

Properties and natural occurrence of maternal-effect selfish genes ('Medea' factors) in the Red Flour Beetle, *Tribolium castaneum*

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Maternally acting selfish genes, termed 'Medea' factors, were found to be widespread in wild populations of *Tribolium castaneum* collected in Europe, North and South America, Africa and south-east Asia, but were rare or absent in populations from Australia and the Indian subcontinent. We detected at least four distinct genetic loci in at least two different linkage groups that exhibit the *Medea* pattern of differential mortality of genotypes within maternal families. Although each *M* factor tested had similar properties of maternal lethality to larvae and zygotic self-rescue, *M* factors representing distinct loci did not show cross-rescue. Alleles at two of these loci, M^1 and M^4 , were by far the most prevalent, M^4 being the predominant type. M^2 and M^3 were each found only once, in Pakistan and Japan, respectively. Although M^1 could be genetically segregated from M^4 and maintained as a purified stock, the M^1 factor invariably co-occurred with M^4 in field populations, whereas M^4 usually occurred in the absence of other *Medea* factors. The dominant maternal lethal action of M^1 could be selectively inactivated (reverted) by gene-knockout gamma irradiation with retention of zygotic rescue activity.

Keywords: maternal effect, *Medea* factor, selfish gene, *Tribolium*.

Introduction

In 1992 we reported the discovery, in wild populations of *Tribolium*, of a class of selfish genes previously unknown in the animal kingdom (Beeman *et al.*, 1992). These genes showed the unusual property of maternal lethality to hatchlings, combined with zygotic self-rescue. They were termed 'Medea' (= *M*) factors, an acronym for *Maternal Effect Dominant Embryonic Arrest*. This mechanism ensures that progeny of a carrier mother survive only if they inherit a copy of the gene from either parent. Such a genetic element is predicted to spread in a population, even in the absence of any selective advantage conferred upon the host (Wade & Beeman, 1994). Subsequently, a genetic disease in mice was shown to be associated with a selfish gene directly analogous to *Medea* (Hurst, 1993; Peters & Barker, 1993), and *Medea*-like maternal and zygotic effects were observed for a region of mouse chromosome 1 bearing a high-copy long-range repeat cluster (Weic-

henhan *et al.*, 1996). In the current work we (i) demonstrate the existence of at least 4 distinct *Medea* loci in wild populations of *T. castaneum*; (ii) confirm the absence of cross-rescue between nonallelic *M* factors; (iii) show that maternal lethal activity of a *Medea* factor can be selectively reverted with retention of rescue activity; and (iv) examine on a global scale the distribution of the two commonly occurring *M* factors in wild populations.

Materials and methods

Description of strains

Five standard laboratory strains, GA-1, M^1 , M^4 *au*, M^1 M^4 and $3P1/au^{14}$, were used to screen field strains for the presence of *Medea* factors. GA-1 is a standard laboratory strain collected in a farmer's corn bin in Georgia (Haliscak & Beeman, 1983) and is apparently devoid of *Medea* alleles. M^1 is homozygous for the M^1 factor (third linkage group) derived from the SP strain from Singapore (Beeman *et al.*, 1992). M^4 *au* is

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homozygous for the visible recessive marker *aureate* (*au*), as well as for the incidental markers, *Abdominal-missing abdominal sternites* (A^{mas}) and *pearl* (*p*), and during the course of this work was found to be also homozygous for M^4 , although it lacks M^1 . $M^1 M^4$ is homozygous for the visible recessive markers *microcephalic* (*mc*) and *jet* (*j*) (Sokoloff, 1962), both incidental to the present work, and during the course of this work was found to be also homozygous for both M^1 and M^4 . $3P1$ and $3P2$ *au* are 3rd linkage group (LG) balancers (Mocelin & Stuart, 1996) that carry the dominant visible marker, *Blunt abdominal and metathoracic projections* (*Bamp*) (Beeman, 1986; Beeman & Stuart, 1990). Both $3P1$ and $3P2$ *au* eliminate crossing-over in a region that includes the *Bamp*, *au* and M^1 loci on LG3. au^{14} is a lethal *aureate* allele, and was used to maintain both $3P1$ and $3P2$ *au* as balanced lethal stocks. These balancer stocks are apparently devoid of M^1 alleles. Most field strains used in this study were collected from farms, grain storage facilities, mills, warehouses and food markets in North and South America, Europe, Africa, the Indian subcontinent, south-east Asia and Australia between 1985 and 1995, and have been maintained in the laboratory on whole wheat flour fortified with 5% brewers' yeast. Additional information is available at the *Tribolium* web site (<http://bru.usgmlr.ksu.edu/bee-man/tribolium.html>).

Detection and differentiation of *Medea* loci

M factors were initially detected in a subset of all field strains by crossing females from each strain with GA-1 males, then testcrossing F_1 females with GA-1 males in single pairs. In the presence of *Medea*, offspring of such F_1 families will segregate 50% *Medea* heterozygotes (viable) and 50% non-*Medea* homozygotes (inviable). Thus, the presence of an *M* factor in the original field strain was indicated by the death of $\approx 50\%$ of the hatchlings from the testcross. Dead hatchlings were never found in control crosses or in crosses involving non-*Medea* segregants. *M* factors diagnosed in this way were then tested for genetic linkage and for cross-rescue. All *M* factors were initially tested for linkage to M^1 on the third linkage group (LG) using the closely linked visible marker *aureate* (Beeman *et al.*, 1992). Additional genetic mapping was carried out using backcrosses of multiple heterozygotes to the multiple homozygous recessive as described (Beeman *et al.*, 1992). Apparent linkage of a new *Medea* with a recessive marker in *trans* was confirmed by separate linkage tests in *cis*. After initial detection and mapping, each apparent *Medea* factor was confirmed by demonstrating that hatchling kill was strictly maternal, and that it occurred only in non-*Medea* progeny. This was accomplished by testing

Medea-bearing males for progeny kill, and by testing surviving progeny of segregating *Medea* females for the presence of *M* factors. Cross-rescue was tested by assessing the zygotic rescue activity of a paternally derived *M* factor in an embryo under the lethal maternal influence of a different *M* factor.

Reversion of M^1 lethality

To confirm that the lethal and rescue activities of *M* factors are controlled by separate (but tightly linked) genetic elements, we tested whether maternal lethal activity of M^1 could be selectively deleted by knockout mutation with retention of intact zygotic rescue activity. Screens for loss of maternal lethal activity were conducted after gamma irradiation of spermatozoa using a Co^{60} source. One-hundred homozygous M^1 *au* males 1–2 weeks of age were irradiated at a dose of 4 kR, then immediately crossed *en masse* to 200 $3P2$ *au/au* virgin females. After 3 days the males were discarded and the females were allowed to oviposit for 1 month. F_1 virgin females heterozygous for $3P2$ *au* and for the treated M^1 *au* chromosome were testcrossed to $3P1/au$ males in single pairs. Revertants of maternal lethality were recognized by the presence of phenotypically *Bamp*, *au* beetles (presumably $3P2$ *au/au*) among the progeny. Because $3P2$ *au/au* progeny did not inherit M^1 , they would not express rescue activity, and thus would normally be killed by maternal M^1 . Putative revertants were tested to rule out false positives derived by recombination between M^1 and the balancer chromosome. Testcross progeny that were phenotypically *Bamp*, non-*au* (presumably M^{1R} *au/3P1*, where R = revertant) were used to establish balanced lethal stocks, or to determine whether the revertant was homozygous viable.

Geographical distribution of *Medea* factors

To survey wild populations more extensively for the presence of *M* factors, separate screenings were conducted for each of three *Medea* categories, namely M^1 , M^4 , and 'all others'. Initially, two individuals were tested for each *Medea* type in each of 123 strains (total = 738 beetles tested). These strains represented 25 source countries in Europe, the Middle East, North and South America, Africa, south-east Asia, Australia and the Indian subcontinent. For most strains, follow-up tests were carried out using 2–5 additional beetles for each *Medea* category (total ≈ 1400 beetles tested). Diagnostic tests were as follows.

(i) To detect M^1 , field strain males were crossed in single pairs with standard $M^1/3P1$ virgin females and the adult progeny were scored for the $3P1$ (= *Bamp*)

phenotype. If the field strain male was either heterozygous or homozygous for M^1 , the maternally derived *3P1* chromosome would be rescued from maternal lethality by a paternal M^1 chromosome. Thus, the presence or absence of *Bamp* progeny indicated the presence or absence, respectively, of a paternal M^1 allele. M^1 was confirmed in females from a subset of positive field strains by demonstrating the presence of M^1 -linked maternal lethal activity. Test females from field strains were crossed to standard M^4 *au* males, and F₁ females (potentially heterozygous for M^1 in trans with *au*) were backcrossed to M^4 *au* males. The presence of an M^1 allele in trans with *au* in the F₁ female would result in the death of almost all homozygous *au* progeny, because *au* is closely linked to M^1 (Beeman *et al.*, 1992). Note that M^4 -derived maternal lethality is completely suppressed in this backcross, as all progeny inherited an M^4 chromosome from their father, resulting in zygotic rescue.

(ii) M^4 alleles were detected by testing the ability of field strain males to rescue the maternal lethality associated with standard $M^4/+$ virgin females. The latter were generated by crossing GA-1 males with M^4 *au* virgin females, then collecting virgin F₁ females. Field strain males that lacked M^4 alleles failed to rescue the maternal lethality associated with M^4 . Crosses involving such males were expected to result in ≈50% survival of progeny. Crosses involving males heterozygous for M^4 should show ≈75% progeny survival, whereas those employing homozygous M^4 males should give rescue of all progeny.

(iii) All other *M* alleles were detected by testing field strains for the presence of maternal lethal activity that was not rescuable by standard alleles of either M^1 or M^4 . F₁ females derived from either of the testcrosses (i and ii) described above were in turn crossed in single pairs with standard $M^1 M^4$ males. Mortality of 50% of the hatchlings indicated the presence in the F₁ female of a field-strain-derived *Medea* factor other than M^1 or M^4 , because the presence in all zygotes of paternally derived M^1 and M^4 factors would preclude any mortality associated with those elements. All standard strains either lack *M* factors (other than M^1 or M^4) or are fixed for the same set of these ‘other’ *M* factors, because all hatchling mortality associated with hybrids between standard strains used here is fully accounted for by the effects of either M^1 or M^4 .

Results

Medea alleles occur at four loci

The M^1 *Medea* factor has been previously described and mapped to the far ‘right’ end of the 3rd linkage group,

within one map unit of the *au* locus (Beeman *et al.*, 1992). The M^1 allele examined in that work was derived from a strain collected in Singapore. New *Medea* factors characterized in the present work were first tested for linkage to *au* to determine whether they were potentially allelic with M^1 . Preliminary tests revealed that several additional strains from south-east Asia carried *M* factors that were closely linked to *au*. One of these (M^2), collected in Peshawar, Pakistan, was unique in that it did not show cross-rescue with M^1 (Table 1). When females doubly heterozygous for M^1 and M^2 in trans were testcrossed to males carrying various combinations of *M* alleles, it became apparent that M^1 and M^2 were closely linked to each other and to *au*, but that neither *M* factor rescued the lethality of the other. A third *M* factor, designated M^3 was also unique, being found in a single strain from Kukisaki, Ibaraki prefecture, Japan. This factor was mapped to the 8th linkage group, ≈11 recombination units from *antennapedia*, away from *squint* (Table 2). The fourth *Medea* gene recognized (M^4) was found in numerous laboratory and field strains from many sources. We have not yet succeeded in mapping M^4 , but it assort independently of the other three *M* loci (data not shown). As expected, M^4 shows normal Mendelian segregation in heterozygous males and the unique ‘selfish’ mechanism (maternal lethal with zygotic self-rescue activity) that is the hallmark of this class of genetic elements (Table 3 and Thomson & Beeman, 1997). In addition to the evidence presented above for the absence of cross-rescue between M^1 and M^2 , we also have extensive evidence

Table 1 The closely linked *Medea* factors M^1 and M^2 do not cross-rescue

Genotype of male parent	M^2 <i>au</i> +		+ <i>au</i> M^1		+ <i>au</i> +		M^2 <i>au</i> M^1	
	<i>au</i>	+	<i>au</i>	+	<i>au</i>	+	<i>au</i>	+
Progeny phenotype								
Number of progeny	255	20	3	400	1	0	78	75
% survival of progeny		47		41		1		87

Males (row 1) were taken from standard, homozygous stocks, and were crossed to $M^2 + +/+ + au M^1$ females. These females were full-sibs, generated from a single-pair $M^2 + +/+ + au$ female × *au* M^1 male. Data for each cross are pooled values from 4 to 5 single pairs. For crosses involving M^2 *au* + or + *au* M^1 males (columns 1–2) we allowed the single pairs to oviposit for 8 weeks prior to determination of progeny phenotypes. Percentage survival data are derived from retests of the same two sets of single pairs, based on number of eggs laid in a 3-day period. For crosses using + *au* + or M^2 *au* M^1 males (columns 3–4), a single, 3-day oviposition period was used both for assessment of progeny phenotypes and for measurement of percentage survival.

Table 2 Assignment of M^3 to linkage group 8, and three-point mapping of M^3

Testcross† (f × m)	Number of testcross progeny of given phenotype				Total	% recomb (M -a)
	a	+	s	a, s		
M +/+ a × + a/+ a	37	210	–	–	247	15
M a/+ + × + a/+ a	251	32	–	–	283	11
+ a/+ a × M +/+ a	56	70	–	–	126	44
M +/+ + as × + as/+ as	0	254	15	32	301	11

Data for 1–3 single pairs were pooled for each testcross. Markers for linkage groups 1–5 and 7 were also tested, but none showed linkage to M^3 (data not shown).

†For each testcross, female genotype is given first.

Abbreviations: a, *antennapedia*; s, *squint*; M , *Medea*³.

Table 3 Segregation of *Medea* factor M^4

Segregation cross†	No. of female progeny of indicated genotype‡	
	M^4 /+	+/+
+/+ × M^4 /+	22	13
M^4 /+ × +/+	32	0

† M^4 heterozygotes used for reciprocal, single-pair testcrosses were full-sibs, generated from a single-pair cross of GA-1 female × M^4 *au* male. For each segregation cross, female genotype is given first.

‡Progeny genotypes were determined by single-pair testcrosses to standard GA-1 (= +/+) males, followed by measurement of percentage survival. A ≈50% mortality rate at the hatching stage indicated the M^4 /+ maternal genotype.

that M^1 and M^4 do not show cross-rescue (data not shown). However, cross-rescue among M^2 , M^3 and M^4 has not been tested.

Selective reversion of M^1 lethality

We screened ≈1000 irradiated M^1 *au* chromosomes for loss of maternal lethal activity and detected three apparent revertants. Two of these proved to be false positives, possibly resulting from recombination be-

tween M^1 and the balancer chromosome. One true revertant (= M^{1R}) was confirmed. A female M^{1R} *au*/3*P2 au* testcrossed to a male 3*P1/au* produced phenotypically Bamp, *au* progeny (testcross 4, Table 4). The latter must have been genotypically 3*P2 au/au*, and would have been killed if the maternal M^{1R} *au* chromosome had intact lethal activity (see control testcross 3, Table 4). Testcrosses 1 and 2 in Table 4 clearly show that a paternally derived M^{1R} *au* chromosome, lacking its own maternal lethal activity, could still rescue progeny from the maternal lethal effect of an intact M^1 chromosome. The revertant chromosome was homozygous lethal and was maintained for several generations as a true-breeding stock over the 3*P1* balancer. However, it was associated with severely reduced fertility and eventually died out in spite of careful husbandry.

World distribution of *Medea* types

We tested 113 strains representing 23 countries for each of three *Medea* ‘types’: M^1 , M^4 and M^X (= all others). These 113 include only unique strains whose geographical origin is well-documented. The results, summarized in Table 5, reveal that M^1 and M^4 are the two predominant types worldwide, M^4 being by far the

Table 4 Selective reversion of M^1 lethality

Testcross (female × male)‡	No. of progeny of given phenotype†				
	B	+	<i>au</i>	B, <i>au</i>	Total
1. M^1 / <i>au</i> × M^{1R} <i>au</i> /3 <i>P1</i>	13	10	6	0	29
2. M^1 /3 <i>P2 au</i> × M^{1R} <i>au</i> /3 <i>P1</i>	22	5	0	9	36
3. M^1 /3 <i>P2 au</i> × <i>au/au</i>	0	37	0	0	37
4. M^{1R} <i>au</i> /3 <i>P2 au</i> × 3 <i>P1/au</i>	4	0	15	4	23

†Diagnostic phenotypes are in boldface type.

‡All testcrosses are single pairs. Crosses 1–2 test whether the M^{1R} *au* revertant chromosome has intact rescue activity. Testcross 3 is a control to verify the presence of maternal lethal activity and zygotic rescue activity on a nonirradiated M^1 chromosome. Testcross 4 is to confirm that the M^{1R} *au* revertant chromosome has lost maternal lethal activity.

most prevalent. Of the 113 strains, 42 (= 37%) representing 14 countries contained M^A , whereas 11 (= 10%) representing nine countries had M^I and 13 (= 12%) representing six countries appeared to carry other *Medea* factors, based on the presence of maternal lethal activity that was not rescuable by either M^I or M^A . Most strains tested from Africa, South and Central America and south-east Asia contained these new *Medea*-like factors, whereas they were absent from North America, Europe, Australia and the Indian subcontinent. However, they appeared to have variable penetrance, and their existence could not be consistently confirmed in subsequent tests. Thus, they are not included in Table 5. Major geographical differences in *Medea* distribution are evident from the data in Table 5. North America and Europe have a high incidence of M^A and a low incidence of M^I ; Australia and the Indian subcontinent have a low incidence of *Medea* factors of either type; whereas South America, Africa and south-east Asia have a high incidence of both types. If the 55 strains from Australia and the Indian subcontinent are excluded, M^A occurs in 38 of 58, or 66% of all remaining strains. M^I occurred in 11 of 28 (= 39%) of all strains tested from South America, Africa and south-east Asia, but in none of 52 strains tested from North America, Europe (including the Middle East), Australia and the Indian subcontinent. In North America we observed a distinct regional nonuniformity in M^A distribution (data not shown). M^A factors were found in all 11 strains originating from the midwest or northern plains, but in none of the five strains originating from the deep south.

M^I always co-occurs with M^A

It is notable that M^A was present in all 11 M^I strains, whereas only 30% of the non- M^I strains carried M^A .

Table 5 World distribution of *Medea* types

Continent†	Number of strains with indicated <i>Medea</i> genes			Total
	$M^I + M^A$	M^A	None	
NA	0	12	6	18
EU	0	8	4	12
AU	0	4	18	22
IN	0	0	33	33
SA	4	3	1	8
AF	2	1	2	5
AS	5	3	7	15
Totals	11	31	71	113

†Abbreviations NA, North America; EU, Europe; AU, Australia; IN, Indian subcontinent; SA, South and Central America; AF, Africa; AS, south-east Asia.

Although M^I was never detected in the absence of M^A in nature, the two could be readily separated by genetic segregation in the laboratory. Purified M^I strains appear to be fully viable and the isolated M^I factor is stable and functional.

Discussion

Although we first reported the existence of *Medea* factors as long ago as 1992, the detailed workings of this unprecedented mechanism for self-propagation of parasitic DNA remain obscure. The present work poses or leaves unanswered a number of intriguing questions. What could explain the patchy distribution of *Medea* elements in nature? Why is M^I never found in the absence of M^A ? Are M factors of recent evolutionary origin? Are they foreign elements or endogenous genes? Are they dispensable? Are they unique to the genus *Tribolium*?

We hoped that the question of whether M genes are dispensable would be answered by reversion analysis. If independently derived revertants of a particular M gene are lethally noncomplementary, then that gene is probably vital. As we obtained only one revertant, this question must be revisited in the future with additional reversion analysis.

The fact that the M^I revertant chromosome had lost maternal lethal activity while retaining zygotic rescue activity confirms the bifunctional nature of this locus. A *Medea* locus could be two separate but closely linked genes (encoding a maternal poison and a zygotic antidote, respectively) that selection favours to be maintained in linkage disequilibrium. Such a gene pair might best thrive in a recombination-suppressed region, for example, near centromeric heterochromatin. Although cytological positioning of *Medea* loci has not been accomplished, M^I and the closely linked M^3 have been recombinationally mapped to one extreme end of metacentric LG3, and thus are probably much nearer a telomere than a centromere.

Until recently all evidence suggested that each M locus is fully independent of all other M loci, i.e. each M factor expresses maternal lethal and zygotic rescue activity independently of other M factors, and rescue activity of a given M factor does not protect against the maternal lethal activity of a different M factor. In 1996, however, we discovered that the hybrid incompatibility factor H (Thomson *et al.*, 1995) interacts lethally with both M^I and M^A . That is, $H/+$ heterozygotes die prior to adult development if they also carry a copy of either M^I or M^A (Thomson & Beeman, 1997). More detailed examination of this phenomenon could give new insight into the *Medea* mechanism.

In geographical regions where *Medea* is at or near fixation, such as M^4 in the midwestern United States, its selfishness is silenced and the element remains hidden. Similarly, it has been reported that sex-ratio distorter is widespread but phenotypically silenced in natural populations of *Drosophila simulans* (Atlan *et al.*, 1997). These observations lend support to the idea that genetic conflicts in general might be more common than often considered (Hurst & Hurst, 1996). It would be remarkable if maternal-effect selfish genes, represented by at least four different loci in the only species to be carefully scrutinized, were not much more widespread than is now apparent.

A full understanding of the *Medea* system will not be achieved without cloning and sequencing at least one *M* locus. Because the *Medea* mechanism appears to be unprecedented, as no homologues are known in other species, and as we have no DNA sequence information, it would seem that positional cloning might be the easiest approach. The feasibility of such a strategy is now under investigation.

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