Retarded development of females, a case of maternal inheritance in a moth, *Ephestia kuehniella*

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**SUMMARY**

Retarded development of females (Symbol: *Rf*) has arisen spontaneously as a new trait in a chromosome mutant strain, which has an autosome fused to the original *W* chromosome. Female imagines with this trait emerge 1–3 weeks later than the males, due to retarded larval development. The proportion of female imagines is low. *Rf* is maternally inherited, it is neither expressed in nor transmitted through males. The evidence suggests that it is a dominant or epistatic factor on the autosomal part of the *W* chromosome-autosome fusion.

1. **INTRODUCTION**

In most organisms maternal inheritance means cytoplasmic inheritance, either true cytoplasmic inheritance or hereditary infection. True cytoplasmic inheritance in animals is mitochondrial in those cases in which the carrier of genetic information has been identified (Beale & Knowles, 1978). However, most cases of maternal inheritance found in animals and studied extensively have been shown to be due to 'pseudoplasmic' inheritance, caused by transmission of an infectious (though not necessarily contagious) agent via the eggs to the subsequent generation (Preer, 1971; Poulson, 1963).

Organisms possessing a *WZ–ZZ* chromosome mechanism of sex determination provide an additional mode of maternal inheritance: inheritance by *W* linkage. This is analogous to paternal inheritance due to *Y* borne genes in organisms with an *XX–XY* sex determining mechanism; and the same reservation applies to both: only very few genes are known on the differential *Y* chromosomes or *Y* chromosome segments or the *W* chromosomes. These are genes which control characteristic and essential functions for the heterogametic sex, such as sex determination and fertility (see, for example, Charlesworth, 1978).

*Ephestia*, like all Lepidoptera investigated, has a *WZ* type of sex determination. The present report describes a new trait in *Ephestia* which fulfils the formal requirement of *W*-linked inheritance: it is restricted to the female sex and is maternally inherited. The new trait arose in a chromosomally abnormal strain which carries a *γ*-ray induced chromosome fusion of an autosome with the *W* chromosome. This allows *W*-linked inheritance of factors located on the original autosome in addition to those located on the original *W* chromosome segment.
2. MATERIAL AND METHODS

The W chromosome mutation $F(A; W)3$, a fusion of the W chromosome with an autosome, was $\gamma$-ray induced (Rathjens, 1974, Traut & Rathjens, 1973). Since then the $F(A; W)3$ strain has been propagated in cytologically controlled single pair cultures, often outcrossed to wild-type males.

All experiments have been performed at room temperature (20–25°C) with single pair cultures, raised in plastic vials of 125 ml volume, which in their turn were kept in larger plastic vials of 500 ml as a means of protection from contamination. Broods were fed with meal prepared from externally sterilized whole wheat grains in a laboratory mill.

As a routine all pairs used for breeding were cytologically examined after successful egg laying. For this purpose Malpighian tubules were fixed in Carnoy's fluid, stained in lactic acetic orcein and examined for the presence and structure of the W chromosomal heterochromatin as an indicator of the state of the W chromosome (Traut & Rathjens, 1973).

Presence or absence of W chromatin was also used for sexing of freshly hatched larvae (Clarke et al. 1975). In this case the whole larvae were fixed in Carnoy's fluid, torn open and stained in lactic acetic orcein. As a control for this type of sexing, imagines of parallel broods, 138 females and 153 males, were sexed by the W chromatin method and proved to be scored correctly.

In the tests for an infectious agent, external sterilization of the eggs was done by immersing them for 90 s in a 1% solution of chloramin T (Merck) either in tap water or in 96% ethanol. They were immediately sucked dry on a Büchner funnel rinsed in tap water and dried again. The antibiotics were administered with the food. For thorough mixing, Aureomycin (3, 0·3 or 0·03 g), Streptomycin (100, 10 or 1 mg), or Penicillin G (10000, 1000 or 100 i.u.) was dissolved in 2 ml distilled water, mixed with 4 g wheat meal and freeze dried. About 1 g of the dry mixture was given to each culture within 24 h before hatching of the larvae; 1–1 1/2 months later additional meal without antibiotics was added.

3. RESULTS

(i) Developmental retardation of females

In the chromosome fusion strain $F(A; W)3$ a new trait, retarded development of females (symbol: $R_f$), appeared spontaneously and was transmitted to the offspring. The characteristic property of this trait is a delayed eclosion of the female adults. The females emerge 1–3 weeks later than the males. This leads to a change in the sex ratio of the moths emerging early in a culture compared to those appearing late. An example of this behaviour is given in Table 1, col. 1.

The delay in the development of the females does not yet appear in the larvae hatching from the eggs. The sex ratio of these larvae is balanced (Table 2, expt 1). The developmental delay and the losses of female larvae therefore must occur later, during larval or pupal development. To decide between these possibilities,
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five broods of the \( Rf \) strain were scored according to stage and sex between 2 and 16 days before eclosion of the first imago (Table 2, expts 2-5). The results demonstrate that male adults not only emerge in advance of the female adults, but males already reach the pupal stage before the females do. The delayed eclosion therefore is caused by a retarded larval development and not (or not only) by delayed development of the female pupae.

Table 1. Eclosion sequence of male and female moths in representative broods from crosses of \( Rf \)-descendants with wild-type animals compared with a wild-type brood: the numbers of emerging imagines are presented in 4-day intervals

| Days | \( Rf \times + \delta \) | \( + \delta \times Rf \) son | \( + \varphi \times + \delta \) |
|------|----------------|-----------------|----------------|
| 1-4  | \( \varphi : \delta \delta \) | 8 : 10 | 3 : 5 |
| 5-8  | \( \varphi : \delta \delta \) | 11 : 10 | 9 : 10 |
| 9-12 | 1 : 29 | 17 : 13 | 16 : 24 |
| 13-16| 1 : 13 | 18 : 15 | 5 : 3 |
| 17-20| 1 : 10 | 5 : 13 | 7 : 8 |
| 21-24| 10 : 7 | 8 : 13 | 10 : 5 |
| 25-28| 8 : 5 | 7 : 6 | 3 : 2 |
| 29-32| 4 : 5 | 6 : 1 | 2 : 7 |
| 33-36| 2 : — | 1 : 4 | 3 : — |
| 37-40| — : — | — : — | — : 2 |
| Σ    | 27 : 109 | 81 : 83 | 58 : 56 |

Table 2. Developmental stage reached by males and females in broods of the \( Rf \) strain at different times between hatching of the larvae and eclosion of the first imago

| Age of the broods: days before emergence of the first imago | Stage and sex |
|----------------------------------------------------------|----------------|
| Expt | No. of broods | Freshly-hatched larvae* | Medium-sized and large larvae† | Pupae‡ |
|------|---------------|----------------|----------------|--------|
| 1    | 10            | 244 : 269     | — : —          | — : —  |
| 2    | 16            |                | 36 : 37        | — : —  |
| 3    | 13            |                | 45 : 27        | — : —  |
| 4    | 5             |                | 130 : 40       | — : 85 |
| 5    | 2             |                | 25 : 1         | — : 38 |

* Sexed according to the presence or absence of \( W \) chromatin.
† Sexed according to the presence or absence of a testis patch; the \( \varphi(?) \)-group includes true females and probably males in a stage before the testes become visible through the dorsal cuticula.
‡ Sexed according to the visible anlagen of the external genitalia.

The overall sex ratio of the moths in \( Rf \) progenies varies considerably, but low female ratios are the rule. Ratios between 0 and 60% females have been found with an average of 26-7%. Since the freshly hatched larvae have a normal sex ratio this reflects a lower viability of the females.
The variation in the overall sex ratio between different Rf broods is statistically significant (\( P < 0.001 \)). This might be due to slightly different conditions in the cultures which could have an enhanced effect on the less viable female sex. It cannot be excluded, however, that part of the variation has also a genetic basis, since the overall sex ratio seems to differ in different sublines of the Rf strain.

Mostly, counting has been given up 1-1\( \frac{1}{2} \) months after the first imago had appeared. This was probably before the slowest imago had emerged. But in some cases counting was continued until no new moths appeared for at least a week. The proportion of females then was only slightly higher than that of a period of 1\( \frac{1}{2} \) months, because only few more adults had appeared.

Most female moths of the Rf broods show varying degrees of disorder of the wing folding. The fore wings are not properly laid back in the normal resting position, so that the hind wings become visible.

### Table 3. Test for transmission of Rf through male and female offspring crossed to non-Rf animals (wild-type, ml- and Us-genotypes)

| Type of cross                  | No. of broods | Rf | Rf(?) | Wild type |
|-------------------------------|---------------|----|-------|-----------|
| Rf daughter × +♂              | 289           | 240| 46    | 3         |
| × ♀× Rf son                   | 9             | 0  | 0     | 9         |
| (+♀× Rf son)♀× +♂             | 8             | 0  | 0     | 8         |

* All broods consisting of at least 20 individuals are included; the average size is 78 ± 33.

A brood was scored Rf or wild type primarily according to the eclosion sequence of the moths. The sex ratio of the adults which emerged during the first 2 weeks was compared to that of the remaining period, using the \( \chi^2 \)-test. A brood was scored Rf when eclosion of females was delayed at the \( P = 0.05 \) significance level. A value of \( P < 0.001 \) was normal in the Rf strain. A brood was judged wild type with \( P > 0.05 \) and a sex ratio close to 1 : 1. Broods with no or a very low proportion of females, in which no statistically significant change in sex ratio with time of emergence occurred, are classed as Rf(?).

(ii) **Maternal inheritance of Rf**

Males and females from Rf broods were crossed to wild-type animals to test the type of inheritance of Rf. Representative examples of these crosses are shown in Table 1, and a summary of all crosses is given in Table 3. Though in some broods the eclosion sequence cannot be inferred clearly due to an extremely low proportion of females it is evident that Rf broods are produced by Rf daughters. Only three exceptional Rf daughters out of 289 crossed to non-Rf males had progenies showing a wild-type eclosion sequence and sex ratio. On the other hand, only wild-type broods are produced when Rf sons are mated to wild-type females. Nearly all daughters transmit the Rf character, sons do not.

Daughters of the +♀× Rf son crosses again produce wild-type broods (Table 3, line 3), while Rf daughters, outcrossed to wild-type males for a period of eight successive generations, do not lose the Rf character. Thus Rf shows a typical maternal inheritance: it is neither expressed in nor transmitted through males.
$W$ chromosomal heterochromatin (arrowheads) in the highly polyploid lobed nuclei of Malpighian tubule cells of (a) a wild-type female imago, (b) an $Rf$ female imago, (c) a female imago of a brood reversed to wild-type eclosion sequence. The scattered heterochromatin in (b) is typical for the presence of a $W$ chromosome autosome fusion while the single $W$ chromatin sphere in (a) and (c) indicates the presence of a $W$ chromosome without an attached autosome.
(iii) Tests for \(W\)-linked inheritance of \(Rf\)

Female meiosis is achiasmatic in *Ephestia* (Traut, 1977). Therefore one cannot make use of crossover events for a genetic analysis. However, if the \(Rf\) character is caused by a genetic factor \(Rf\), located on the autosomal segment of the chromosome fusion, a breakage of the chromosome fusion should result in a removal of the sex specific effects. Breakage of the \(W\) chromosome-autosome fusion (Rathjens, 1974) or breakage and reunion of the \(W\) with a different autosome do occur in other fusion strains (Traut & Weith, 1980).

To select for exceptional daughters in the \(Rf\) strain 23 female moths which appeared as the first females in their respective broods were isolated in brother-sister pairs. Five gave progenies which were wild-type in eclosion sequence and sex-ratio, and three females taken from these wild-type progenies in turn produced wild-type progenies. This demonstrates a lasting reversion to the wild-type state.

One can infer the presence of a \(W\) chromosome-autosome fusion from the appearance of scattered \(W\) chromosomal heterochromatin in the highly polyploid nuclei of the Malpighian tubule cells, while the presence of a wild-type \(W\) chromosome or at least a \(W\) chromosome without an attached autosome is indicated by a single \(W\) chromatin body per cell (Traut & Rathjens, 1973). Cytological inspection of the Malpighian tubules revealed the uniform presence of wild-type heterochromatin in the females of four of the five exceptional wild-type progenies (Plate I). The \(W\) chromosome-autosome fusion obviously was broken in these cases. And consequently the previously fused autosome with any factors located on it could have freely recombined again with the sex chromosomes. The detected wild-type behaviour of these broods therefore is evidence in favour of (i) a \(W\)-linked inheritance of \(Rf\); (ii) an association of \(Rf\) with the fused autosome.

In the last of the five wild-type broods cytological control of the Malpighian tubules of the mother and daughters revealed the presence of a mosaic of both wild-type \(W\) chromatin cells and scattered \(W\) chromatin cells.

The same is true for two of the three exceptional broods which appeared in the \(Rf\) \(\varphi \times +\delta\) crosses (see previous section and Table 3, first line).

(iv) Tests for a putative infectious agent

Since most cases of maternal inheritance of a sex specific character in animals are caused by infectious agents of various kinds, viruses, bacteria, spiroplasms and microsporidians (Leventhal, 1968, Bulnheim, 1977, Martin *et al.* 1973, Williamson & Whitcombe, 1974), several experiments were designed and performed to test this alternative explanation.

To prevent the transmission of a putative symbiont on the external surface of the chorion, egg batches from \(Rf\)-mothers were treated with chloramin. Curing from a putative infectious agent was attempted by feeding freshly hatched larvae with aureomycin, streptomycin or penicillin. None of these treatments inhibited the transmission of \(Rf\) nor was any brood cured from the \(Rf\) phenomenon (data not shown).
Experimental transmission of the putative infectious agent from Rf females to wild-type animals was attempted by injection of Rf hemolymph into wild-type ♀ moths or larvae, by transplantation of the germarium of Rf ♀ moths into wild-type ♀ larvae, and by feeding of Rf tissues to wild-type larvae. None of the progenies produced by these animals showed any change in the direction of Rf properties (data not shown).

Since all attempts to cure the Rf Ephestia or to transmit the Rf character to non-Rf lines have failed, one cannot reasonably assume a hereditary infection as the cause for the retarded female development.

4. DISCUSSION

The most probable interpretation for the origin and inheritance of the Rf trait is a mutation on the autosomal part of the W chromosome-autosome fusion present in this strain. The fusion chromosome acts as a neo-W chromosome and like Rf is transmitted through the female line. Rf is assumed to be a dominant or epistatic factor that depresses viability and development of the larva. Location on the W chromosome explains sex specificity as well as maternal inheritance of Rf.

A full proof of the presented interpretation requires a genetic marker for this specific autosome. The autosomal markers a, b, ml, t, and Us have been tested: they are not linked to the W chromosome-autosome fusion in the Rf strain (data not shown). As the chromosome number in Ephestia is large (n = 30) there is only a limited chance of finding a gene on the autosome in question among the genetic markers available (for a review see Caspari & Gottlieb, 1975).

Maternal inheritance of a female-specific trait by W chromosome linkage has been experimentally produced in a few cases in Lepidoptera. These may serve as models for the one described here. In all cases a mutant W chromosome is involved, which carries either a translocated autosomal segment or a translocated Z chromosomal segment or a whole autosome fused to the W chromosome. The respective genes are located on the originally autosomal or Z chromosomal part of the mutant W chromosome. An experimental sex dimorphism of the wing pattern in Ephestia (Traut & Weith, 1980) is caused and transmitted in this way. Similarly, strains with a sex specific colour of the embryonic serosa (Strunnikov, 1975) or a sex specific larval pattern (Tazima et al. 1975) have been constructed in the silkworm Bombyx mori.

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