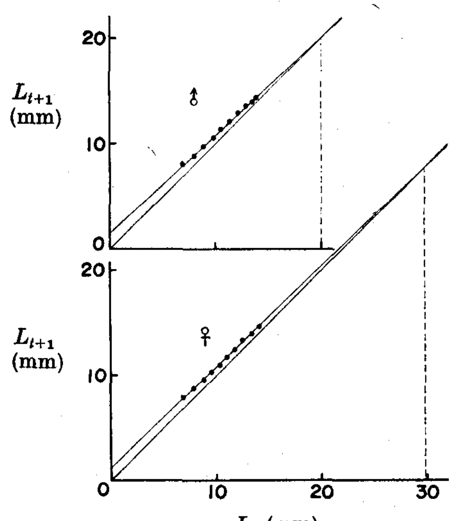

Fig. 2. Walford's diagram for mean body lengths.

This fish is ovo-viviparous. If it is assumed that the above growth curves are fit in the embryonic period as well, the value of t_0 may denote approximately the time of embryonic growth, for the periodic parturition has already been observed in this species. According to the correlogram analysis regarding the weekly number of newborn fry by a senior author, Hamai (unpublished), the reproductive cycle of this strain of the guppies was probably about five weeks under the temperature condition of 22–24°C, and further for the other strains 4.04 weeks (25.5°C)[2] and 4.44 weeks (22.2°C)[3] have been observed. The value of t_0 in the male is similar to the period of reproductive cycle, but the female t_0 is slightly larger. Consequently t_0 may be merely a corrective term, and

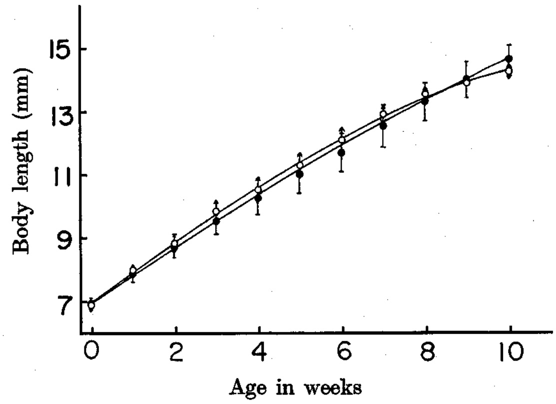

Fig. 3. Growth curves of body length expressed by von Bertalanffy's growth equation. Open circles show mean body lengths of the male, and closed circles those of the female. Vertical lines with arrowheads represent 95% confidence intervals of the means.

it may be the case where the embryonic growth pattern differs from that of the immature period after birth. Moreover, it seems that the value of L_∞ are evaluated somewhat lower than the actual values, since some errors might be comprised in those estimated values, because these growth equations were calculated from only the values of body lengths until 10 weeks of age and those after 11 weeks were not used in calculation; or there might be a possibility of existence of a breaking point between the pre-adult and adult growth curves.

Differential growth to sexual dimorphism

From a point of view of relative growth, as several external characters bore sexual differentiations through growth in the immature guppies, the lengths of body parts, *AL*, *AO*, and *BO* (Fig. 1), were taken into account (Fig. 4). Although in the female the lengths of these body parts draw a straight line in relation to the body lengths, in the male they are curvilinear or divided into two straight lines breaking at about the 12.5 mm body length, therefore the sexual differences become remarkable over this size. Whereas the sexual difference in *BO* is relatively small, and below the 12.5 mm body length it is hardly seen (Fig. 4). Owing to these facts *BO* was taken aside from the question now. But slight sexual differences appear on *AL* and *AO* even below the 12.5 mm body length, and in addition the relation between each of these parts and the body length are reversed by the sex, i.e. *AL* of the female is always larger than that of the male, on the contrary *AO* of the female is always smaller than that of the male, so that the ratio of those values must emphasize the sexual differences. The ratios, *AO*/*AL*, are always higher in the male than in the female (Table 2), and the sexual differentiation of these ratios become markedly recognizable after the body length has reached about 11 mm

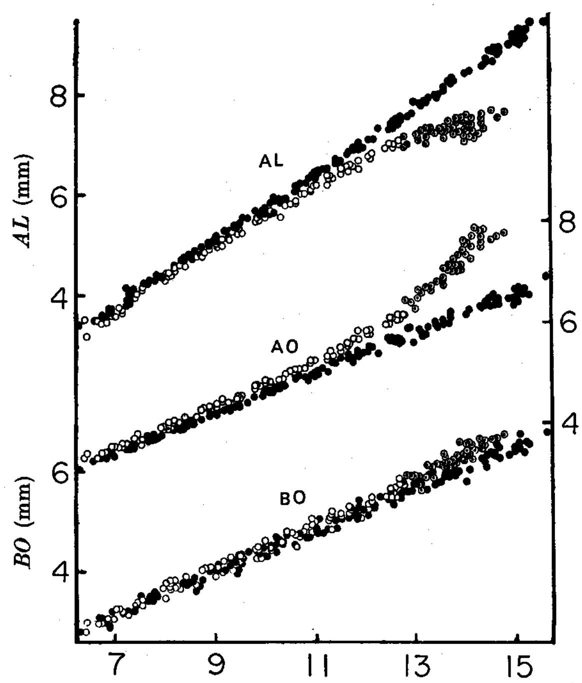

Fig. 4. Relation of body parts to body length. Open circles show individual values of male, closed circles those of female, and dotted circles those of males with black spots on their tails.

(Fig. 5), which corresponds with the body length of 5-week-old guppies in average (Fig. 3).

This fact implies that the anal fin of the male is situated more anteriorly than that of the female. The average values of AO/AL and their 95% confidence intervals at each age every week are shown in Table 2. These ratios fall down until the 5th week for both sexes, however after this age though those of the female continue to decrease somewhat slowly until the 10th week, on the contrary those of the male rise up continuously from the 6th week to the end of the experiment. Extremely significant differences were observed in the average ratios, AO/AL, between the male and the female every week, especially through the later periods (Table 2). The weekly changes of the ratio, AO/AL, were calculated as follows, t being the age in weeks (Fig. 6):

For the male $AO/AL = 0.9441 - 0.0492\,t + 0.0059\,t^2$,

and for the female $AO/AL = 0.8823 - 0.0264\,t + 0.0010\,t^2$.

The occurrence of remarkable differences between sexes is, therefore, in the 5th week, after which period the sexual difference gradually becomes visible macroscopically at a different situation of the anal fin.

Table 2. *Mean ratios, AO/AL, at each age and their comparisons between male and female. Comparison of means performed by the t-test in the case of equal σ and by the method of Cochran-Cox in the case of unequal σ. Variance ratio tested by the F-distribution. Number of individuals measured was 26 (male 12, female 14) every week.* **: below 5% significance level, **: below 1% significance level.*

Age (weeks)	Averages and their 95% confidence intervals		Variance $\times 10^3$		Variance ratio	t	t'
	male	female	male	female			
0	0.957±0.021	0.898±0.012	1.1263	0.4498	2.5040	5.4409**	
1	0.885±0.011	0.846±0.010	0.3152	0.2912	1.0824	5.7032**	
2	0.869±0.010	0.824±0.008	0.2720	0.1947	1.3972	7.5406**	
3	0.852±0.009	0.806±0.010	0.2220	0.2991	1.3474	7.1910**	
4	0.841±0.008	0.792±0.010	0.1715	0.3099	1.8070	7.7341**	
5	0.840±0.005	0.778±0.007	0.0735	0.1647	2.2408	14.2125**	
6	0.858±0.007	0.767±0.012	0.1313	0.4287	3.2650*		14.1260**
7	0.895±0.016	0.751±0.010	0.6036	0.3295	1.8379	16.7833**	
8	0.934±0.021	0.738±0.010	1.0824	0.2890	3.7453*		18.6135**
9	0.990±0.027	0.726±0.009	1.8145	0.2195	8.2665**		20.4334**
10	1.029±0.024	0.711±0.010	1.4516	0.2971	4.8859**		26.6779**

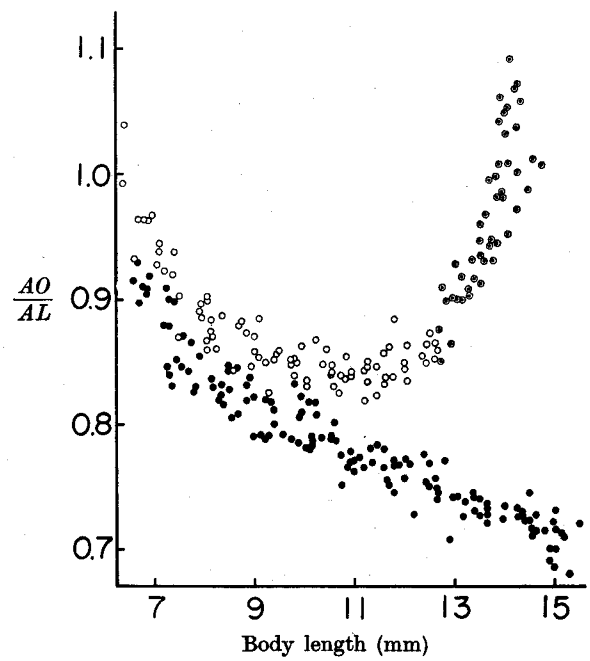

Fig. 5. Relation between *AO/AL* and body length. Marks are the same as those in Fig. 4.

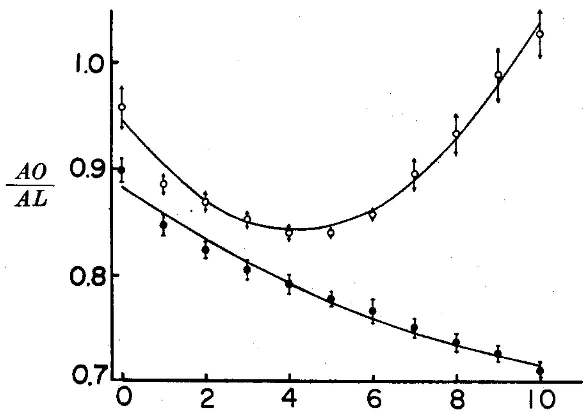

Fig. 6. Weekly average values of AO/AL. Open circles: male, closed circles: female. Mathematical representation of curves is given in text.

Manifeststion of external secondary sex characters

The appearance processes of external secondary sex characters, viz. the shape of the anal fin, the black spots on the body sides, and others, which have been already known in general, are shown in Table 3. At first the third ray in the anal fin became longer and thicker than the other rays after 5.9 weeks in average (11.8 mm body length), next the black spots appeared on the caudal zone of the body side (6.7 weeks, 12.7 mm body length), and at last red or orange, or variegated colouration appeared (8.7 weeks, 13.8 mm body length). The caudal fin also differentiates both sexes, however it was not treated here because of difficulties in measuring multifarious forms of the male caudal fin.

The growth of the anal fin length is shown in Table 4 and Fig. 7. During the period from the time at birth to the 5th week, no significant difference was

Table 3. *Appearance processes of external secondary sex characters in male. Number of observations is 12.*

Item	Age (weeks)			Body length (mm)		
	Range	Average	Variance	Range	Average	Variance
The third ray in the anal fin became larger.	5-7	5.9	0.7992	11.5-12.4	11.8	0.0525
Black spots appeared.	6-8	6.7	1.1876	12.5-14.0	12.7	0.1137
Colouration	8-10	8.7	0.9803	13.6-15.2	13.8	0.1079

Table 4. *Mean anal fin lengths, A, at each age and their comparisons between male and female. Procedure of comparison and number of fish observed are the same as those in Tables 1 and 2.*

Age (weeks)	Averages and their 95% confidence intervals (mm)		Variance × 10		Variance ratio	t	t'
	male	female	male	female			
0	1.41±0.07	1.47±0.07	0.1213	0.1333	1.0989	0.6746	
1	1.75±0.04	1.72±0.08	0.0412	0.1891	4.9858**		0.7299
2	1.96±0.07	1.95±0.11	0.1259	0.3587	2.8491*		0.1667
3	2.13±0.10	2.12±0.11	0.2481	0.3612	1.7204	0.1446	
4	2.33±0.12	2.27±0.14	0.3648	0.5777	1.8714	0.6960	
5	2.54±0.17	2.39±0.15	0.6713	0.6786	1.1947	0.4672	
6	3.11±0.28	2.55±0.14	1.8856	0.6088	3.0972*		3.9548*
7	3.76±0.32	2.67±0.14	2.5544	0.6214	4.0910**		6.7998**
8	4.12±0.15	2.84±0.14	0.5697	0.5952	1.0448	13.3646**	
9	4.22±0.09	2.97±0.15	0.1942	0.6384	3.2874*		15.9236**
10	4.25±0.10	3.09±0.12	0.2247	0.4384	1.9510	15.9795**	

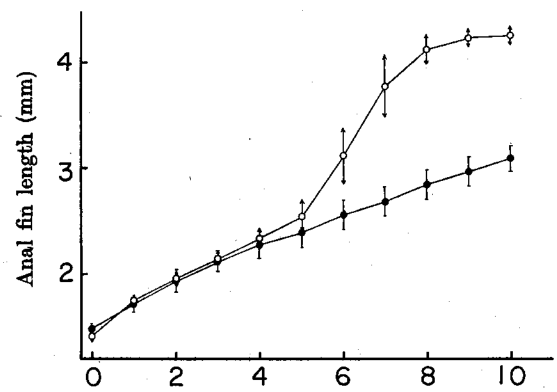

Fig. 7. Growth of anal fin length. Marks are the same as those in Fig. 3.

observed in the average lengths of the anal fin between the male and the female, but extremely significant differences were detected in the advanced period after the 6th week. The anal fin length of the male quickly grows larger than that of the female during the 6–8th weeks, and after the 9th week the growth rate slows down. In contrast with this male growth pattern of the anal fin, the female one is monotonous and demonstrates nearly a straight line till the 10th week excluding the first one or two weeks (Fig. 7). It must therefore be considered that the maturity of the male guppies has considerably advanced during this period of the 6–8th weeks. The relative growth of the anal fin toward the body length may support this fact more clearly.

The relative growth of the anal fin may be exactly expressed by a sigmoidal

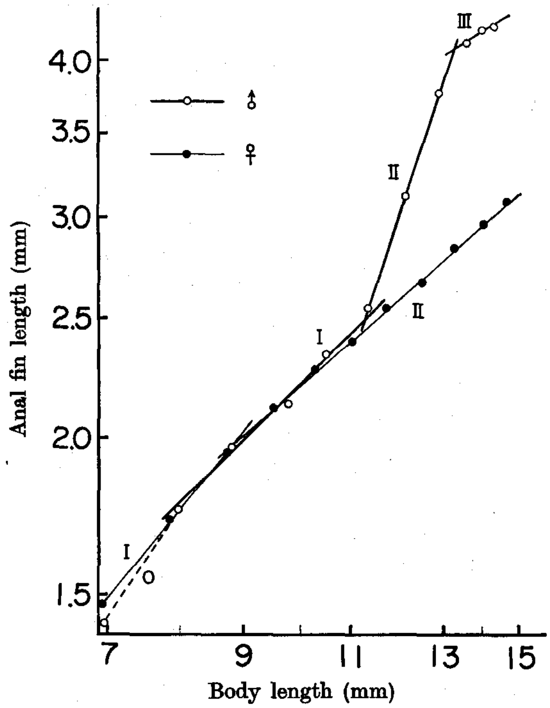

Fig. 8. Relative growth of anal fin length to body length. The Roman numerals show the allometric stages.

Table 5. *Values of the constants in the allometric equation,* $y=kx+B$, *where* $y=\log A$, $x=\log L$, *and* $B=\log b$. *Differences between k's were tested by* $t=(k_1-k_2)\{(n_1+n_2-4)/(\Delta_1+\Delta_2)(1/S_{x1}+1/S_{x2})\}^{1/2}$, *where* $\Delta_i=S_{yi}-k_i^2S_{xi}$, *S being the sum of squares, when* $\sigma_{k1}=\sigma_{k2}$, *and* $t=(k_1-k_2)/(s_{k1}^2+s_{k2}^2)^{1/2}$, *where* s_{ki}^2 *is variance of* k_i, *when* $\sigma_{k1}\neq\sigma_{k2}$.

Sex	Stage	Range of age in week	n	$k\pm$(95% confidence interval)	log *b*	Test of difference between *k* *t*
Male	O	0- 1	2	1.52		I♂–II♂ : 14.43**
	I	1- 4	4	1.015±0.103	−0.671	II♂–III♂ : 12.90**
	II	5- 7	3	3.032±0.188	−2.793	I♀–II♀ : 9.54**
	III	8-10	3	0.597±0.390	−0.059	I♂–I♀ : 2.40
						III♂–II♀ : 1.78
Female	I	0- 2	3	1.231±0.086	−0.870	I♂–II♀ : 2.06
	II	3-10	8	0.878±0.011	−0.535	II♂–II♀ : 28.05**

or more complicated curve in the case of the male and by a slightly convex curve in the female, but approximately it can be represented by dividing into four allometric stages in the male and into two allometric stages in the female (Table

5 and Fig. 8). Between the successive stages significant differences in the equilibrium constants of allometry, k, are proved in both sexes. The male shows a tachyauxetic k-value, 1.52, calculating approximately from $(\log A_1 - \log A_0)/(\log L_1 - \log L_0)$ in the 0 stage (the first week), and in the following I stage (1–4th week) its relative growth ratio (k) falls down to an isauxesis. The reason why this falling-down occurs during the earliest period in the male is unknown, but from the fact that between the I stage of the male and the I or II stage of the female no significant difference of k is found (Table 5), it may be considered that the relative growth is not so variable at least till the 4th week between the male and the female. After the 5th week a sudden change in the allometry occurs in the male demonstrating a remarkable tachyauxesis, and after having attained 8 weeks of age the allometry reverts to a slow state being rather a bradyauxesis, which shows no significant difference comparing with the II stage of the female. The II stage of the male corresponds, of course, to the period of an abrupt growth of the male anal fin as shown in Fig. 7, by which maturity is clearly completed, accordingly, the III stage of the male may denote a relative growth in the adult form after maturity has been attained.

The processes of form changes in the anal and the ventral fins were observed by purusing the same individuals (Fig. 9). In the male (individual No. 8) the third ray of the anal fin began to grow thick and long surpassing the other rays after the 6th week, accompanying an elongation of the ventral fin. In the male the ratio, A/L, jumped up to a greater value of 0.24–0.31 after the 6th week from the value of 0.20–0.22, which had been shown by the male before this period and by the female throughout 10 weeks (Fig. 9). The elongation of the ventral fin in this male individual occurred a little later from the beginning of a rapid growth of the anal fin, and this period might probably be the 8th week. The black spots appeared on the tail sides during the 7th week, and the body coloured during the 9th week. In contrast with the male, the female individual (No. 3) did not show any change in the fin shape and the body colour throughout the whole period. Therefore, it may be reasonably said that the first obvious external sex differentiation appears at about the 6th week after birth.

Discussion

Excluding the newborn fry (the 0th week), the body length of the male was slightly larger than that of the female in the immature period through the 1–8th weeks, although no significant difference was detected (Fig. 3). In the newborn fry both sexes have almost the same body length in average. But, when the fish attains 9–10 weeks of age, the above relation begins to reverse, and throughout the adult periods the female grows strikingly larger than the male. It is also found from the values of L_∞ in the growth equations. It is also valid in the other strain of this species[4]. Bodily excellence in the adult female is widely seen in many other fish species too.

Although no significant difference was detected in the mean body length between both sexes, as mentioned above, in fact, there was found an interesting

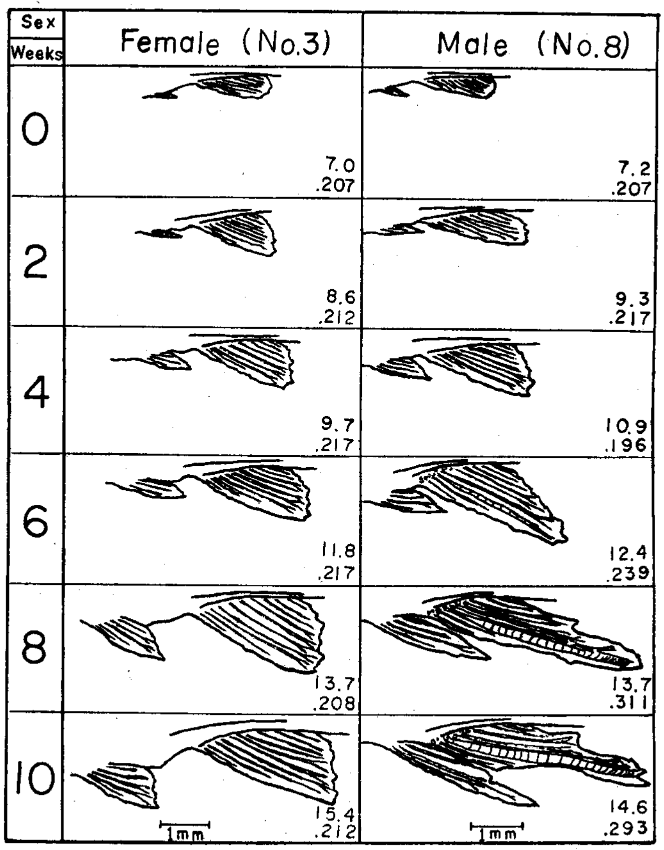

Fig. 9. Diagramatic sketch representing form changes of ventral and anal fin. In each column, the upper figure shows the body length in mm and the lower shows the ratio of anal fin length against body length, A/L, at the week concerned.

phenomenon that remarkable differences occurred in the variance ratios between both sexes after the 5th week and in the patterns of weekly changes in variances (Table 1). In the male the variance of the body length changes so wavily that it rises up to higher values during the 3-6th weeks and thereafter falls down to lower values during the 8–10th weeks nearly equal to the initial weeks. In the female, higher values are found from the 4th week, and there is a tendency that, after the highest value is attained at the 7th week, the variance falls down, however from the 4th to the 10th week no significant variation appears. The coefficient of variation shows also a similar tendency of change to that of the variance (Table 5 and Fig. 10). It is not clear, from the present experimental data, what has caused these changes of variance, but it is naturally accepted that both sexes have the same relatively small variances at the early periods which may increase

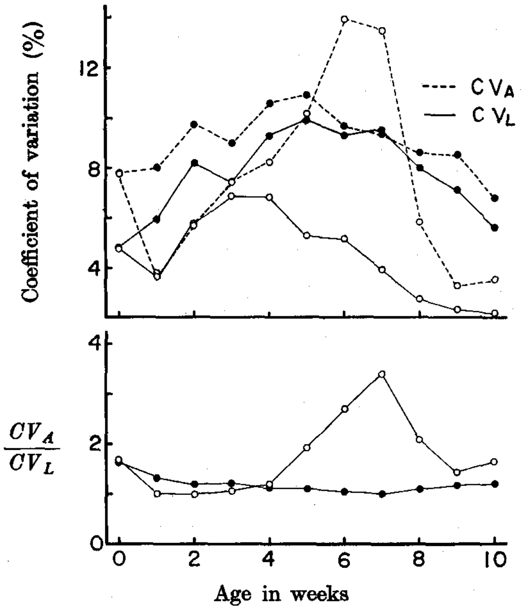

Fig. 10. Weekly changes of coefficients of variation of anal fin length (CV_A) and of body length (CV_L), and their ratios (CV_A/CV_L) in male (open circles) and female (closed circles).

as the guppies grow or advance in age, i.e. the individual variation extends with age. The pattern of increase in the variance till the 4th week may be due to the above consideration for both sexes. But the pattern of decreasing variance after the 5th week cannot be explained by these considerations. Thereupon a hypothesis may be necessary to the explanation for this phenomenon, i.e. in the course of maturation the growth is greatly affected by a large variation of maturation velocity or intensity with variable physiological conditions according to each individual, and accordingly, the time from the beginning to the completion of maturation is very variable, even though the maturity size is relatively definite in a narrow range. Here the so-called biological minimum size is meaningful.

It should be taken into account that the commencement of the maturity in the male occurs at the time that the ratio of AO/AL in the male shifts from descent to ascent (Fig. 5 and 6) and that significant differences appear in the mean lengths of the anal fin between the sexes (Fig. 7). The synchronism in the sudden changes of position, size and shape of the anal fin regarded as a reproductive organ suggests quick maturity of the male. On the contrary, the female does not show such a critical period. The continuous decreasing of AO/AL in the female perhaps means a continuous enlargement of the body cavity, accordingly a perpetual development of the ovary. This proved by the increase of litter size along with the growth of body (Hamai, unpubished).

In the male the variance of the anal fin length rises up from lower values in the 0–4th weeks to higher values in the 5–8th weeks and then falls down to lower values in the 9–10th weeks almost similarly to those of the initial weeks, whereas in the female the ascent in the initial periods occurs as well as in the male but there exists no significant change during the later half periods (Table 4). These changing patterns in the variance are nearly the same as those of the body length. These facts demonstrate clearly the variability of growth during the period of maturation. Therefore, the growth of the body length synchronizes with that of the anal fin, and probably individual variations of the former are parallel to those of the latter, consequently the hypothesis derived from the growth pattern of the body length may hold good to that of the anal fin.

The above considerations are also convinced from the coefficients of variation (Table 6 and Fig. 10). For the sake of contrast between the relative growth and the relative variation, the ratio of the coefficients of variation of the anal fin length (CV_A) versus those of the body length (CV_L) is most useful, and the changes of the ratio, CV_A/CV_L, may demonstrate very clearly the period of maturity, corresponding to the allometric stages in both sexes. That is to say, it is easily recognizable that in the male the O stage has a decrease of CV_A/CV_L, the I stage shows almost constant ratios, the II stage accompanies an extremely remarkable rise of the ratios, and the III stage shows a slight decrease of the ratios, and in the female the changes of CV_A/CV_L are very little in general, however the I stage shows a slight decrease of the ratio, and the II stage shows almost a constant ratio. From these facts it may be well deduced that the maturation causes the large variation of growth in the male of the sexually dimorphic species.

Table 6. *Coeffients of variation* (*CV*) *and their ratios.*

Age (weeks)	Male			Female		
	Coefficient of variation (%)		CV_A/CV_L	Coefficient of variation (%)		CV_A/CV_L
	L	*A*		*L*	*A*	
0	4.75	7.82	1.65	4.87	7.85	1.61
1	3.76	3.67	0.98	5.95	7.99	1.34
2	5.81	5.72	0.98	8.20	9.72	1.19
3	6.87	7.40	1.08	7.41	8.97	1.21
4	6.82	8.20	1.20	9.32	10.59	1.14
5	5.29	10.20	1.93	9.91	10.90	1.10
6	5.17	13.96	2.70	9.32	9.67	1.04
7	3.95	13.44	3.40	9.47	9.34	0.99
8	2.74	5.79	2.11	7.99	8.60	1.08
9	2.32	3.30	1.42	7.08	8.51	1.20
10	2.19	3.53	1.61	5.58	6.78	1.22

The male anal fin is situated anteriorly when compared to that of the female throughout the immature period since the newborn fry, so that the discrimination of sex about living bodies of the pre-adult guppies could be possible to some extent, even though it is considerably difficult. The anal fin of the male is an intromittent organ. Before the time of copulation, the male guppies pursue the female from behind[5)], therefore it may be advantageously adapted for the purpose that the anal fin of the male be located more forward on the body. As shown in Fig. 5 and 6, the position of the anal fin changes continuously, and its individual variations are partially overlapped between both sexes at a time from birth to about the 11 mm body length, even though the sexual differences are clear in the mean values during this period. Therefore the discrimination of sex may be accurate by the *AO*/*AL* ratio or by the relative position of the anal fin after the 5th week or about the 11 mm body length, even if it becomes possible even earlier. From the viewpoint of the elongation of the male anal fin it is validly possible after the 6th week or about the 12 mm body length.

At any rate, even though it is difficult to discriminate the sexes from the external characters of the fry body, for sure the sexual dimorphism already appears externally from the time of birth, and continues the differentiation during the immature periods till the commencement of maturity, thereafter it is markedly emphasized and a great discrepancy occurs between both sexes. The spots and colours are only indications at later or adult periods.

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