

HERMAPHRODITISM WITH SELF-FERTILIZATION, AND THE MONTHLY EXTRUSION OF UNFERTILIZED EGGS, IN THE VIVIPAROUS FISH *LEBISTES RETICULATUS*

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THIS communication is a preliminary report on two independent phenomena: first, functional hermaphroditism, resulting in self-fertilization, has been observed in a fish and eighteen of her descendants; secondly, in all but two families in which more than one female became adult, one or more fish has extruded groups of unfertilized oocytes into the surrounding water. Such extrusions have occurred in fish unrelated to the hermaphrodites. Both kinds of abnormal parturition were discovered by keeping virgin females alone in small glass vessels (usually in 600 c.c. of water). The baby fish were always isolated before puberty and usually within 24 hr. of birth.

Tables 1 and 2 show that the ages of both the extrusion of the first group of oocytes and the first fatherless birth are not only variable, but also often

higher than the median ages of death. Also some fish (not chosen at random) are removed from the populations either to be mated for genetical experiments or killed for histological examination. Therefore a meaningful estimate of the frequencies of fish which perform either of these two abnormal parturitions, and comparison of these frequencies between families, involves statistical procedures the assumptions of which require discussion. A full analysis was delayed until the population here discussed and kept in the Department of Biometry, University College, Gower Street, London, was discarded in July 1957.

Lebistes reticulatus is a typical poeciliid fish in which internal fertilization is effected by copulation. The anal fin of males develops into an intromittent organ

Table 1

1 Parentage	2 No. of families	3 No. of females surviving 100 days	4 Median ages at death	5 No. of self-fertile females	6 No. of females extruding oocytes	7 Corrected median age at first oocyte extrusion
Original stock	5	30	500-500	3	0	∞
Females outcrossed to males from stock B	3	01	500-500	0	21	300-300
Fatherless and grandfatherless (females descended from outcross to stock B)	17	121	400-400	6	40	400-400
Females outcrossed to <i>Macrulatus</i> males	0	81	500-500	0	41	400-400
F ₁	1	10	300-300	0	1	252
Males outcrossed to females from <i>Macrulatus</i> stock	5	04	900-1,000	0	41	300-300
<i>Macrulatus</i> stock and F ₁ B male × <i>Macrulatus</i> female	7	68	400-400	0	15	400-400

Table 2

Self-fertile mother			Fatherless family					
	Age at birth of first litter (days)	Age when removed from population	Ref.	No. of litters	No. of births dead	No. of births alive	No. of adults	
							Female	Intersex
<i>D9</i> proposita	210	510m ±	<i>D99</i>	1	0	1	1	0
<i>DD25</i> fatherless	197	357m ±	<i>J</i>	1	0	3	3	0
<i>D19</i> "	185	540d	<i>K</i>	1	0	5	5	0
<i>DD22</i> "	206	367m ±	<i>L</i>	1	0	1	1	0
<i>P3 F</i> , with stock <i>B</i>	184	236d	<i>a</i>	2	0	17	12	0
<i>P8</i> "	184	404d	<i>b</i>	2	0	8	8	0
<i>P25</i> "	206	407d	<i>c</i>	4	0	105	31	1
<i>P26</i> "	204	632m	<i>t</i>	2	0	9	8	0
<i>P29</i> "	215	531d	<i>e</i>	1	0	24	6	0
<i>P29</i> "	650	683d	<i>n</i>	1	2	10	6	0
<i>P41</i> "	663	888d	<i>o</i>	1	0	17	6	0
<i>S7</i> "	769	770m - 848d	<i>x</i>	2	7	20	3	1
<i>S2</i> "	687	538m ±	<i>v</i>	1	2	20	15	2
<i>d20</i> fatherless intersex	215	443d	<i>θ</i>	9	47	25	19	0
<i>d1</i> "	230	473d	<i>μ</i>	4	3	9	7	0
<i>d5</i> "	379	380m ±	<i>ε</i>	1	0	4	4	0
<i>F7</i> "	277	378d	<i>ι</i>	1	0	1	1	0
<i>#7</i> "	317	318d	<i>κ</i>	1	0	1	1	0
<i>#5</i> "	140	415d	<i>phi</i>	2	0	8	2	0

during the first eight weeks of life. Sperm from a single copulation can sire up to eight litters^{1,2}. These are born at approximately monthly intervals^{1,2} (but see also Fig. 4), and the young are 5-6 mm. long at birth.

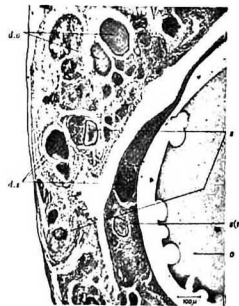
Leistes males are bright coloured and variable; wild-type females are monomorphic and have a typical telostean shape. Usually the males are heterogametic (or XY) and the females homogametic (or XX). XX and YY males and XY females can be functionally normal^{1,2}.

Nineteen individuals all related to one another have produced fatherless offspring. Eighteen of these were superficially normal females. The anal fin of the nineteenth developed into an intromittent organ coincidentally with the birth of its first litter. With this it made courtship movements but did not sire

any young on unrelated females. It was found to contain testicular tissue by M. J. Hollingsworth, now of the University College of Ghana, Achimota, who failed to discover any testicular tissue in two other self-fertile mothers, *P8* and *S7*. However, Prof. J. Z. Young, of University College, London, discovered testicular tissue in the self-fertile females *S7* and *#7* (Figs. 1 and 2). I wish to thank both those colleagues, and their assistants, for this histological co-operation.

The self-fertility is, therefore, assumed to be due to hermaphroditism and not to parthenogenesis, as was stated previously^{1,2}.

The self-fertile proposita *D9* occurred in an inbred aquarium stock given me by Maurice Wilson, now of Queen Mary College, London. This was segregating for alleles of the loci *Flavus*¹, *blond*² and *gold*¹, all affecting pigmentation and scorable on females. These were used for diagnosing paternity when self-fertile or related females were mated. The sex ratio among certainly sired offspring was normal. There

Fig. 1. Section of ovotestis of $\mu 7$

Key: *d.o.*, developing oocytes; *d.s.*, developing seminiferous tubules; *s*, follicle of mature oocyte; *e*, seminiferous tubule containing mature sperm; *e'*, ruptured seminiferous tubule liberating sperm (this rupture is not certainly an artefact of preparation and it may be by such means that the sperm and the oocyte come into contact).

Fig. 2. Another section of the ovotestis of $\mu 7$. [Key as for Fig. 1.]

is no evidence for self-fertilization first occurring in an animal after it had been in the company of a male.

Table 1 shows the incidence of the self-fertility and oocyte extrusion in the animals born in the collection. The first column gives a classification of the animals in terms of parentage.

Sired and fatherless females are classed together as the original 'stock' (excluding twenty-one females including the propogita, because they were kept in aquaria where young, but not oocytes, could have been detected). Females (self-fertile, and fatherless and sired daughters and grand-daughters of self-fertile females) were outcrossed to males from two stocks, for which I wish to thank Dr. Caryl Haskins, of the Carnegie Institution of Washington. Stock *B* was descended from a recent capture in Trinidad; the *Maculatus* stock was a subculture of that produced by Winge, and thus X_3Y_{Ma} in his nomenclature⁴. Sons, both fatherless and sired, of self-fertile females derived from outcrosses to *B* were crossed to females from the *Maculatus* stock, that is, X_3X_3 .

In Table 1, column 2 shows the number of families in each of the six groups, and column 3 the number of females who survived 100 days. Column 4 is a rough estimate of their median age of death. The families within a given group are not homogeneous among themselves.

Column 5 shows the number of females which have been self-fertile. The absence of self-fertile females among the progeny of *Maculatus* males, compared with the progeny of stock *B* males, is significant after allowance has been made for the detailed longevity of the females tested. Column 6 gives the number of females which extruded oocytes (excluding six which extruded them synchronously with full-term young, two which had previously given birth to fatherless litters and four which had previously given birth to sired litters).

Table 2 shows some details about the self-fertile mothers and their fatherless litters, and the extract from the pedigree given in Fig. 3 shows the relationship of the parents of the five sired families (*D*, *DD*, *P*, *S* and *Z*) in which self-fertile individuals have segregated.

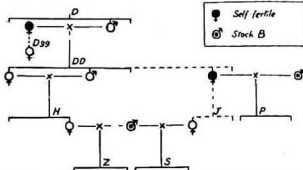


Fig. 3. Part of pedigree showing relationship of parents of the five fatherless families in which hermaphrodites segregated.

The range of the mothers' ages at the birth of their first fatherless litter means that some apparent females must die before they become old enough to manifest their hermaphroditism by a parturition, and perhaps before any testicular tissue has developed. Such variation in expression would prevent any Mendelian ratios being observed even if the hermaphroditism were due to a change of allele at a single locus. It also makes it difficult to compare such a change with the non-functional hermaphroditism which seems characteristic of the development of lower vertebrates⁴.

The third column shows the age at which the mother was removed from the population either by death (*d*), killing (*k*) or introducing a male (*m* + if producing sired offspring, or *m* - if subsequent litters were recognized as products of self-fertilization on the evidence provided by their alleles for the three pigment genes).

Within the fatherless families there is positive correlation between the number of litters, the size of these litters, and the number of miscarriages and deaths before puberty; that is, as would be expected, the greater the development of the testis tissue the greater the number of conceptions but also the greater the disturbance of the follicular pregnancies.

DD20, the one certain fatherless offspring born to *D9* after it had been mated, was in its second sired litter born when *D9* was 694 days old. The second litter of *X8* was produced when the mother was 293 days old, an extrusion of unfertilized oocytes having

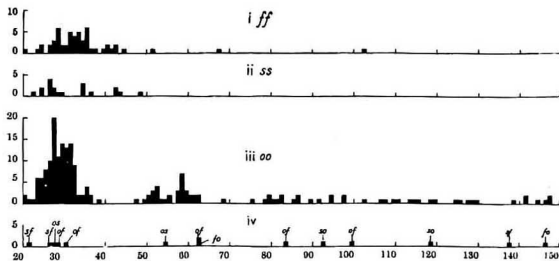


Fig. 4. Distribution of intervals between births

Absolute, length of interval in days. Ordinates, number of intervals. *f*, fathered litter; *s*, fatherless litter; *o*, oocyte extrusion (for omissions see text)

intervened at 265 days. All other intervals between fatherless litters are shown in histogram II of Fig. 4. This distribution is statistically indistinguishable from that observed for the production of sired litters (histogram I). This confirms the histological findings that the testis tissue is transitory and usually does not re-develop.

I have discussed elsewhere¹ the significance of the increased expression of self-fertility in the F_1 generation between two inbred stocks which is unexpected on certain interpretations of heterosis. Table 2 corrects several previously published errors in addition.²

The last three columns of Table 2 give the superficial sex of the adult products of self-fertilization. Among the animals scored as females, many more than the five who were self-fertile must be presumed to be actually or potentially hermaphrodites. This sex ratio suggests that the hermaphrodites are XX , which is supported by the phenotypes and the sexual history of the four males. All were under-colored, and no Y chromosome empty of alleles for male epigenic patterns has yet been reported. Three were sterile, though one of these had histologically normal testes. The fourth provided evidence for its XX constitution by siring only females, sixteen of which were by a *Maculatus* stock female.

Among fatherless fish, the three colour alleles segregated in the 3:1 ratio characteristic of selfing in hermaphroditic plants.

The hereditary factor responsible for making XX zygotes develop into hermaphrodites is not X -linked, and is not an autosomal recessive allele either with or without a delayed zygotic effect. Because the crosses were made with the *Maculatus* stock which suppresses their penetrance, we do not know whether the determiners are inherited differently through males or females; that is, I have not discovered whether hermaphroditism is dependent on an autosomal allele with dominant but variable penetrance, or on a cytoplasmic factor comparable with those influencing hermaphroditism in some species of plants.³

Histogram III of Fig. 4 shows the distribution of 200 of the 222 intervals observed between two extrusions of oocytes. The 22 observed intervals ranged in length from 165 to 447 days. The distribution of these intervals is significantly different from that of the intervals between births. 126 (that is, more than half) ranged between 21 and 39 days, and this group did not deviate significantly from the normal distribution. Its mean was 29.4, and its standard deviation 3.4. This is significantly lower than the mean observed for sired litters, which is 33.0, with standard deviation 5.16 even when the three extremes are neglected. The next group of thirty-five intervals between oocyte extrusions ranged from 48 to 69 days, again normally distributed among themselves with a mean of 67.1 and standard deviation of 4.6. These groups are called 'single' and 'double' intervals, respectively. The range of the single intervals is too great for groups to be apparent if higher multiples of these do occur among the longer intervals between extrusions.

Table 3 shows that (except for the oldest two fish) the greater the age of a fish at its first extrusion of oocytes the greater the mean number of its subsequent extrusions. Omitting from consideration inadequately tested animals, 73 females first extruded before 300 days but not one extruded more than five times; though of the 84 which began later, eight

Table 3

Age at first extrusion	Mean No. of extrusions	Ratio of single intervals to all intervals
100-199	2.18 ± 0.19	0.450 ± 0.076
200-250	2.41 ± 0.22	0.433 ± 0.064
300-350	2.65 ± 0.34	0.587 ± 0.060
400-450	2.90 ± 0.38	0.714 ± 0.121
500-550	3.15 ± 0.45	0.815 ± 0.135
600-650	3.3 ± 0.44	0.809 ± 0.104
700-750	4.0 ± 0.48	0.877 ± 0.076
800-850	3.25 ± 0.32	0.805 ± 0.089
900-999	1.9 ± 0.5	0.0 (1 interval)

produced six to ten groups of eggs. This is unexpected, because the earlier in its life a fish extrudes oocytes the longer is the expected (and observed) period before its death in which subsequent extrusion could occur. This correlation is partly explained by the observation that the greater the age of a fish at its first extrusion of oocytes, the higher is the proportion of single intervals between consecutive extrusions to all such intervals.

Extrusion reveals a failure of the follicles to re-absorb unfertilized oocytes. In the females which begin this extrusion late in life and then extrude regularly, this failure may be described as a senility and is then mainly dependent on a monthly rhythm of oocyte maturation⁴. In the animals that extrude earlier, and afterwards extrude intermittently or not at all, other causes of ovarian inadequacy must be suspected, some of which seem transitory. In some families, perhaps in all, the females which extrude oocytes live longer (for example, a mean increase of more than 300 days) than their sisters who do not. This suggests that extrusion itself compensates for failure of other processes, and prevents the animals becoming 'egg bound'.

Histogram IV of Fig. 4 shows the distribution of the intervals between two different kinds of parturition. Two of 201 and 740 days and 3 of 210, 340 and 385 days are omitted. This histogram reveals that oocyte extrusion is not evidence that the ovary has become incapable of pregnancy. Also, when it is borne in mind that the interval before a sired birth depends on when the male was introduced as well as on the mother's receptivity, and that the senile oocyte extrusion may not occur until many months after sperm has been exhausted, it is seen that oocyte extrusion coincides reasonably well with the rhythm of other births. It has also occurred six times with them. Therefore, the breakdown of the follicular walls may be a failure of an incipient pregnancy because the oocyte was not fertilized; that is, the extrusions seem more comparable to the menstruation than to the ovulation of mammals.

The gross synchronizing regulations which take place in the follicles just before term⁵ provide an explanation of the significantly longer intervals observed between fertile parturitions and perhaps for the positively skew distribution of these intervals.

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⁴ Winge, O., and Dillmann, E. *Hereditas*, 1, 68 (1947).
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¹⁰ Casperl, E., *Advanc. Genet.*, 3, 1 (1948).