216. *Unstable Multilunar Modifying Genes in the Silkworm, Bombyx mori.*^{*)} I

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(Comm. Dec. 12, 1967)

Introduction. Since about twenty years ago, I had a special interest in unstable genes of the silkworm and I accumulated the pertinent experimental data, collecting, on the other hand, the literature on this subject. So far as I am aware, the definition of genetic instability has not yet been settled, and many cases of various kinds have been treated under this name, the observed organisms covering a wide range from higher animals and plants to micro-organisms. Though the classification of all known cases according to the causes of instability is difficult, they may be roughly divided into the following five groups: a) genic, b) chromosomal, c) cytoplasmic, d) developmental, and e) physiological.

The results of Lilienfeld (1929), Demerec (1932), Imai (1934), Malinowski (1935), Sirks (1950), Tanaka (1951, 1954, 1959, 1961, 1962), Lindegren (1953, 1956), Hazelwood *et al.* (1955), Shifriss (1955), Brink (1956, 1958), Zamenhof *et al.* (1956, 1958 a, b, c), Ishikawa (1957), Smith *et al.* (1957), Sand (1957), Sand *et al.* (1960), Lewis (1958), Schwarts (1958, 1960), Moav *et al.* (1960), Sand (1957), Sand *et al.* (1960), Sandler *et al.* (1960), Moav (1961), Nagai (1962), Babos *et al.* (1962), Scholtissek (1962), Barnett *et al.* (1963), Dawson (1964), Frydenberg (1964), Grigg (1965), Alderson (1966), Alderson *et al.* (1966), Darlington (1966), Nasim (1966), Ryasty (1966), and Fincham *et al.* (1967) seem to be due to genic instability.

Some are more related to chromosomal instability as shown by McClintock (1953), Watanabe (1954), Hinton (1955), Snoad (1955), Britton *et al.* (1956), Frost *et al.* (1959), Smartt (1960), Murray *et al.* (1964), Ball (1966), Croft (1966a, b), Hughes *et al.* (1966), Judd (1967), Morse (1967), and Newmeyer *et al.* (1967). Ephrussi (1951) reported a cytoplasmic effect on the unstable state of the yeast cell. Developmental instabilities have been observed by Bankowska (1956) and Sakai *et al.* (1965a, b). The cases of Schaffner (1937), Avi-Dor (1953) and Shifriss (1956) may be classified as at least partly physiological.

^{*)} Contribution from the Silk Science Research Institute, Hyakunin-machi, Shinjuku, Tokyo.

Notwithstanding that genetic instability covers heterogeneous cases as to their nature, they seem to imply many important problems on the structure and action of genes in higher as well as in microbial organisms.

Materials. Our multilunar strains come from two sources, one was the material sent by Dr. S. Ishiwata of Kyoto Sericultural College in 1915, while the other material was given by Mr. D. Kasai, Director of Fukushima Branch, National Sericultural Experiment Station, in 1917. Both materials are of Chinese origin. Since then, they have been continuously bred in the author's laboratory, and have given rise to various strains by selection.

Characteristics of the marking. The multilunar marking consists of a number of more or less brownish, somewhat rounded spots on the dorsal side of the larva. The spots are present on 3rd, 5th, and 8th segments¹⁾ without exception, being superposed upon the "eye-spot" in the 2nd segment. Various multilunar types are distinguished by the number and distribution of spots on 4th, 6th, 7th, 9th, and 10th segments.

The multilunar marking develops only when $+^p$ gene (normal marking) co-exists with L , the major gene for this character. When the larva is homozygous for p (no-marking or plain) instead of $+^p$, the marking shows only a faint trace of the characteristic spots which is hardly perceptible even for skilled eyes.

Symbols. In the description of multilunar types, 2nd, 3rd, 5th, and 8th segments can be excluded because they exhibit the spots without exception, but the spots are always absent on the 1st segment. Thus, a type furnished with spots on all abdominal segments is denoted by L_{467910} and a type in which the spots are absent from all abdominal segments, except 5th and 8th, by L_0 , and so on.

The spots are usually paired on both sides of the dorsal median line, but they are not unfrequently single. If special exactitude is needed, we represent an unpaired spot by putting a dot upon the segment number, for instance, $L_{467910\cdot}$. In most cases, however, paired and unpaired spots are not distinguished from each other and only the segment numbers where the spots occur are given.

I have assumed two groups of multilunar modifiers: L-developing and L-inhibiting modifiers. L-developing modifiers are indicated by D_4, D_6, D_7 , etc. and L-inhibiting modifiers by I_4, I_6, I_7 , etc. The method of testing modifier constitutions will be mentioned later.

1) Larval segments of the silkworm are, for the sake of simplicity, counted from the 1st thoracic segment down to the 7th abdominal segment. The 1st abdominal segment is our 4th segment, and our 10th segment means 7th abdominal segment.

Fig. 1. Several multilunar types (1)

a) L_{167910} b) L_{167910} c) L_{467910} d) L_{167910} e) L_{4679}

Numerals on the left represent segment numbers.

Fig. 2. Several multilunar types (2)

f) L_{467} g) L_{46} h) L_{467} i) L_{67} j) L_{467} k) L_{46} l) L_6 m) L_0

Stability of the major gene. The multilunar marking is a product of the major gene L and a number of modifiers. In contrast to very unstable L -modifiers, the major gene L is perfectly stable. During fifty years of rearing, I have observed more than hundred generations of multilunar strains, but have never met either with spontaneous or induced mutations of L , while rather frequent mutations have been found in other larval markings such as normal ($+^p$), moricaud (p^M), striped (p^S), zebra (Ze), and so on.

Instability of L -modifiers. In order to avoid the deleterious

effects of strict inbreeding in the silkworm, we preferred a method of moderate inbreeding for the maintenance of various strains as follows: Different multilunar types in a culture (lot) are separated, counted and recorded. The standard type of the strain is selected, and mated *inter se* to obtain several batches of eggs. Some three batches are taken and each batch is cut into three pieces. The larvae hatched from three one-third pieces of the batches are mixed together and reared together in the same lot. This process is repeated in each generation. Table I shows the results for the latest ten generations of several strains.

The table clearly shows that stability widely varies by the strain. L_{467910} is perfectly stable, while L_0 comes next, proving that there is no positive relation between number of spots and stability.

Table I. Actual numbers and percentages of standard types in latest ten generations of various strains

Standard type	L_0		L_6		L_{46}		L_{67}		L_{467}		L_{4679}		L_{46710}		L_{467910}	
Strain	l_6		l_5		l_{14}		l_{54}		l_1		l_4		l_{2a4}		l_2	
Generation	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
1	329 334	98.5	171 258	66.3	43 316	13.6	152 211	72.0	123 327	37.6	291 368	79.1	105 489	21.5	296 296	100.0
2	270 295	91.5	298 328	90.9	173 422	41.0	122 132	92.4	154 227	67.8	84 264	31.8	52 358	14.5	290 290	100.0
3	290 292	99.3	146 260	56.2	104 264	39.4	336 349	96.3	170 173	98.3	189 235	80.4	73 362	20.2	352 352	100.0
4	269 305	88.2	293 376	77.9	110 247	44.5	133 136	97.8	107 141	75.9	196 301	65.1	50 461	10.9	450 450	100.0
5	329 347	94.8	36 258	14.0	21 269	7.8	227 233	97.4	239 246	97.2	33 359	9.2	95 382	24.9	304 304	100.0
6	45 50	90.0	55 102	53.9	65 309	21.0	164 280	58.6	170 180	94.4	111 175	63.4	27 363	7.4	336 336	100.0
7	411 416	98.8	157 266	59.0	104 251	41.4	181 248	73.0	110 119	92.4	236 266	88.7	19 235	8.1	434 434	100.0
8	355 421	84.3	162 266	60.9	82 307	26.7	142 300	47.3	222 237	93.7	326 363	89.8	51 345	14.8	107 107	100.0
9	234 287	81.5	119 165	72.1	184 259	71.0	202 256	78.9	304 328	92.7	437 498	87.8	40 212	18.9	417 417	100.0
10	175 180	97.2	34 78	43.6	237 350	67.7	139 140	99.3	220 223	98.7	119 139	85.6	79 481	16.4	344 344	100.0
Average	92.4		59.5		37.4		81.3		84.9		68.1		15.7		100.0	
Order	(2)		(6)		(7)		(4)		(3)		(5)		(8)		(1)	

The numerator of fraction shows the actual number of standard type larvae and the denominator shows the total number of larvae in the same lot.

The percentages indicate the degree of stability of each strain. "Order" means the order of stability, from higher to lower, the order of instability is naturally just reversed.

Stability depends, it seems to me, upon the degree of balance of the acting various multilunar modifier genes. L_{467910} involves the maximum number of L-developing modifiers, but has no L-inhibiting modifiers. It is perfectly homogeneous so far as to modifiers are concerned. All L-developing modifiers act in the same direction, and perhaps are firmly united together. L_0 is thoroughly free of L-developing modifiers, but it may or may not involve some L-inhibiting modifiers such as I_9 and I_{10} , as was proved by gene analysis, though they cannot be phenotypically distinguished. It is somewhat heterogeneous. This seems to account for the fact that L_0 takes second place to L_{467910} in stability. Other multilunar types are mixtures of various modifiers, or much more heterogeneous of them, whence come their instabilities.

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