of the other solid organs, such as the liver. There did not appear to be any specific localization of either genotype lineage in this organ.

A random pattern of patch distribution observed in the lungs was unrelated to structural elements in this organ. The patches were uniform in size and shape and were similar to those in the liver.

The cortex and medulla of the ovary had a random patchy distribution of cells of the two genotypes. The graafian follicles were unstained, consistent with the absence of MHC antigens in germ cells (Fig. 2F). Chimera 0911 had mature follicles and numerous primary and secondary follicles. Its oviduct contained an ovulated germ cell demonstrating germinal vesicle breakdown and corona radiata. Of the eight live births, five were female and three were male. All four of the unequivocal chimeras were female. Two of the three surviving females have mated with male Holtzman rats and have given birth to a total of 18 pups in six litters.

There are three systems that allow histological examination of chimeric tissues other than the liver (*8, 9, 16*). Interspecific chimeras suffer the disadvantage of having disparate genetic backgrounds with possible effects on normal cell assortment in the organogenetic phase of development. This does not affect the function of at least the vital organs of such interspecific chimeric animals in any determinate manner. The use of congenic strains that vary genetically only at or near the marker locus obviates this limitation. Morphometric analyses of specific organs in chimeric mice have been limited to the liver (*17*). Our results in the rat liver tend to support the conclusions of these studies concerning average patch size, patch distribution, and the relation between the proportion of the two genotypes present and the patch size.

The combination of MHC antigens in congenic animals marked histologically with monoclonal antibodies provides a useful addition to the already widespread developmental applications of mammalian chimeras and may provide new insights into the organogenesis of some viscera. This may be particularly true of the thymus, for which evidence of oligoclonal development, which has important implications for the tempo of T-cell repertoire formation and the induction of self-tolerance, is currently derived solely from the radiation chimera model. It is possible that accessibility of the thymus to immigrating cells in that situation is atypical, and it is important to evaluate this possibility in normal animals. The system we have described allows histological examination and study of organogenesis of mosaic rat tissues.

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Microorganism Mediated Reproductive Isolation in Flour Beetles (Genus *Tribolium*)

Abstract. *Reproductive isolation is induced by microorganisms in diverse geographic strains of the flour beetle* Tribolium confusum *(Coleoptera:Tenebrionidae). The incompatibility between populations is due to nongenetic cytoplasmically inherited factors. Males of infected strains produce no progeny when crossed with females of noninfected strains; however, they produce "normal" numbers of progeny when crossed with infected females. Males from noninfected strains show no reproductive isolation. Infected strains of* T. confusum *can be cured when tetracycline or other antibiotics are added to the flour medium. "Cured" strains become partially reproductively isolated from all noncured strains including the source strain.*

Incompatibility between populations due to nongenetic factors is of interest because of its possible role in the process of speciation (*1–3*) and because of its potential application to the control of pests (*2*). In the mosquito *Culex pipiens*, cytoplasmic incompatibility is mediated by the prokaryotic microorganism *Wolbachia pipientis* (*1*). Males not containing *Wolbachia* can mate with any female, whereas those containing *Wolbachia* are reproductively isolated from females not containing the microorganism. The antibiotics tetracycline, erythromycin, and spectinomycin inhibit prokaryotic and mitochondrial protein synthesis by binding to the ribosome (*4*); thus *Wolbachia* infected mosquito larvae raised in water containing tetracycline are cured of the microorganism.

In 1961, Stanley (*5*) reported that females of the McGill black *Tribolium confusum* (flour beetle) strain were reproductively incompatible with two other strains, although males of this strain were not. Moreover, McGill black females were also incompatible with the hybrid males produced by crossing McGill black males with females of other strains. Cawthon and Mertz (*6*) discovered a similar phenomenon in the bI *T. confusum* strain. He showed further that the infertility exhibited a cytoplasmic pattern of inheritance: bI females were infertile with F_1, F_2, and F_3 backcross hybrid males derived from mating bI males with Chicago strain females. [The bI strain was derived from the Chicago strain by inbreeding (*7, 8*).]

In 1979, the mating compatibilities of 12 strains of *T. confusum* at the University of Chicago were surveyed. Six of the strains, including bI and Chicago, had been in the laboratory for more than 20 years. The other six strains had been recently collected from stored grain

Table 1. Proportion of successful single pair matings in standard medium when adults were reared on standard medium or 0.125 percent tetracycline medium.

Medium	Male parent					
	Standard medium			Tetracycline medium		
	bSM*	b-Circle A*	bI	bSM	b-Circle A	bI
Female parent						
Standard						
bSM*	9/9	10/10	9/10	9/10	10/10	9/10
b-Circle A*	8/10	10/10	10/10	9/10	9/10	10/10
bI	0/10	0/9	9/9	9/9	10/10	10/10
Tetracycline						
bSM	0/9	0/10	10/10	10/10	8/10	10/10
b-Circle A	0/10	0/10	10/10		9/10	9/10
bI	0/10	0/9	10/10	10/10	10/10	9/10

*Indicates an "infected" beetle.

products in Spain, Japan, Yugoslavia, and Illinois. (We use the term "b-Circle A" to identify the Illinois strain.) Five replicate pair matings were set up with virgin males and females for every possible combination of within and between strain crosses (12 male types × 12 female types × 5 replicates per cross = 720 matings). The survey revealed that, while bI and b-Circle A were interfertile, females of these two strains always failed to produce progeny when crossed with males from any of the other ten strains. No partial incompatibilities were detected.

When tested again in 1983, b-Circle A females were interfertile with males of other strains; but b-Circle A males were no longer compatible with bI females. Females from the b-Circle source population maintained at the University of Illinois at Chicago by Professor David Mertz are completely infertile with males of all other tested strains except bI. In summary, the interstrain crosses demonstrate that (i) bI females are incompatible with males of wild populations from geographically dispersed portions of the species distribution; (ii) a recently collected wild strain, b-Circle, shows mating incompatibility similar to that previously reported for the laboratory strains bI and McGill black; (iii) strains that demonstrate incompatibility with other strains are completely interfertile; and (iv) reproductive compatibility can be naturally acquired (b-Circle A) but when this occurs the strain is no longer interfertile with other incompatible strains.

We selected three strains of *T. confusum* for further study: (i) bSM, a laboratory hybrid derived from the mass mating of the bII, bIII, and bIV cannibalism strains (*8*); (ii) b-Circle A; and (iii) bI. We reared beetles from egg to pupa on both standard medium (95 percent by weight whole wheat flour and 5 percent brewer's yeast) and medium supplemented with 0.125 percent crystalline tetracycline hydrochloride. Using virgin adults from both types of medium, we established ten replicate single pair matings of each possible cross on the standard medium. The presence of tetracycline in the rearing medium has no effect on the incompatibility displayed by bI females (Table 1), and males of b-Circle A and bSM become interfertile with bI females when reared on tetracycline medium. Furthermore, females of b-Circle A and bSM reared on tetracycline medium become reproductively incompatible with b-Circle A and bSM males reared on the standard medium. Thus, we can induce the same pattern of reproductive incompatibility seen in the McGill black, bI, and b-Circle strains in otherwise reproductively competent strains by rearing them on medium containing tetracycline. Additional experiments have shown that (i) similar results are effected by simply husbanding adults for 20 days on 0.125 percent tetracycline medium; (ii) "cured" strains remain fertile in future generations; (iii) partial cures are obtained with very low dosages of tetracycline (0.005 percent, by weight); and, (iv) similar low dosages of erythromycin and spectinomycin also effect partial cures.

Table 2. Mean numbers of offspring ($\bar{X}$) produced by single pairs of adults in 66 days on standard flour medium. Adults had been raised in either standard medium or 0.125 percent tetracycline medium (T). A separate analysis of variance on $\ln(X)$ was performed for each strain, and no significant differences were detected at the 0.05 level; S.D., standard deviation.

Male	Female	N	$\bar{X}$	S.D.
bSM	bSM	9	52.67	15.38
bSM-T	bSM-T	10	67.80	18.75
bSM-T	bSM	9	64.33	17.68
b-Circle A	b-Circle A	10	52.30	8.73
b-Circle A-T	b-Circle A	9	63.89	17.41
bI	bI	9	54.78	6.10
bI-T	bI-T	9	55.11	14.21
bI-T	bI	10	56.50	11.33
bI	bI-T	10	53.60	12.09

We interpret these observations as indicating that most strains of *T. confusum* harbor a microorganism. Infected males are not reproductively competent with cured females but infected females can successfully reproduce with either cured or infected males. Furthermore, the numbers of offspring produced by cured and uncured pairs are not significantly different (Table 2). (In a survey of five laboratory and nine recently collected strains of *Tribolium castaneum*, we found no evidence of partial reproductive isolation and none has ever been reported. In addition, none was induced in any strain of this species when adults were reared on 0.1 percent tetracycline medium for 20 days.)

We conclude that most populations of *T. confusum* harbor a microorganism whose loss can induce a nongenetic partial reproductive isolation from other infected strains. This phenomenon in the order Coleoptera, one of the most speciose of all taxa, appears to be the first report of such a phenomenon outside the Diptera. This finding enhances the likelihood that extranuclear heredity or "endosymbiont intracellular inclusions" (*9*) may play an important role in the evolution of eukaryotes.

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