FURTHER OBSERVATIONS ON *LYTHRUM SALICARIA*

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Received October 15, 1931

In an earlier paper (EAST 1927), it was shown that the inheritance of heterostyly in *Lythrum salicaria* can not be described in a complete and satisfactory manner by the hypothesis that mid-styled plants are conditioned by a factor M , that short-styled plants are conditioned by a factor A which is epistatic to M , and that long-styled plants are conditioned by the action of the two recessive allelomorphs am . It is true that Mrs. BARLOW (1923), Miss VON UBISCH (1925), and I have studied crosses where the results fit the assumption that only these two factors are involved; but Mrs. BARLOW has also studied several crosses in which the results could not be interpreted so simply; and later, working with plants which came from Mrs. BARLOW's cultures, I have observed a similar complexity of behavior. There is no difficulty in accounting for Shorts. Shorts, in the experience of all workers on *Lythrum*, are conditioned by the factor A , which is always found in the heterozygous condition. And the factors A and a are distributed in the regular orthodox manner. The disturbing feature of the situation is the behavior of certain Mids which consistently throw about 10 percent more "Long" gametes than would be expected on the two-factor hypothesis. For example, as I reported earlier, Longs crossed with pollen from certain "homozygous" Mids gave 19 Longs to 191 Mids, while these same "homozygous" Mids, crossed with pollen from Longs, gave 9 Longs to 121 Mids. Additional data from similar crosses now make the total figures read as follows: "homozygous" Mids used as males on Longs have given 50 Longs to 438 Mids, and Longs used as males on "homozygous" Mids have given 22 Longs and 200 Mids. Thus about 10 percent of both the megaspores and the microspores of these so-called "homozygous" Mids carried the factors for long-style.

These facts, together with consistent data from other crosses, were interpreted (EAST 1927) by assuming that the particular Mids with which we were dealing owed their existence to two duplicate linked factors M_a and M_b , which have a lethal effect if in the homozygous condition and show a crossover value of 10 percent. Under such an assumption four types of Mids may exist, namely

$$(1) \frac{M_a M_b}{m_a m_b}, (2) \frac{M_a m_b}{m_a M_b}, (3) \frac{M_a m_b}{m_a m_b}, \text{ and } (4) \frac{m_a M_b}{m_a m_b}.$$

Type 2 is the "homozygous" Mid under consideration. Types 3 and 4 contain only one factor for Mid and should show no complexity in their behavior.

A reference to the data reported in my earlier paper will show the reader that nearly all crosses involving plants other than certain descendants of those which were received from Mrs. BARLOW conformed in their behavior to the simple two-factor hypothesis. In fact, the way that the behavior of the majority of plants fits this idea is rather remarkable, considering the fact that we are dealing with a species where the plants are highly self-sterile, where plants bearing flowers of like forms are highly cross-sterile, where the percentage of abortive pollen is large, and where the germination of the seeds is poor. From the consistency of the results under the conditions which obtain, therefore, one may conclude that ordinarily pollen and seed elimination are not sufficiently selective to render unsatisfactory the simple two-factor interpretation of heterostyly. We have only the behavior of the aberrant Mids to explain. Altogether there were five of these so-called "homozygous" Mids, though only two were tested in numerous crosses. Their behavior was consistent with the balanced lethal hypothesis. No critical proof of the balanced lethal condition has been offered, however; it is merely a plausible theory. And it will probably remain only a plausible theory, for critical evidence could be offered only by testing the genetic constitutions of such a large number of plants that the game would hardly be worth the candle.

Since the earlier data were reported, I have been able to make a number of additional observations, some of which appear to be worthy of record. (1) Attempts at a cytological analysis of *Lythrum* material have resulted in failure. The species is highly unsatisfactory for such work. The chromosomes, of which there are apparently 24 pairs in each form, are very small, rendering it practically impossible to say whether the aberrant Mids are different from ordinary Mids. (2) It is impossible to say whether the families which should show high percentages of non-viable zygotes on the balanced lethal hypothesis actually do differentiate themselves from families where no lethal combinations are present. A large number of germination tests were made; but in all types of combination, with one exception, the percent of seeds germinating varied from 7 to 60. The one exception is a set of four crosses where homozygous Mids having presumably but one factor were involved. Here the germination was rather high, being in fact between 65 and 80 percent. Perhaps the fact is significant. (3) Results from some 40 additional families where presumably only one Mid factor was involved show that in such cases behavior is normal. I will report only

the results from families where the germination of seeds was high and the resulting populations were large enough to give some confidence in the evidence.

Plants from families where but one Mid factor was suspected were selfed. Selfing is difficult, of course, and the number of plants obtained is usually small; but 14 Mids gave results which indicated that their constitution was of this type. One of these Mids was crossed four times with Longs. The data are found in table 1. These families are all somewhat larger than one usually obtains; and, as I have already remarked, the germination of the seeds from which they came was between 65 and 80 percent, showing that there was little possibility of a selective elimination of zygotes. *The results show that homozygous Mids can be obtained which throw no gametes carrying Long.*

TABLE 1
Results from crossing Longs with homozygous Mids having but one factor.

NUMBER	ORIGIN	CROSS	RESULT		
			L	M	S
1401	LC×1208-21	L×M	0	162	1
1402	1208-21×LC	M×L	0	151	0
1403	1208-21×LB	M×L	0	95	0
1315	1208-21×1202-49	M×L	1	99	0

In addition to these cases, twenty-one crosses were made between Longs and heterozygous Mids supposed to contain but one factor. Most of the resulting populations were too small to give significant figures, and may be disregarded. There were eight families, however, where the numbers are reasonably large (table 2). Families 1501, 1505, 1506, and 1509 are

TABLE 2
Results from crossing Longs with heterozygous Mids having but one factor.

NUMBER	ORIGIN	CROSS	RESULT		
			S	M	L
1501	LA×MA	L×M	1	58	58
1505	1401-45×1408-43	M×L	0	61	66
1506	1408-43×1401-45	L×M	0	42	56
1509	1311- 2×LA	M×L	1	151	152
1516	LA×1311-20	L×M	0	29	16
1518	1311-69×LA	M×L	0	25	18
1503	1401- 1×1408-43	M×L	0	31	9
1504	1408-43×1401-1	L×M	1	127	25

clearly good one to one ratios. They total 332 Longs to 312 Mids. Families 1516 and 1518 show an excess of Mids, since there are 54 Mids to 34 Longs; but this excess is not enough to make it probable that we are dealing with additional variables. In Families 1503 and 1504, coming from reciprocal matings, there is, on the other hand, a sufficient excess of Mids to make one suspect some complicating factor or factors. The figures are 158 Mids to 34 Longs. I shall speak of this matter again a little later.

From these data and from the other published records (one may pick out obvious cases in the papers of Mrs. BARLOW, Miss VON UBISCH, and EAST), it is clear that the Mids of *Lythrum salicaria* are ordinarily conditioned by a single factor that behaves in the normal manner.

It is only necessary, then, to account for the behavior of the aberrant type of Mid. I have seen five plants definitely of this type. I suspect that some six or eight others that I have observed and some three or four of those that Mrs. BARLOW has observed, have the same constitution. Thus all of those found are from samples of a single stock. We do not know, therefore, whether these aberrant Mids are rare in nature or not. Miss VON UBISCH has not observed them in her German material, and apparently no such type has been observed in the relatively few plants of other trimorphic species that have been investigated. At all events, they are not common.

I stated, in 1927, that Mids having both representatives of the duplicate factors postulated to account for the aberrant individuals ought to be more frequent in nature than those having but one. This was simply a slip of the pen, as a careful reading of the paper will show that what I had in mind at that time was the relative frequency of individuals having duplicate genes in the populations under consideration, these populations being the progeny of plants assumed to have duplicate genes.

As I have already stated, the data concerning the behavior of these aberrant Mids are not of a type to establish the duplicate factor hypothesis. Nevertheless, there are reasons for believing that this hypothesis is the most plausible of those which will fit the facts. One can not utilize any system of independent multiple factors, of linkage without balanced lethals, or of multiple allelomorphs. One can also rule out nearly all of the common forms of reduplication, inversion, and similar types of abnormal chromosome distribution. They will not fit the facts.

It is possible to account for the *origin* of the duplicate linked factors $M_a - M_b$ at 10 units distance from each other by one of the rarer forms of abnormal chromosome distribution; but when this condition has once arisen, normal chromosome distribution is required if the segregations

actually found are to be explained satisfactorily. Duplicate linked factors would be produced if a piece of a chromosome containing the gene M were inserted in an homologous chromosome at the proper point. The mere apposition and continued union of two homologous chromosomes, on the other hand, would not fit the requirements. Attachment at homologous ends presumably would lead to a situation similar to that in the attached-X stock of *Drosophila*; attachment at non-homologous ends probably would result in crossover values higher than 10 percent. Such speculations are unnecessary, however, for the origin of the duplicate linked factors postulated in the balanced lethal hypothesis is not in question. Genes having the same or similar functions are known to exist in various species. So far as I know, no linked duplicate genes have been described; but it is not unreasonable to assume that they may exist.

Unless I have overlooked some of the facts, then, there are only three ways of explaining the behavior of our aberrant Mids, non-disjunction, mutation, and balanced lethals.

If, because of unequal chromosome distribution, a trisomic condition arose in which there were factors $M-M-m$, then unless unknown variables were present to increase the complexity of the situation, the gametes formed would be MM , Mm , Mm , M , M and m . Self-fertilization of this type of homozygous Mid would give 1 Long to 35 Mids. Crossed with Longs, there would be 1 Long to 5 Mids. This condition may possibly be what we have in Families 1503 and 1504 of table 2. I am not inclined to favor this hypothesis, however, for a reason beyond that of OCCAM's test of the validity of hypotheses. In order to obtain the results actually found, other than those in Families 1503 and 1504, one would have to suppose that the non-disjunction $Mm-M$ occurs about three times as frequently as the non-disjunction $MM-m$. One can not deny the possibility that such an assumption accords with the facts. It has been used, I believe, by other geneticists to account for abnormal ratios. Yet evidence is accumulating that non-disjunction results from loose-pairing or non-pairing of chromosomes, and is, therefore, just the opposite of what the term implies. It is a mechanism that is not exact and produces ratios that are not very consistent. This does not accord with our experience where over 50 families involving three plants of the aberrant "homozygous" Mid type have given very consistent results. The consistency of these results—that is to say the uniformity with which these aberrant "homozygous" Mids produce 10 percent of Long gametes—appears to demand a mechanism at least as exact as crossing over, for crossing over itself is a more variable procedure than most biological processes.

The second possibility of interpreting the behavior of these interesting Mids is mutation. Two different correspondents have called my attention to the fact that the simplest interpretation of our data is to assume that a factor M conditioning Mid mutates to m about ten times per 100 gametes. This hypothesis had been considered before the publication of my 1927 paper but had been abandoned for the following reasons. Unless it be assumed that the aberrant type of Mid is an ephemeral condition which Mrs. BARLOW happened to discover at exactly the right moment, a convincing interpretation of its genetic behavior should provide for survival. Since there is no evidence that m ever mutates to M at a higher rate of frequency than is to be expected for any chance mutation and since there is no evidence that natural selection favors Mids, it is obvious that such a high rate of mutation from M to m would soon result in the elimination of all mid-styled plants. Now no such process appears to be going on. On the contrary, plants of the three style-lengths have about the same frequency in nature. Perhaps there is some deficiency of Shorts, but Longs and Mids have been found in approximately equal numbers in the various censuses that have been made. These censuses are as follows (see VON UBISCH 1925).

DARWIN, in England, 148 Longs: 135 Mids: 110 Shorts; KEPPERT, in Germany, 64 Longs: 36 Mids: 44 Shorts; VON UBISCH, in Germany, 161 Longs: 191 Mids: 109 Shorts; EAST, in Massachusetts, U.S.A., 134 Longs: 151 Mids: 122 Shorts.

Both the two-factor hypothesis and the balanced lethal hypothesis satisfy the requirement that plants of the three forms come to equilibrium at approximately equal frequency, if self-sterility, cross-sterility of like forms, random mating, and random survival are assumed. For example, with 48 plants of which 12 are $aamm$, 8 are $aaMm$, 4 are $aaMM$, 3 are $Aamm$, 6 are $AaMm$, and 3 are $AaMM$ —giving equal numbers of Longs, Mids, and Shorts—after 10 generations of random mating the figures are 11.42 $aamm$, 11.44 $aaMm$, 1.04 $aaMM$, 6.03 $Aamm$, 5.02 $AaMm$, and 1.04 $AaMM$.

Similar results flow from the balanced lethal hypothesis. Three generations of random matings are shown in table 3, assuming that types $\frac{M_a m_b}{M_a m_b}$

and $\frac{m_a M_b}{m_a M_b}$ are viable, but not types $\frac{M_a M_b}{M_a m_b}$, $\frac{M_a M_b}{m_a M_b}$ or $\frac{M_a m_b}{M_a M_b}$. As the calculations are rather tedious, I have not carried the problem any further.

It is sufficient to see that there is a general trend toward equilibrium when the three forms of plants are approximately equal, and to note that the single factor Mids will be about nine times as numerous as the duplicate factor Mids.

TABLE 3

*Results of random matings of different types of *Lythrum salicaria* under the balanced lethal hypothesis crossovers 10 percent.*

CONSTITUTION	FREQUENCY IN GENERATION NUMBER			
	1	2	3	4
<i>aamambmamb</i>	16	11.153	13.685	14.306
Total Longs	16	11.153	13.685	14.306
<i>aaM_am_bm_am_b</i>	4	8.564	7.408	7.459
<i>aam_aM_bm_am_b</i>	4	8.564	7.408	7.459
<i>aaM_am_bM_am_b</i>	2	0.479	0.465	0.397
<i>aam_aM_bm_aM_b</i>	2	0.479	0.465	0.397
<i>aaM_aM_bm_am_b</i>	2	1.981	1.317	0.998
<i>aaM_am_bm_aM_b</i>	2	0.959	0.930	0.793
Total Mids	16	21.026	17.993	17.503
<i>Aam_am_bm_am_b</i>	6	6.040	6.744	7.319
<i>AaM_am_bm_am_b</i>	2	3.451	3.543	3.419
<i>Aam_aM_bm_am_b</i>	2	3.451	3.543	3.419
<i>AaM_am_bM_am_b</i>	1	0.479	0.465	0.397
<i>Aam_aM_bm_aM_b</i>	1	0.479	0.465	0.397
<i>AaM_aM_bm_am_b</i>	2	0.959	0.631	0.443
<i>AaM_am_bm_aM_b</i>	2	0.959	0.930	0.799
Total Shorts	16	15.818	16.321	16.193

Another argument which makes the "mutation" theory unworthy of serious consideration derives from the fact that there are Mids, originating from the same stock as the aberrant Mids, which throw no Longs. It would be strange to assume that the factor *M* is mutating rapidly in some individuals and is constant in others.

There is left the balanced lethal interpretation of the behavior of the aberrant Mids. The evidence taken as a whole, including that presented earlier (EAST 1927), makes it the most plausible theory to hold, though I do not claim that it is established beyond reasonable doubts.

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