Assisted Suicide of a Selfish Gene

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Medea (M) factors and the hybrid incompatibility factor (H) are involved in two incompatibility systems in flour beetles that were previously thought to be independent. M factors are a novel class of selfish genes that act by maternal lethality to nonself. The H factor causes the death of hybrids with a paternally derived H gene and previously uncharacterized maternal cofactors. We now find that M factors exhibit their selfish behavior only in the absence of the H factor. Furthermore, we show that the previously uncharacterized maternal cofactors required for H-associated hybrid inviability are identical to M factors. We propose that incompatibility between H strains and M strains is due to suppression by the H factor of the self-rescuing activity of the lethal M genes. This interaction has the effect of converting M elements from selfish into self-destructive or "suicidal" genes. M factors are globally widespread, but are conspicuously absent from India, the only country where the H factor is known to occur. Such a mechanism could prevent the spread of selfish M elements by establishing an absolute barrier to hybridization in the boundary between M and non-M zones.

Studies of hybrid incompatibility have led to the discovery of a variety of intriguing and unexpected genetic adaptations. Examples include complementary lethal gene interactions associated with speciation (Coyne and Orr 1989; Hutter et al. 1990; Orr 1995), microorganism-mediated cytoplasmic incompatibility (Breeuwer and Werren 1990; Wade and Stevens 1985), transposable element-mediated hybrid dysgenesis (Kidwell et al. 1977), and a novel class of maternally acting selfish genes called Medea (M) elements (Beeman et al. 1992; Wade and Beeman 1994). Recently we described a case of hybrid incompatibility in *Tribolium castaneum,* manifested in crosses between any of several strains from India and certain strains collected in a variety of other countries (Thomson et al. 1995). Genetic analysis of one of the purified Indian strains indicated that it was homozygous for a dominant, autosomal, hybrid incompatibility factor, H. H factors were lethal to hybrids in the presence of unknown, maternally derived cofactors. We report here that these H-incompatible cofactors are identical to M elements.

Materials and Methods

Description of Strains

All *T. castaneum* strains are maintained at the Grain Marketing and Production Research Center (Manhattan, Kansas). The T-1 strain is an isofemale subline of the T strain, the latter having been collected in Puranpur village, Bareilly District, Uttar Pradesh, India, in June 1988 by S. N. Tiwari. T-1 is fixed for the H factor, and was used as the standard H strain. None of the other strains used in this work carry H factors, but some are H incompatible and carry M factors. The terms "H incompatibility" and "T-1 incompatibility" are used interchangeably in this work. These terms refer to cofactors in strains that are incompatible with the H-bearing T-1 strain, and should not be confused with the H factor itself. M^1 and M^4 are independently assorting Medea factors that do not cross-rescue (Beeman et al. 1992 and unpublished observations). *3P1* is a balancer chromosome for the third linkage group (LG3) (Mocelin and Stuart 1996), including the M^1 locus. This balancer carries the dominant visible mutant marker *Blunt abdominal and thoracic projections* ($Bamp^{27}$), and is maintained opposite the lethal LG3 marker *black* of Scott Thomson (b^{ST}). $Bamp/b^{ST}$ is devoid of M factors. The C strain (Thomson et al. 1995) was collected in Argyle, Manitoba, Canada, in October 1988 by N. D. G. White. The *mas p au* and *mc j* strains are of North American origin and have been reared in the laboratory for

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more than 20 years. The *antena bifurcada* (*ab*) strain was collected near Bogota, Colombia (Vasquez and del Castillo 1985), and has been reared in the laboratory for more than 10 years. C, *mas p au, mc j,* and *ab* are all incompatible with T-1 at 25°C. C and *mas p au* are each fixed for M^4 but are devoid of other M factors. The SP strain (Beeman et al. 1992) and the *mc j* and *ab* strains are each fixed for both the M^1 and M^4 M factors (Beeman et al. 1992 and unpublished observations). SP-1 is derived from hybridization and backcross of the SP strain into the GA-1 strain. SP-1 is fixed for M^1 but is devoid of M^4. GA-1 is a standard laboratory strain of American origin (Haliscak and Beeman 1983) and has been in continuous culture for over 15 years. GA-1 is compatible with T-1, C, *mas p au,* SP, SP-1, *mc j,* and *ab,* and is devoid of Medea factors.

Segregation of H Incompatibility

In order to monitor segregation of female factors required for incompatibility with H males, small mass-crosses or backcrosses to the GA-1 strain (five virgin beetles of each sex) were set up in 118 ml glass bottles containing 35 g of culture medium (19:1 v/v whole wheat flour:brewer's yeast). Crosses or backcrosses were incubated at 32°C for 5 weeks to generate test progeny. To detect the presence of incompatibility factors in F_1 hybrids or in segregating individuals from backcrosses, virgin adult female progeny approximately 1 week old were used for single-pair testcrosses to T-1 (= H/H) males in 4 dr shell vials at 25°C. Development is much more rapid at 32°C than at 25°C, but incompatibility between T-1 and either C or *mas p au* is expressed more strongly at the lower temperature. Vials were incubated for 12 weeks prior to assessment of progeny viability.

Cosegregation of Medea and H Incompatibility

For testing cosegregation of the M^4 M factor and H incompatibility, *mas p au* females (homozygous for M^4 and incompatible with H) were crossed to GA-1 males, and F_1 males heterozygous for M^4 and for H incompatibility were backcrossed to GA-1 females in single pairs. Segregating female backcross progeny were scored both for the presence of Medea and for H incompatibility by sequential testcrosses. The presence of M^4 was first assessed by pairing virgin females with standard GA-1 males, then measuring the percent progeny survival from a 3 day oviposition period as described (Beeman et al. 1992). The same females were then scored for the presence of H incompatibility by re-pairing with standard T-1 males for 1 week to displace sperm from the previous mating, followed by a 1 week oviposition period and measurement of progeny survival. For testing cosegregation of M^1 and H incompatibility, we used the SP strain as the source of M^1. Since this strain was fixed for both M^1 and M^4, the latter was first removed by consecutive outcrosses to GA-1, followed by specific selection for M^1 in the segregating progeny. The repurified M^1 strain thus generated is referred to as SP-1. The balancer chromosome *3P1* (Mocelin and Stuart 1996) was used as a marker for the M^1 factor in *trans.* SP-1 females were mass-crossed to $3P1/b^{ST}$ males, and male $M^1/3P1$ progeny were outcrossed to GA-1 females in single pairs. Segregating female progeny of this outcross were separated into *3P1* (non-M^1) and wild-type (M^1) categories and tested for H incompatibility as described for M^4.

Cosegregation of M^1- and M^4-Incompatible H Factors

To determine whether a single H factor was responsible for incompatibility with M^1 and M^4, we segregated H factors by crossing T-1 males to GA-1 females, then backcrossing hybrid males to GA-1 females. We continued the backcross for five generations, with selection of H-bearing male progeny each generation after testcrosses to M^4 females. Male progeny of the sixth backcross generation (presumably a mixture of H/+ and +/+) were testcrossed sequentially to *mas p au* (= M^4) and SP-1 (= M^1) females in single pairs. Eggs were counted and percent progeny survival measured after 12 weeks at 25°C (M^4 testers) or 5 weeks at 32°C (M^1 testers).

Results

Segregation of H Incompatibility

As previously shown (Thomson et al. 1995), strains GA-1 and C were compatible and incompatible, respectively, with T–1 because of a lethal interaction between an H factor from the T-1 strain and uncharacterized maternal incompatibility cofactors from the C strain. In the current work, all 25 GA-1 females, testcrossed in single-pair matings to T-1 males, produced healthy adult progeny (Table 1, cross 1). No inviable progeny were found in any of the 25 families. In contrast, only inviable progeny were produced from each of 25 similar testcrosses between C females and T-1 males (Table 1, cross 2). Maternal incompatibility was transmitted by either sex as a dominant trait. F_1 hybrid females from reciprocal crosses of C with GA-1 were fully incompatible with T-1 males (Table 1, crosses 3 and 4). Incompatibility segregated as a simple Mendelian autosomal trait when F_1 hybrid males from either reciprocal cross were backcrossed to GA-1 females. The female progeny from either backcross were a 1:1 mixture of incompatible and compatible types (Table 1, crosses 5 and 6). However, when F_1 hybrid females of a cross between GA-1 and C were backcrossed to GA-1 males, all adult female progeny tested were incompatible (Table 1, cross 7), that is, incompatibility appeared to segregate in hybrid males but not in hybrid females. Seven consecutive generations of backcrosses to GA-1 males were performed and faithful maternal transmission of incompatibility was observed throughout (data not shown). When male siblings of these nonsegregating females were backcrossed to GA-1 females, the incompatibility factor again segregated 1:1 in the female progeny (Table 1, cross 8). These results indicated that an M element in C females is closely linked or is identical to a factor that confers incompatibility with H males. C is known to be fixed for the M^4 factor, but lacks M^1 (unpublished observations).

Table 1. Genetic segregation of a female cofactor required for incompatibility with the male H factor

Hybridization or segregation cross[a]	Number of progeny in each segregating class[b] (N = 25)	
	Compatible	Incompatible
1. G control	25	0
2. C control	0	25
3. C × G	0	25
4. G × C	0	25
5. (C/G) × G	11	14
6. (G/C) × G	13	12
7. G × (G/C)	0	25
8. [G × (G/C)] × G	13	12

[a] Male is given first in each cross; for example, (C/G) × G indicates male C crossed with female G, then an F_1 male backcrossed to a G female.

[b] Twenty-five virgin female progeny from each cross were testcrossed in single-pair matings with T-1 males and compatibility was assessed after 12 weeks incubation at 25°C. Compatible = all progeny developed into viable adults (no dead forms detected). Incompatible = all progeny died in preadult stages.

Cosegregation of M^4 and H Incompatibility

To confirm that M^4 is associated with H incompatibility, we tested for cosegregation of the two activities in *mas p au,* an M^4 strain that is unrelated to C but shows

Table 2. Temperature sensitivity and reciprocality of H incompatibility in five strains[a]

Cross[b]	Medea	Terminal stage of hybrid development[c] 25°C	32°C
mpa × T-1	M^4	Viable[d]	Viable
T-1 × *mpa*	M^4	L4	Viable[d]
C × T-1	M^4	Viable[d]	Viable
T-1 × C	M^4	L4	Viable[d]
mcj × T-1	$M^4 + M^1$	L2	L2
T-1 × *mcj*	$M^4 + M^1$	L2	L4
ab × T-1	$M^4 + M^1$	L2	L2
T-1 × *ab*	$M^4 + M^1$	L2	L4
SP-1 × T-1	M^1	ND[e]	L3

[a] Data are based on five single-pair crosses. All pairs of a given type gave the same result. Data for crosses involving C were taken from Thomson et al. 1995.

[b] Male is given first in each cross.

[c] Terminal stage is given as larval instar of longest-surviving hybrid larvae, for example, L2 = second larval instar.

[d] Some hybrid adults were poorly viable.

[e] ND = not determined.

Table 3. Cosegregation of M^1 and incompatibility with T-1 males

Number of tested females[a]	Terminal stage of progeny development in crosses to T-1 males[b] Phenotype of tested females	30°C	25°C
20	*Bamp* (M^1 absent)	Adults[c]	Adults[c,d]
13	Wild type (M^1 present)	L4	L3

[a] Females were progeny of a single-pair cross of M^1/*3P1* male × GA-1 female. All 33 female progeny from this cross were scored for the dominant *3P1* (= *Bamp*) phenotype, then tested for incompatibility by crossing to T-1 males. *3P1* is tightly linked with M^1 in trans.

[b] Terminal developmental stages of progeny produced by single-pair crosses after 6 weeks (30° C) or 12 weeks (25°C) incubation.

[c] All adults were tremorous and/or malformed.

[d] At 25° C, a few of these crosses produced mature larvae (L5) but no pupae or adults.

the same type of temperature-sensitive, unidirectional incompatibility with the T-1 strain (Thomson et al. 1995) (see Table 2). The M^4 factor in the *mas p au* strain cosegregated with H incompatibility. Among 35 segregating female progeny of a cross between M^4/+ males and +/+ females, 22 carried the M^4 allele, as determined by the presence of maternal lethal activity to a portion of the progeny in testcrosses to GA-1 males. All 22 were also fully incompatible with T-1 males. In contrast, all 13 segregating females that lacked the M^4 allele were fully compatible with T-1 males. Since meiotic recombination occurs in *Tribolium* males as well as in females, this result indicates that H incompatibility is closely linked to M^4 on the same chromosome.

Two Types of H Incompatibility

During screening of additional strains we discovered that T-1 incompatibility in the *mc j* and *ab* strains has novel properties not observed in the C or *mas p au* strains. This new type of incompatibility is bidirectional, that is, hybrids derived from either reciprocal cross are fully inviable. Bidirectional incompatibility was also observed between SP-1 males and T-1 females (Table 2). We will hereafter refer to the previously described incompatibility as type 1 and the new type as type 2. The *mc j, ab,* and SP-1 strains all carry the M^1 factor (unpublished observations). From these observations we postulated that type 1 H incompatibility is encoded by M^4, whereas type 2 H incompatibility is encoded by M^1.

Cosegregation of M^1 and H Incompatibility

Like M^4, the M^1 factor also cosegregates with H incompatibility (Table 3), although M^1 and M^4 assort independently. Among 33 segregating female progeny of a cross between M^1/3P1 males and +/+ females, 13 carried the M^1 allele, as indicated by their non-*Bamp* phenotype. All 13 were also fully incompatible with T-1 males, at either 25°C or 30°C (all progeny in all 13 testcrosses died as immature larvae at either temperature). In contrast, all 20 non-Medea siblings were at least partially compatible with T-1 males. All 20 testcrosses produced mature larvae at 25°C, and most produced adults at this temperature. At 30°C all progeny survived to the adult stage, although their viability was reduced. However, unlike the case with M^4, a portion of the female incompatibility in the M^1 strain appeared to be independent of M^1 itself. We infer from these observations that M factors are themselves a primary cause (although perhaps not the only cause) of H incompatibility, and we postulate that different, independently acting and unlinked M factors interact lethally with the same H factor.

A Single H Factor or a Closely Linked Set Interacts Lethally with Both M^1 and M^4

Previously we mapped all H activity to a single region of linkage group 9 (Thomson et al. 1995). We have not rigorously tested the possibility that the T-1 strain might carry more than one H gene, each one specific for a single Medea system. To determine whether a single H factor is responsible for incompatibility with both M^1 and M^4, we segregated H factors by crossing T-1 males to GA-1 females, then backcrossing hybrid males to GA-1 females. Segregating male progeny from the sixth backcross generation were then tested for incompatibility (i.e., the presence of an H factor), first with M^4 and subsequently with M^1. Incompatibility was manifested by the presence of inviable progeny in testcross families. Inviable progeny of M^4 testers were malformed or moribund pupae or adults. Inviable progeny of M^1 testers were stunted, developmentally arrested larvae (approximately third instar-sized after 5 weeks). Of 23 male backcross progeny tested, 14 were H positive when tested with either M^1 or M^4 testers, while 13 were H negative with either tester. No recombinants were found. These results indicated that the same H factor or a closely linked set of H factors from the T-1 strain interacts lethally either with M^1 from the SP-1 strain or with M^4 from the *mas p au* strain.

Discussion

Possible Mechanisms by Which H and M Interact

Previously (Beeman et al. 1992) we argued that the simplest explanation for M behavior is that each M gene encodes two distinct activities, namely a maternal lethal activity that kills non-M-bearing progeny in the early larval stages, and a zygotic rescue activity that protects M-bearing progeny from this same maternal lethal effect. A rescue factor is active only against the lethal factor encoded in the same gene complex, that is, nonhomologous M factors (such as M^1 and M^4) do not rescue each other's lethality. The observations described in the current work are explained if the H factor is a dominant, zygotically acting suppressor of the M-encoded rescue activity. Although M-encoded rescue is locus specific, suppression of rescue encoded by the H locus is active in at least two distinct M systems and could be generally functional in all M systems.

One of the best-studied examples of lethal interaction of nonhomologous genes in insects is the hybrid inviability system

of *Drosophila* (see Orr 1995, 1996; Orr et al. 1997). In this system an X-linked gene in *D. melanogaster* is incompatible with one or more autosomal genes in other species in the *melanogaster* complex. None of the relevant genes has been cloned, but the time of hybrid death (just prior to pupation), mosaic analysis of somatic clones, and other evidence implicates a defect in mitosis. In the M-H system in *Tribolium* the time of hybrid death ranges from egg hatch to postadult eclosion, depending upon M allele and temperature. In general, there is little similarity between the M-H interaction in *Tribolium* and any other known system of hybrid incompatibility in the animal kingdom. A mechanism based on complementary lethal interaction such as that proposed for the *Drosophila* system does not incorporate the unique selfish behavior of M factors known to occur in the absence of H.

M^1 Lethality Acts Both Maternally and Zygotically

M^4 appears to interact with H primarily through a maternal mechanism. All progeny of a heterozygous or homozygous M^4 mother suffer temperature-sensitive hybrid inviability if they inherit an H allele from their father. The reciprocal cross produces a relatively mild degree of reduced viability in only some progeny. In contrast, the interaction of M^1 with H appears to be largely zygotic, since any zygotic combination of M^1 and H results in death of immature larvae, regardless of parentage. However, maternal influences are not completely lacking in the interaction between M^1 and H, since a paternally transmitted M^1 in combination with a maternally transmitted H results in death in the first or second larval instar at 32°C, whereas hybrid progeny derived from the reciprocal cross survive to the third or fourth larval instar (Table 2). Note that the incompatibility of the M^1-H interaction is more severe in hybrids derived from H females, whereas that of the M^4-H interaction is more severe in the reciprocal cross.

If H acts by suppression of M's self-rescue of its own lethal effect, as suggested above, then M^1 lethality must act zygotically as well as maternally. That it acts maternally has been previously documented (Beeman et al. 1992). That it might also act zygotically is suggested by the bidirectionality of the lethal interaction between M^1 and H. Discovery of suppressor or revertant mutations that eliminate the incompatibility between M and H could provide insight into the true mechanisms by which the two genes interact.

Natural Distribution of M and H Factors

Although hybrids between M and H strains are inviable, both types occur naturally. M factors are globally widespread (Beeman et al. 1992 and unpublished observations) and are predicted to spread through populations in the absence of deleterious effects (Wade and Beeman 1994). We have identified large geographic areas that appear to be fixed for either M or non-M alleles. As might be expected, M and H have not been found to coexist in the same locales. Indeed, M factors are conspicuously absent from India, the only country known to harbor H populations in the wild (unpublished observations). A more detailed account of the natural occurrence and global distributions of compatibility types will be the subject of a separate article.

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