but due to anatomical constraints much homoplasy resulted. Pettigrew argues that "functionally obscure" neurological features are less apt to be under selective constraints and similarities between primates and megabats are due to recent common ancestry rather than convergence (*7*). Our ϵ-globin noncoding DNA data strongly oppose the "flying primate" view of wing convergence during the descent of two separate bat lineages, in support of the classical hypothesis of a monophyletic Chiroptera and a common origin of mammalian flight.

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Maternal-Effect Selfish Genes in Flour Beetles

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A previously unknown class of dominant, maternal-effect lethal *M* factors was found to be widespread in natural populations of the flour beetle, *Tribolium castaneum*, collected on several continents. Such factors are integrated into the host chromosomes at variable locations and show the remarkable property of self-selection by maternal-effect lethality to all hatchlings that do not inherit a copy of the factor itself. Offspring are rescued by either paternally or maternally inherited copies. The *M*-bearing chromosome is thereby perpetuated at the expense of its non-*M* homolog. *M* factors that map to different regions of the genome do not rescue one another's maternal-effect lethality. Factors expressing these properties are predicted to spread in a population, even in the absence of any additional selective advantage. Similar factors also occur in the related species *T. confusum*.

Much of an animal's genome may be composed of parasitic or "selfish" DNA that serves no immediate function for the host but rather exploits the host for its own propagation (*1*). Parasitic DNA may thrive and spread by replicative transposition, by segregation distortion, by mechanisms involving supernumerary chromosomes (*2*) or by other non-Mendelian mechanisms. We report a novel mechanism by which a selfish gene (or gene complex) may facilitate its own propagation at the expense of its unselfish homolog. We show evidence for the widespread distribution in nature of a chromosomally integrated factor that confers maternal-effect lethality to all progeny that do not inherit a copy of the factor itself. To our knowledge similar mechanisms are unknown in the animal kingdom.

In a screen for hybrid dysgenesis (*3*) between geographically diverse strains of *T. castaneum*, we found numerous cases of reciprocal hybrid female semisterility. One example of such bidirectional, female-specific semisterility, observed in hybrids between strains collected in Singapore (SP) and the United States (US) (*4*), is documented in Fig. 1A. Crosses within each strain and reciprocal crosses between strains are fertile. F_1 hybrid males from either interstrain cross are fertile when backcrossed to females from either parental strain. F_1 hybrid females from either interstrain cross are fertile when crossed to SP males, but semisterile when crossed to US males. This semisterility is due to a preadult mortality rate of 50 to 80% among the

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progeny. Finally, F_1 hybrid females crossed to hybrid males show a more modest reduction in fertility, manifested by a progeny mortality of about 25 to 50%.

The semisterility exhibited by hybrid females crossed to US males is also expressed by all of their daughters when similarly mated (Fig. 1A). This faithful maternal transmission persists for many generations of such backcrosses. However, the observations that hybrid females derived from either reciprocal cross are semisterile in crosses to US males and that hybrid males transmit the basis of semisterility to some of their daughters show that this trait is not strictly maternally inherited.

These observations are consistent with the hypothesis that the SP strain is fixed for an autosomal dominant factor that acts maternally to cause the death of any progeny which do not inherit it (Table 1 and Fig. 1B). Such a mechanism for genetic self-selection appears to be unprecedented. We designate this factor *Medea* (M), an acronym for maternal-effect dominant embryonic arrest. We designate chromosomes not showing M behavior with a "+" symbol, although we do not know whether such chromosomes carry an alternative allele or entirely lack a homologous locus.

M/+ females have no apparent abnormalities in ovarian morphology or oogenesis. They show normal fecundity, and fertilization and embryonic development appear normal on gross examination. However, when M/+ females are mated to +/+ or M/+ males, a subset of progeny dies during a period ranging from just prior to larval hatching through the second larval instar (5). The doomed larvae appear normal with respect to external morphology but become sluggish, uncoordinated, or paralyzed before death.

We mapped the M factor derived from the SP strain by outcrossing M/+ females to males homozygous for various recessive variants, and then backcrossing F_1 daughters to males of the paternal genotype. The expectation is that linkage of a visible mutant marker to the M locus will result in a decrease in the predicted 1:1 ratio of mutant to wild-type progeny because of preferential maternal-effect lethality to progeny inheriting the marked (= non-M) homolog. (This strategy assumes that all laboratory stocks lack *Medea* factors, an assumption consistent with results obtained to date.) M assorted independently of all linkage groups tested except the third. Mapping experiments (5) (Table 2) involving four visible markers on linkage group (LG) 3 showed that M is located about 1 unit to the right of *aureate* (*au*), making it the rightmost known marker for this linkage group. LG 3 appears to reside on the longest autosome, a metacentric (6). The *au* region of LG 3 is strongly disfavored as a site for radiation-induced, semisterility-associated rearrangements (7), but the position of this region on the chromosome (whether telomeric or otherwise) is unknown.

The observation that M *au*/+ + females crossed to + *au*/+ *au* males yield only *aureate* progeny (excepting rare recombinants whose genotypes were confirmed by progeny testing) provides direct evidence for the hypothesis that the ensuing semisterility is due to the death of zygotes inheriting the non-*Medea* maternal locus, and also demonstrates the complete penetrance of lethality. We also crossed M *au*/+ + females to M *au*/M *au* males, and found the 1:1 ratio of *aureate* and wild-type progeny expected when no semisterility occurs (Table 3). When these same females were recrossed to + *au*/+ *au* males, the expected mortality of the non-*aureate* progeny class was observed (Table 3).

Although these data suggest strongly that the zygote's maternally derived M chromosome can mediate zygotic rescue, it is not clear from the data in Table 3 whether the paternally derived zygotic rescue factor is associated with paternal chromosomes, sperm cytoplasm, seminal fluid, or possibly other sources. To provide direct evidence that zygotes are rescued from maternal-effect lethality by paternally derived M chromosomes as well as maternally derived ones, we crossed M *au* +/+ *au* *Bamp*^{27} males with M + +/+ *au* + females, then analyzed progeny phenotypes. If rescue were not mediated by the paternally derived M chromosome, then the *Bamp*, *aureate* phenotypic class should be equal in size to all other classes. The results (Table 4) are consistent with the hypothesis that the paternally derived M chromosome itself confers rescue, but are inconsistent with the possibility that other chromosomes or extrachromosomal factors mediate such rescue. The observed frequency of *Bamp*, *aureate* progeny (Table 4) matches that predicted from normal meiotic recombination between M and *Bamp* in the male with the assumption that only those sperm containing recombinant M *au Bamp* chromosomes, and not sperm containing + *au Bamp* nonrecombinant chromosomes, confer rescue to zygotes that do not happen to inherit an M factor from their mother. Progeny receiving a single, maternally derived M fac-

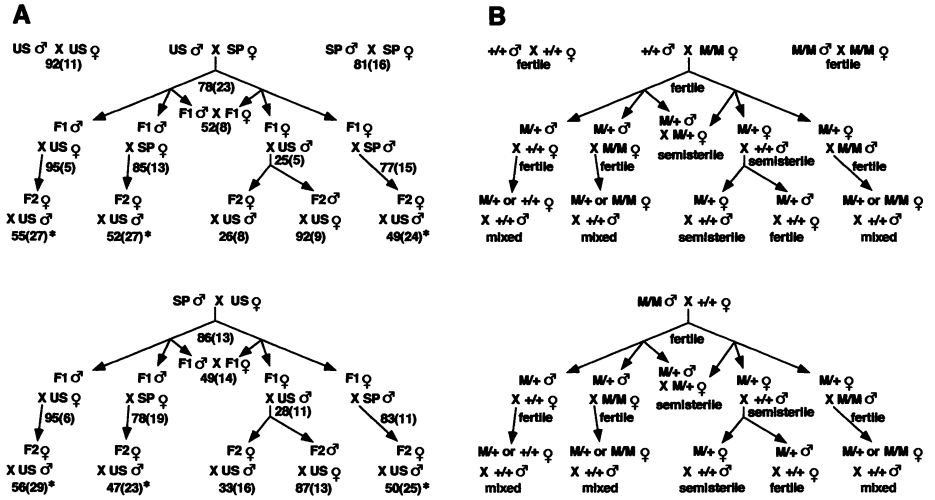

Fig. 1. (**A**) Fertility (expressed as percentage of survival of progeny) for various crosses from parental strains derived from the United States (US) and Singapore (SP). Each datum is the mean (standard deviation in parentheses) of 50 single-pair crosses. Fertility determinations were based on pooled data, usually from two consecutive 3-day oviposition periods (total, 6 days oviposition yielding about 50 to 60 eggs per female) or, in some cases, on a single 3-day oviposition period, with sexually mature young adults, 2 to 3 weeks old. Asterisk indicates progeny groups in which both fertile and semisterile daughters appear to be segregating. (**B**) Behavior of the *Medea* (*M*) factor in crosses between *M*/*M* and +/+ strains. The flow diagram is redrawn from (A) and reflects the assumption that *M* is fixed in the SP strain and is absent from the US strain. Designation of crosses as "fertile," "semisterile," or "mixed" suggests our interpretation of the data in (A), and is consistent with our proposed self-selection mechanism. Note that crossing *M*/+ males to +/+ females produces a mixture of semisterile (*M*/+) and fertile (+/+) daughters.

Table 1. Interactions of *Medea*-encoded cytotype and genotype. *M* cytotype implies *M*/+ or *M*/*M* maternal genotype. The + cytotype implies +/+ maternal genotype. Zygotic genotype of *M*/*M* is impossible with + cytotype.

Cytotype	Genotype	Phenotype
M	*M*/*M*	Viable
M	*M*/+	Viable
M	+/+	Lethal
+	*M*/+	Viable
+	+/+	Viable

tor (phenotypically *Bamp*, excepting recombinants), as well as progeny receiving M factors from both parents (phenotypically wild type, excepting recombinants), were also rescued, as expected (Table 4).

Although the death of +/+ progeny of M/+ mothers occurs with complete penetrance, in some cases the rescue of M/+ or M/M progeny does not. For example, in Fig. 1A the mean percentages of progeny survival in crosses involving M/+ (= F_1) or M/M (= SP) females were somewhat lower than the model predicts. This could have occurred because a portion of the M-bearing females deposited a higher than average dose of lethal product in their eggs, resulting in the death of a significant portion of their M/+ or M/M progeny. However, in other genetic backgrounds rescue was nearly complete (see Table 3).

Medea factors in strains sampled from dispersed natural populations show diversity in both their genomic location and interactions. The one carried by the SP strain (designated M^1) represents the most commonly found type worldwide. So far we have found M^1 in several strains collected within the last 5 years in Asia, Africa, and South America. In all cases the factor maps to the same position, approximately 1 unit to the right of *aureate* on LG 3. However, not all factors that map to this region are M^1. M^2, derived from a strain collected in Pakistan, neither rescues nor is rescued by M^1 (5), although the two are closely linked. M^2 maps 4 units to the left of *au* (toward the *Bamp* locus), or about 5 units from M^1. In addition, many M^1-bearing strains harbor another M factor different from M^1 or M^2, again by the criterion that they do not provide rescue from one another's maternal-effect lethality. Such factors have been found in association with M^1 in strains collected on all three continents where M^1 has been found. Conversely, not all M-bearing strains carry M^1 (for example, the M^2-bearing strain does not). It is not yet known how much heterogeneity exists among these additional factors in genomic location or interaction class, but they assort independently of LG 3.

Maternal effects have previously been implicated in interspecific hybrid sterility or inviability within the genus *Drosophila* (8). Hutter *et al.* (9) described mutations in *Drosophila melanogaster* that rescued the embryonic lethality of certain such hybrids. Such mechanisms have been interpreted as "complementary lethal" systems in which certain alleles at one locus are lethal in the presence of certain alleles at other (normally complementary) loci. Build-up of such complementary lethal systems is considered to be an important mechanism for the evolution of reproductive isolation (expressed as hybrid inviability) during speciation in both animals and plants (10). Cases of reproductive incompatibility in animals that combine maternal and zygotic influences appear to involve multilocus interactions and occur in hybrids between separate species or clearly distinct subspecies. In contrast, the *Medea* system appears to be entirely controlled by any one of several autonomous and independently functioning loci in a species that shows no other evidence of subdivision into genetic races.

A predicted consequence of the maternally controlled self-selection mechanism just described is that a *Medea* factor introduced into a wild-type population, even at a low frequency, should gradually invade the population and become fixed, even in the absence of any selective advantage beyond the immediate survival conferred by resistance to its own maternal lethal effect (11). This prediction is based on the assumption that no loss of fitness is associated with the M factor other than the reduced number of offspring that directly reflects the death of +/+ progeny of M/+ mothers. Although the invasive nature of M factors has not yet been demonstrated in laboratory populations, we have observed that most M-bearing individuals are equal to wild-type in rate of development, fecundity, longevity, and mating success (5).

M-like factors are not restricted to *T. castaneum*. We tested four strains of *T. confusum* from four Asian countries and found evidence of an M-like factor in a strain from Thailand. This factor conferred dominant, female-specific semisterility that was faithfully transmitted to all surviving daughters by heterozygous mothers but that segregated in heterozygous males (5). Horizontal transmission of M factors between these two species may have occurred through an infectious agent, but could not have occurred by normal gene flow in recent evolutionary time, because crosses between the two species are entirely sterile. The existence of M-like factors in two congeneric species that cannot interbreed raises intriguing questions about the distribution of such elements more broadly within the genus and at higher taxonomic levels. The *Medea* system could be viewed as an un-

Table 2. Mapping of an Asian *Medea* (*M*) factor on the third linkage group (LG). Percentage of crossover (in cis or trans) between *M* and the indicated visible LG 3 marker was tested by backcrosses to appropriate homozygous recessive (non-*Medea*) testers. In crosses involving *lod*, both parents were homozygous *pearl*. Data indicate percentage of visible mutant (trans tests) or wild (cis tests) phenotype in progeny. Male data merely reflect percentage of segregation of visible mutant gamete, and not recombination, because the *M* phenotype being scored is maternal effect lethality to non-*M* progeny. Recombination occurs equally in the two sexes in *Tribolium*. ND, not determined.

Genotype of test parent	Male		Female	
	Apparent crossover (%)	Progeny scored (no.)	Apparent crossover (%)	Progeny scored (no.)
M *+/+ au*	46	273	1	8926
M au/+ +	ND		0	241
M *+/+ Bamp*	46	383	9*	509
M Bamp/+ +	52	702	9	1455
M *+/+ lod*	46	384	15	387
M lod/+ +	50	606	21	802
M *+/+ b*	50	141	43	295
M b/+ +	50	307	41	241

*Confirmed by test crosses. All *Bamp* recombinants appeared mosaic or aneuploid. Several apparent somatic mosaic *Bamp* progeny had wild-type (non-*Bamp*) germlines.

Table 3. Meiotic segregation of *Medea* in females. Fifteen *M au*/+ + females (dams) were tested individually. Data are expressed as means ± 1 standard deviation. Each female was crossed first to a pair of *M au*/*M au* sires, then to a pair of + *au*/+ *au* sires. Fertility is defined as percentage of survival of progeny and is based on all eggs laid during a 3- to 6-day oviposition period. Progeny phenotypes are based on counts from all progeny produced in the first 3 days of oviposition after a 3-day premating interval.

Sire genotype	Fertility (%)	Progeny of given phenotype (no.)	
		+	*au*
M au/*M au*	93 ± 3	22 ± 4	21 ± 5
+ *au*/+ *au*	46 ± 12	0 ± 0	16 ± 5

Table 4. Zygotic rescue by paternal or maternal *Medea* factors. Test cross was *M au* +/+ *au Bamp* male × *M* + +/+ *au* + female (five of each sex). The *Bamp au* phenotypic class (in boldface) stems from meiotic recombination between *M* and *Bamp* in the male. All other classes derive from noncrossover chromosomes. Data represent two complete ovipositions. Predicted number of progeny is based on the assumption of tight linkage between *au* and *M*, 13% recombination between *au* and *Bamp* (the published value), and 297 total progeny.

Progeny phenotype	Observed progeny (no.)	Predicted progeny (no.)	Maternal chromosome	Paternal chromosome
+	105	99	*M* + +	*M au* +
Bamp	94	99	*M* + +	+ *au* *Bamp*
au	85	86	+ *au* +	*M au* +
Bamp au	13	13	+ *au* +	**M au Bamp**
Total	297	297		

usual strategy for the self-propagation of selfish DNA that can also function as a postzygotic isolating mechanism to facilitate an ongoing process of speciation.

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A Conformation of Cyclosporin A in Aqueous Environment Revealed by the X-ray Structure of a Cyclosporin-Fab Complex

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The conformation of the immunosuppressive drug cyclosporin A (CsA) in a complex with a Fab molecule has been established by crystallographic analysis to 2.65 angstrom resolution. This conformation of CsA is similar to that recently observed in the complex with the rotamase cyclophilin, its binding protein in vivo, and totally different from its conformation in an isolated form as determined from x-ray and nuclear magnetic resonance analysis. Because the surfaces of CsA interacting with cyclophilin or with the Fab are not identical, these results suggest that the conformation of CsA observed in the bound form preexists in aqueous solution and is not produced by interaction with the proteins.

Cyclosporin A (CsA), an immunosuppressive drug that acts as an inhibitor of T cell activation (*1*), forms a complex in vivo with cyclophilin (CYP) (*2*), a cis-trans isomerase, and can inhibit its activity in vitro (*3, 4*). The molecular mechanisms of immunosuppressive activity are not understood. Furthermore, the relation between immunosuppressive activity and inhibition of rotamase activity has recently been questioned [for review, see (*5, 6*)]. Knowledge of the conformation of the drug that is recognized by CYP is central to an understanding of its mechanism of action. Two crystallographic (*7, 8*) and two nuclear magnetic resonance (NMR) analyses in different apolar solvents (*9*) of isolated CsA yielded superposable structures, suggesting that CsA is a rigid molecule with a distinctive main-chain conformation. The conformation of CsA in aqueous solution has never been studied because of its poor solubility. Two NMR studies of CsA in a complex with CYP yielded, however, a conformation of CsA with a totally rearranged backbone conformation (*10, 11*). The cis peptide bond found between residues MeLeu^9 and MeLeu^{10} (Me, *N*-methyl) in free CsA was replaced by a trans peptide bond in the conformation of CsA in interaction with CYP.

Is the radical conformational rearrangement of the whole CsA molecule a result of its binding to CYP (*12, 13*)? Although some conformational adaptation may occur upon interaction of CsA with an antibody combining site, such drastic changes are not expected from its free conformation in solution. We have established by crystallographic analysis to 2.65 Å resolution the structure of CsA in a complex with the Fab of an antibody and analyzed its interaction with the antibody combining site.

The monoclonal antibody to CsA R454511, which was chosen for crystallographic analysis of an Fab-CsA complex, is an immunoglobulin G1/κ, known from immunochemical data to strongly recognize residues 2 through 5 and 11 and weakly recognize residues 1 and 9 of CsA (*14*). Messenger RNA sequencing of its variable region revealed an unusually long H3 loop (*15*). The structure of CsA in the Fab R454511–CsA complex, derived here from our crystallographic analysis, is shown in Fig. 1A. The difference electron density map clearly shows the shape of the CsA cycle as well as the location of side chains. The conformation is different from that of the isolated form (Fig. 1B), also obtained by x-ray diffraction (*8*). However, the conformation of CsA in interaction with the antibody closely resembles that recently observed, with NMR analyses, in a complex with CYP (*10, 11*). The conformation of isolated CsA (Fig. 1B) contains two antiparallel β strands formed by residues 11 to 3 and 4 to 7. All four nonmethylated main-chain nitrogens are involved in internal hydrogen bonds with carbonyl groups. In the structure of CsA shown in Fig. 1A, no internal hydrogen bonds are formed. Instead, main-chain nitrogens and carbonyls are available for hydrogen bonding to the protein or to the solvent. The internal structure of the cycle is mainly hydrophobic because most main-chain *N*-methyls, as well as the side chain of MeVal^5, point to the inside of the cycle.

The connection between residues MeLeu^9 and MeLeu^{10} is best fit by a trans peptide bond, as in the conformation of CsA in interaction with CYP (*10, 11*), in contrast to the cis peptide bond found in isolated CsA (Fig. 1B). Although topologically similar overall, the conformations of CsA bound to the Fab or to CYP (*10, 11*) are not identical. For example, when CsA is bound to the Fab, MeBmt^1 does not fold back onto the molecule as when CsA is bound to CYP (MeBmt, 3-hydroxy-4-methyl-2-methylamino-6-octenoic acid). Differences, however, are small compared to those of the totally unrelated conformations of CsA in complex with the Fab (Fig. 1A) and isolated CsA (Fig. 1B).

The antibody is unlikely to distort CsA through a rotamase activity that would mimic CYP. Indeed, the binding modes of the Fab and of CYP are different: CYP is known from NMR experiments to recognize residues 1 through 3 and 9 through 11 of CsA (*10, 11*). Crystallographic analysis of the Fab-CsA complex clearly shows that residues 9 and 10 and the side chain of residue 1 are not involved, whereas residues 11 and 2 to 5 and the main chain of residue 1 are critical for binding (Fig. 2). These latter residues are also those found from immunochemical data to be important for recognition (*14*).

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