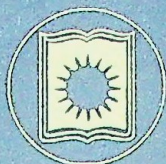


**AN ESTIMATE OF CYTOPLASMIC INCOMPATIBILITY
AND ITS USE IN THE CONTROL OF A STORAGE
PEST, *TRIBOLIUM CASTANEUM* (HERBST)**



**A THESIS SUBMITTED IN FULFILMENT OF
REQUIREMENTS FOR THE DEGREE OF MASTER OF
PHILOSOPHY IN ZOOLOGY**

BY

KAZI MD. FAISAL HOQUE
B.Sc. (Hons.), M.Sc.

**DEPARTMENT OF ZOOLOGY
UNIVERSITY OF RAJSHAHI
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UNIVERSITY OF RAJSHAHI**

JULY 2000

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DECLARATION

I do hereby declare that this dissertation contains no material which has been submitted for the award of any other Degree or Diploma from this or any other University. To the best of my knowledge, it contains no material previously published or written by any other person(s), except when due reference is made in the text.

Dated: 26/07/2008
Department of Zoology
University of Rajshahi


Kazi Md. Faisal Hoque



CERTIFICATE

This is to certify that the dissertation titled "An Estimate of Cytoplasmic Incompatibility and Its Use in the Control of a Storage Pest, *Tribolium castaneum* (Herbst)", submitted by Mr. Kazi Md. Faisal Hoque for the award of the Degree of Master of Philosophy in Zoology, is based on the results of his own investigation carried out in the Genetics Research Laboratory of this Department under my supervision. The results which are embodied in this thesis or part thereof have not been previously presented for any other Degree.

Dated: 26/7/2010

Dr. M. Saiful Islam
Professor & Supervisor

SUMMARY

Using purebred and hybrid crosses involving four indigenous (*viz.* CHI, DHA, KHU and RAJ) and four exotic (*viz.* BLA, CTC, FSS and KAN) strains of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae), cytoplasmic incompatibility (CI) relationships between the strains have been established. An analysis of the observed incompatibility, where the percentage of egg-hatch data was considered as an index, demonstrate that of 52 crosses, 34 (65.38%) were compatible, 12 (23.08%) were partially incompatible and 6 (11.54%) were incompatible. The results suggest that two of the indigenous strains *viz.* KHU and RAJ and three of the exotic strains *viz.* CTC, FSS and KAN might be infected with RMO (rickettsia-like microorganisms). Backcross data, on the other hand, indicate that the two indigenous (KHU and RAJ) and one exotic (BLA) strains are perhaps uninfected.

Effect of tetracycline treatment in the food medium and its consequence on the expression of CI in *T. castaneum* demonstrate that when males reared on untreated food are mated with the females reared on a similar medium, all the infected strains show compatibility. But when untreated males are paired with females reared on tetracycline-treated food, all the crosses exhibit incompatibility. Alternatively, when males reared on treated medium are crossed to females reared either on untreated or treated medium, all the cross-matings become compatible. This indicated that the tetracycline treatment might have 'cured' the infected males from the RMO, if any. Apparently fecundity of

the experimental insects was not affected by the tetracycline treatment.

Effects of 254 nm and 366 nm UV radiation on the expression of CI in *T. castaneum* were evaluated. Results showed that when both parents were untreated, 19 of the crosses were fully compatible, while 6 crosses were partially incompatible. When untreated males were crossed with 254 nm UV-treated females, 12 crosses were incompatible, whereas the rest 13 crosses were partially incompatible. Contrary to the results on untreated males, when treated males were paired either with untreated or treated females all 50 crosses were compatible, suggesting that 254 nm UV treatment on males resulted the strains free of any RMO. Data on the effect of 366 nm UV radiation on the expression of CI in *T. castaneum* appear to be a bit erratic. Untreated males mated to untreated females mostly were compatible except six crosses that exhibited partial incompatibility. But when untreated males were paired with 366 nm UV-treated females, the results were quite different. Six crosses were incompatible and the rest 19 crosses were partially incompatible. But unlike 254 nm treatment, 366 nm-treated males paired with untreated females gave rise to 6 incompatible, 6 partially incompatible and 13 compatible crosses. As with 254 nm UV treatment, 366 nm radiation also yielded all compatible crosses when both parents were treated.

Inferences drawn from the above results were utilized to verify if cytoplasmically incompatible exotic males (CTC, FSS and KAN) could be used to regulate the population of apparently

uninfected, indigenous females (CHI and DHA). Keeping the ratios of indigenous females to males constant, the proportion of exotic males was gradually increased from 1 to 3 and the consequence was recorded in terms of fecundity, percent egg-hatch and the number of adult progenies. Results of such a frequency-dependent experiment demonstrate that for CHI female x CTC or FSS or KAN male crosses, both the percentage of egg-hatch and the number of adult progenies decrease compared to controls. Similar results were obtained for DHA female x CTC or FSS or KAN male crosses except that the extent of decrease was slightly lower than that recorded for CHI females. These findings indicate that the indigenous CHI strain could effectively be regulated by the exotic CTC male, provided that sufficient males are released.

Mating competitiveness values (CVs) of the three exotic males of *T. castaneum* viz. CTC, FSS and KAN were estimated from the percentage of egg-hatch data originated from the frequency-dependent experiment described above. The estimated CVs of the CTC males competed with CHI females and males were 0.60, 0.46 and 0.37 at 1:1:1, 1:1:2 and 1:1:3 ratios respectively. Those of the FSS and KAN males competed each with CHI females and males were 0.36, 0.14 and 0.29, and 0.15, 0.34 and 0.43 respectively. CTC, FSS or KAN males, on the other hand, when competed with DHA females and males, gave slightly different CVs. These were 0.21, 0.18 and 0.18, 0.23, 0.18 and 0.21, and 0.34, 0.24 and 0.23 respectively at the three ratios, suggesting that the exotic males performed better with the indigenous CHI strain than did with DHA strain.

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Kazi Md. Faisal Hoque

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ABBREVIATIONS USED IN THE TEXT

BLA	= Black
CHI	= Chittagong
CI	= Cytoplasmic incompatibility
CIM	= Cytoplasmic incompatibility microorganisms
CTC	= CTC-12
DHA	= Dhaka
FSS	= FSS-II
KAN	= Kano
KHU	= Khulna
nm	= Nano metre
PCR	= Polymerase chain reaction
RAJ	= Rajshahi
RH	= Relative humidity
RLO	= Rickettsia-like organisms
RMO	= Rickettsia-like microorganisms
UV	= Ultraviolet

CHAPTER ONE

INTRODUCTION

INTRODUCTION

1.1 Pest status of *Tribolium castaneum*

Flour beetles of the genus *Tribolium* include over 30 described species feeding on a variety of stored commodities throughout the world (Sokoloff, 1972; 1974). They have been associated with grains at least since early Egyptian times (Andres, 1931). These are found whenever flour and cereal products are manufactured or stored. The larvae are yellowish mobile grubs which burrow into flour, damaged grains, cereals, dried fruits, ground nuts or spices. Being tolerant of wide temperature extremes, flour beetles are widespread species of pest insects. Owing to their importance and availability, these species are of interest to ecologists, physiologists and economic entomologists as experimental insects.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is one of the major storage pests of Bangladesh and is abundantly associated with stored grains of different cereals (Alam, 1971; Khan & Mannan, 1991). It lives in almost any kind of flour, cracked grain, breakfast food or meal. The beetle is unable to feed on whole cereal grains because their mouth parts are not adapted to attacking large and hard pieces of food (Chapman, 1931). The species also lives on chocolate, red pepper, various kinds of nut and specimens in insect collection (Good, 1936). A list of specific foods in which these beetles are

found was compiled some hundred years ago (Chittenden, 1896; 1897) and the list includes whole wheat flour, bleached and unbleached white flour, bran, rice flour, cornmeal, barley flour and oatmeal. About 20% of annual production is damaged by storage insects in our country (Mondal & Port, 1994). That is why it is very important to protect food from insect damage not only for economic losses alone but also for health reasons.

Much of the damage done by *T. castaneum* is directly related to the kernel of the grain. It contaminates more than it consumes. The contamination results from the presence of living or dead insect parts, cast exuviae, egg shells, pupal cases, faecal matter, noxious and persistent odour and webbing of food (Khan & Mannan, 1991). In the last decade *T. castaneum* has been described as a 'serious secondary pest' in food stores throughout the warmer parts of the world (Hill, 1983). Infestation is apparent by the appearance of adults on the surface of the grain. There is extensive damage to previously holed or broken grains, or grains damaged by other pests. Damage is done by both larvae and adults.

Economic entomologists throughout the world spend a great deal of time and effort in attempts to determine the presence of beetles in stored-products, check their infestations and design better and safer methods to bring them under control. At the same time, the ease in rearing these insects in the laboratory has attracted the attention of ecologists, nutritionists, physiologists and more recently geneticists to these beetles.

Various insecticides are used for controlling insect pests which have many side effects. Since chemical insecticides are conventionally used for controlling insect pests in most part of the world (Schofield, 1993), growing concerns over the environmental consequences, the resulting constraints regarding the use of non-selective and persistent insecticides, coupled with the development of insecticide resistance by target species, appearance of secondary pests due to destruction of natural enemies (parasites and predators of the pests) and steadily increasing costs, have brought about intensified efforts to develop genetic and other control agents for the suppression of pestiferous insects (Lacey & Undeen, 1986; Knowles, 1994). Considerable interest, therefore, has recently been developed in utilizing self-propagating genetic methods of population control. One of such methods entails the introduction of a genetic material called cytoplasmic incompatibility (CI) into the target pest population causing a partial or complete breakdown in gamete formation (Collins & Paskewitz, 1995).

1.2 Cytoplasmic incompatibility (CI)

Cytoplasmic incompatibility (CI) has been defined as a functional incompatibility between a maternally inherited cytoplasmic milieu and a male element (Clements, 1992). It is a widespread phenomenon among plants and animals, involving self-replicating extrachromosomal factors of a variety of types. One of the most investigated instances is that of *C. pipiens*, in which crosses between populations from different geographical locations may be sterile in one or both directions. The sterility

that occurs when incompatible strains of an insect cross is expressed as the production of non-hatching but mostly embryonated eggs by inseminated females. Most authors have recognized three categories of cross, although their definitions have varied. However, the following definitions are consistent with current usage (Clements, 1992). (1) *Compatible crosses*: larvae hatch from $\geq 50\%$ eggs laid and the sex-ratios are normal; (2) *Partially compatible crosses*: embryonic development starts in a substantial percentage of eggs but these have an intermediate hatch rate ($\leq 50\%$) and adults that develop include males and females; and (3) *Incompatible crosses*: embryonic development proceeds in a good number of eggs but larvae hatch from only 0 to $\leq 1\%$ of eggs and adults, if any, that develop (of course, parthenogenetically) are always females.

As early as 1937 it was found that crosses of *C. pipiens* L. from different geographical areas sometimes produced eggs that did not hatch (Marshall & Staley, 1937). Recognized by Laven (1951) as a possible means of controlling mosquitoes, the phenomenon was soon studied extensively. Laven (1967) showed that the factor(s) causing incompatibility were maternally transmitted through the cytoplasm, often as many as half of the eggs laid were dead with haploid embryos in them and mating and insemination of the females appeared to be normal. Although various hypotheses of the cause of incompatibility were proposed (Smith-White & Woodhill, 1954; Laven, 1967; Jost, 1971; Yen & Barr, 1971), only Yen & Barr (1973) offered experimental evidence in support of their hypothesis. After a rickettsia-like microorganism, *Wolbachia pipientis* Hertig was found in the

cytoplasm of *C. pipiens* eggs (Yen & Barr, 1971), the microorganism was determined to be the cause of incompatibility through crosses of infected (with *Wolbachia*) and tetracycline-treated, aposymbiotic or 'cured' strains by Yen & Barr (1973, 1974). Soon after this, Subbarao and colleagues reported variations in CI properties in populations of *C. p. fatigans*. The Paris and Delhi strains were in general incompatible while the Delhi strain was polymorphic for its crossing properties, the majority were bidirectionally incompatible and the minority unidirectionally incompatible with the Paris strain (Subbarao et al., 1974). Variant incompatibility properties in *C. pipiens* complex involving the Delhi, Paris, Prague, Hamburg and Bangkok strains were further analysed by Subbarao et al. (1977a,b).

1.3 A brief survey of literature on CI

CI was first described in mosquitoes and studied in detail by Laven in the 1950s. Laven (1956) observed that crosses between different strains of mosquitoes sometimes failed to produce progeny, or produced progeny only when crossed in one direction i.e. crosses of males from one strain with females from another produce progeny, but not the reciprocal cross. Laven (1967) showed that cytoplasmic factors were involved. This led to propose a new hypothesis of the cause of CI in *C. pipiens* (Yen & Barr, 1971) and the isolation of *Wolbachia* microorganisms as the causative agent of incompatibility by Yen & Barr (1973). The proximate cause of CI in different insects, therefore, was due to presence of a bacterium *Wolbachia pipientis* first identified by Hertig in 1936. These bacteria, variously been named as

rickettsia-like microorganisms or RMO by Clements, (1992), rickettsia-like organisms or RLO by Beard et al., (1993) and cytoplasmic incompatibility microorganisms or CIM by Breeuwer & Werren (1993), fall in the alpha subdivision of Proteobacteria and family Rickettsiaceae of the order Rickettsiales. It is a Gram-negative bacteria and are delimited by three membranes. The internal structure consists of ribosomes, slightly smaller than those of the surrounding cytoplasm and a network of nucleic acid filament. In the ovary they are found in oocyte and nurse cells and in spermatocytes of the testes (Clements, 1992).

Following the early mosquito work, CI was found not only in several other insects, including *Nasonia*, plant hoppers, raisin moths, weevils and *Drosophila*, but also in an isopod (Crustacea), showing that the phenomenon is not restricted to insects. Soon after incompatibility was described in *Drosophila*, major advances were made in understanding both the population dynamics of incompatibility systems and the molecular evolution of incompatibility factors. Molecular markers for the agent were constructed by O'Neill et al. (1992) and used to investigate the nature and distribution of incompatibility factors in populations and among species. These markers also enabled phylogenetic analyses of *Wolbachia* and their hosts.

1.3.1 CI in mosquitoes

In the 1950s, several workers observed that crosses among strains of mosquitoes often resulted in no adult offspring or relatively few adult offspring. Crossing relationships between

some European, Asian and American strains of *Culex* indicated that complex incompatibility patterns exist (Laven, 1967). Strains do not appear to fall into discrete incompatibility groups, but instead can be characterized only by their crossing patterns with several other strains. Thus incompatibility between strains may be bidirectional in which no or few progeny are produced in both sets of reciprocal crosses between populations. Incompatibility may also be unidirectional, when males from one strain are incompatible with females from the other strain, but the reciprocal cross appears fertile. Studies by Laven (1956, 1967) indicated strict maternal inheritance of the factor causing incompatibility. In a series of papers, Subbarao *et al.* (1974; 1977a,b) described the variation and segregation of CI in *C. pipiens* complex. Genetic and phenotypic studies on the dynamic nature of the CI system in this species were reported by French (1978). In addition to the crosses between geographically isolated populations of *C. pipiens*, incompatibility has also been found in crosses between strains from the same populations (Barr, 1980; Wright & Wang, 1980; Magnin *et al.*, 1987). Apart from incompatibility in *C. pipiens* linked to rickettsia-like microorganisms by Yen & Barr (1973), other cases of incompatibility in mosquitoes were linked to a *Wolbachia*-like organisms in *Aedes* species (Wright & Barr, 1980). Other factors unrelated to bacterial infection, however, have also been associated with incompatibility in this species complex (Meek & Macdonald, 1984). Arunachalam & Curtis (1985) and Shahid & Curtis (1987) integrated CI with radiation for genetic control in the *C. pipiens* complex. CI in mosquitoes involving cytoplasmotypes, restorer genes and the dynamics of cytoplasmic

inheritance have been reviewed by Clements (1992). Recent data on other insects suggest that infections causing incompatibility may involve more than one strain of *Wolbachia* (Breeuwer *et al.*, 1992; Sinkins *et al.*, 1995). There is a strong possibility that incompatibility is controlled by different genes in different *Wolbachia* strains and several hypotheses, interpretations based on nuclear factors, cytoplasmic factors and interactions between these, have been proposed to account for such incompatibility (Hoffmann & Turelli, 1997).

1.3.2 CI in *Tribolium*

Unidirectional incompatibility in *T. confusum* was initially detected in crosses between two strains named McGill Black (bl) by Stanley in 1961 and was later confirmed in other strains by Wade & Stevens in 1985. Females from these strains produced inviable eggs when mated with males from other strains. The ability of males to cause incompatibility was shown to be cytoplasmically inherited. *Wolbachia*-like microorganisms in the ovaries of *T. confusum* strains were detected but no infectious organisms were found in three laboratory strains of *T. castaneum* (O'Neill, 1989). Further work on *T. confusum* accompanied by the presence of *Wolbachia* and its curing with an antibiotic was reported by Stevens (1989). Using polymerase chain reaction (PCR) assays, O'Neill *et al.* (1992) studied a range of insect species including *T. confusum* and concluded that CI may be a more general phenomenon in insects than is currently recognized. Report on a multi-strain single-pair mating assay designed to look for variation in incompatibility type (Fialho & Stevens,

1996) indicated that *Wolbachia* infections cause a common incompatibility type across strains of *T. confusum*. Though the presence of any rickettsia-like microorganisms in *T. castaneum* is not yet confirmed, Islam et al. (1997) have recently reported that an incompatible cross is possible between a local (RAJ) and an exotic (FSS-II) strains of the beetle. In addition, cross-matings among several indigenous and exotic strains of *T. castaneum* by the same authors show the presence of both partial and complete incompatibilities in this species.

1.3.3 CI in *Nasonia*

In the hymenopteran parasitic wasp *Nasonia vitripennis*, incompatibility was first reported by Saul (1961), who demonstrated that the causative agent was maternally transmitted. It is one of the few species in which the cytogenetic mechanism of CI is known. Report by Breeuwer & Werren (1990) showed that in *N. vitripennis*, the paternal chromosomes form a tangled mass in a fertilized egg and are eventually lost. The same report also revealed that the cytoplasmic microorganisms are associated with complete bidirectional incompatibility between *N. vitripennis* and a closely related sympatric species, *N. giraulti*. Later it was found that *Nasonia* individuals may harbour single, double or even multiple infections (Breeuwer et al., 1992). It was subsequently confirmed by Breeuwer & Werren (1993), who demonstrated that compatibility type was independent of the host genotype and was based on differences between cytoplasmic bacteria of the two *Nasonia* species.

1.3.4 CI in *Drosophila*

In *Drosophila*, CI was first described by Hoffmann et al. (1986) in *D. simulans* from California and by Hoffmann (1988) in *D. melanogaster* from Australia. Electron microscopy identified the causative agent of incompatibility in this species as being a *Wolbachia*-like microorganism (Binnington & Hoffmann, 1989), the latter being present in ovaries and testes of naturally infected *D. simulans*. Backcrosses indicated that the infection carried by type 'R' flies was maternally inherited (Hoffmann & Turelli, 1988), but when infected males were mated to uninfected females (incompatibility type 'W'), infected progeny were produced at a very low frequency, suggesting a paternal inheritance. Findings by Turelli & Hoffmann (1991) indicate that CI in *D. simulans* is spreading rapidly at a rate of more than 100 km per year and populations in which the infection was rare have become almost completely infected within three years. Laboratory experiments by Hoffmann et al. (1994) show that infection by *Wolbachia* in *D. melanogaster* is not associated with a reduction in fecundity. Moreover, infections that do not cause incompatibility have been isolated from natural populations of *D. simulans* which is widespread in Australian, Ecuadorian and Floridan populations (Hoffmann et al., 1996). Apart from *D. simulans* and *D. melanogaster*, CI has also been reported in the species like *D. mauritiana*, *D. sechellia*, *D. recens*, *D. orientacea*, *D. auraria* and *D. ananassae* (Giordano et al., 1995; Hoffmann & Turelli, 1997). Recent studies by Hoffmann et al. (1998) in *D. melanogaster* suggest that the *Wolbachia* infection probably

provides a fitness benefit for the host in order to persist in populations. Clancy & Hoffmann (1998), on the other hand, showed that environmental factors may influence the expression of CI in *D. simulans* and the environmental effects may be mediated by the density of the RMO causing infections.

1.3.5 CI in other insects

Evidence of CI has been obtained in three other insects viz. the almond moth, *Ephestia cautella* (Kellen et al., 1981), the small brown plant hopper, *Laodelphax striatellus* (Noda, 1987) and the alfalfa weevil, *Hypera postica* (Hsiao & Hsiao, 1985a,b). However, crossing data from several other insects suggest that *Wolbachia* are present in 10-20 percent of all insect species (Werren et al., 1995), and numerous additional cases of CI are expected to be detected in future (Hoffmann & Turelli, 1997).

1.4 Mechanism of the expression of CI

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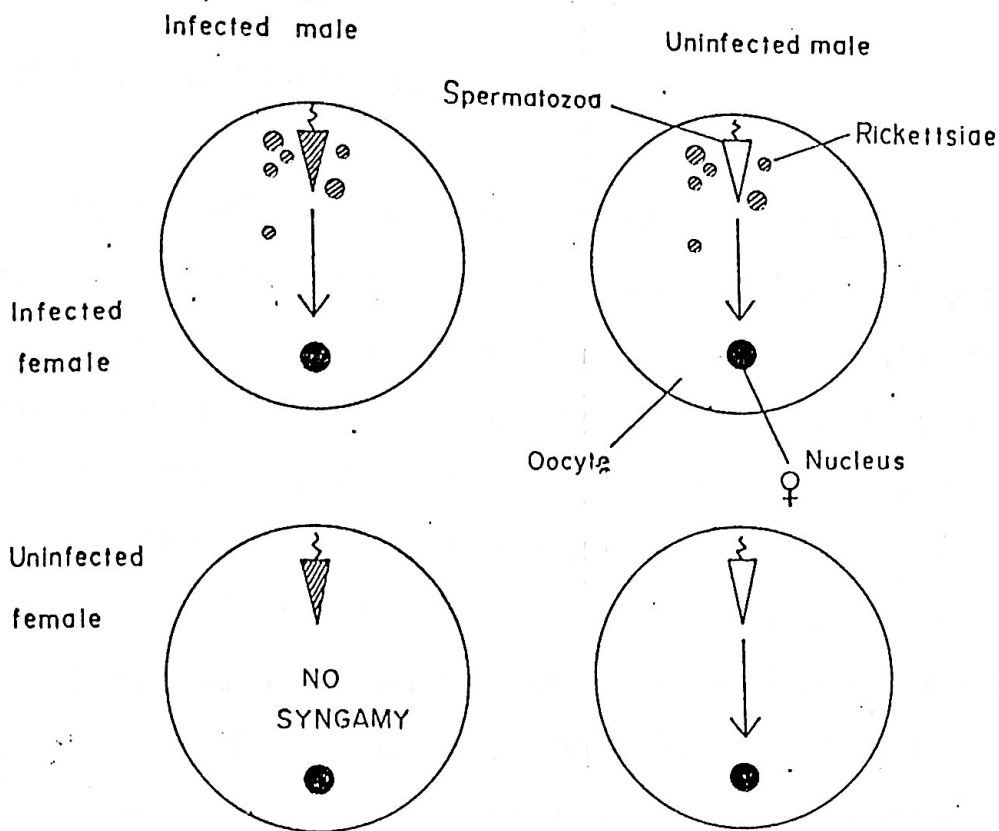
In *Culex pipiens*, incompatible sperms appear to enter eggs and induce the two meiotic divisions, but the sperms do not fuse successfully with the female pronuclei (Jost, 1971). This results in haploid egg, which may show limited development before the embryo dies unless diploidy is restored by rare parthenogenesis. There is very little embryo development in incompatible crosses with *Drosophila* (O'Neill & Karr, 1990), suggesting that *Wolbachia* effects generally occur early in development. In the haplodiploid species *Nasonia vitripennis*, *Wolbachia* disrupt the condensation of chromosomes from the male parent at the first mitotic

division of an embryo, as a result, these chromosomes become a tangled mass and fragment (Ryan *et al.*, 1985). The reason why chromosomes fail to condense and embryos die is unclear. Since *Wolbachia* are not present in mature sperm (Yen & Barr, 1973; Binnington & Hoffmann, 1989), they must exert their effect on immature sperm which is then carried over to the egg during fertilization. Moreover, it has been observed that the expression of incompatibility is influenced by the density of bacteria in cells (Hoffmann *et al.*, 1986; Binnington & Hoffmann, 1989; Breeuwer & Warren, 1993). Solignac *et al.* (1994) found a correlation between bacterial density in sperm cysts and levels of incompatibility based on 24 *D. melanogaster* strains from around the world. These findings suggest that bacteria determine the compatibility status of eggs as they mature. Breeuwer & Warren (1993) proposed a 'bacterial dosage' model and suggested that unidirectional incompatibility results from the relative dose of bacteria in males *versus* females. Incompatibility will be strong when the dose in the males is higher than that in the females. But it should be borne in mind that dosage alone is insufficient to account for all aspects of CI, such as bidirectional incompatibility and superinfection (Hoffmann & Turelli, 1997).

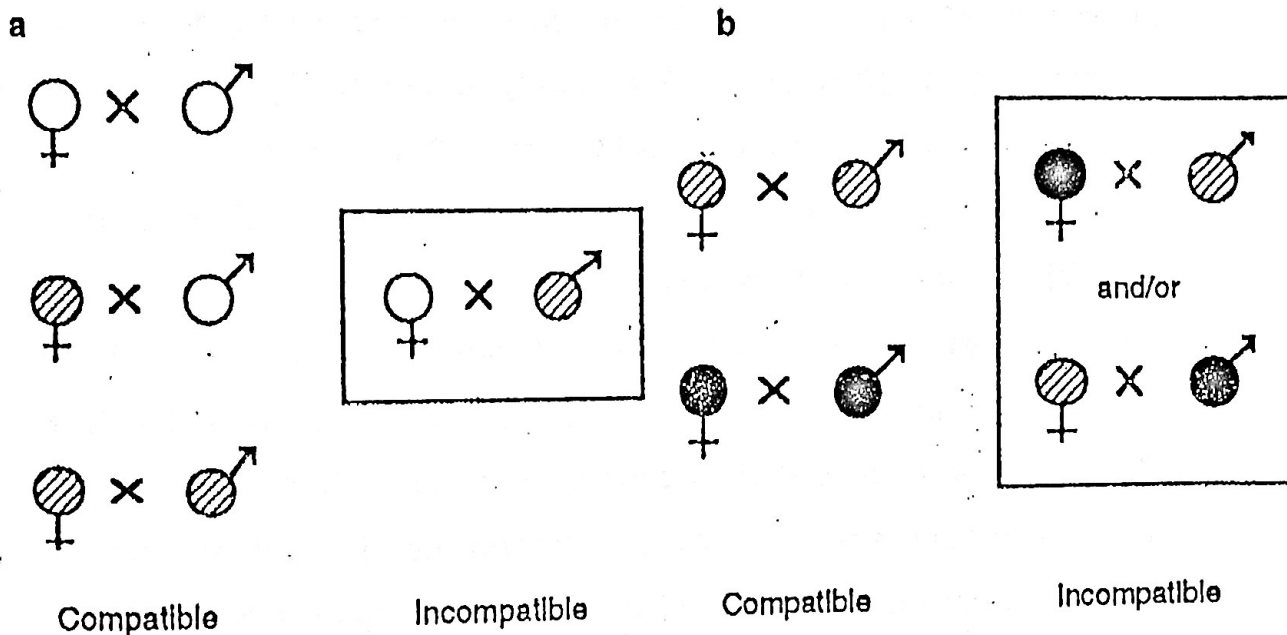
Unidirectional incompatibility is typically expressed when male insects infected with *Wolbachia* are crossed with uninfected females of the same species. The reciprocal cross (*i.e.* infected female x uninfected male), as well as crosses within a given infected strain (*i.e.* infected female x infected male), are fully compatible and produce normal numbers of surviving progeny. This has been explained by Rousset & Raymond (1991) as

follows (Diagram 1A): A mass of *Wolbachia* is present under the micropyle of infected eggs, where the spermatozoon enters. The fusion of gametic nuclei normally occurs when neither or both parents are uninfected, so wolbachiae are not needed for fusion to occur. Fusion also occurs when the male is uninfected and the female is infected. But when the mass of wolbachiae is absent, and the male is infected, the spermatozoon stops its progression, that means, syngamy is not achieved. The paternal *Wolbachia*, therefore, induce incompatibility, and the maternal *Wolbachia* restore fertility.

Bidirectional incompatibility is found when infected individuals from different insect strains, usually from different geographical localities are crossed. The resulting incompatibility can be expressed in both of the reciprocal crosses *i.e.* infected female of strain I x infected male of strain II and infected female of strain II x infected male of strain I (Diagram 1B). The patterns of incompatibility observed between such infected populations (commonly called 'crossing types') appear quite complex in some species (Beard *et al.*, 1993). Bidirectional CI is a more rarely observed phenomenon that has been reported in only a few insect hosts like *C. pipiens* (Laven, 1967; Magnin *et al.*, 1987), *D. simulans* (O'Neill & Karr, 1990) and two parasitoid wasp species of the genus *Nasonia* (Breeuwer & Werren, 1993). In this system, compatibility type appears to be independent of host genotype and is likely to be related to differences in the bacterial strains (Fialho & Stevens, 1996).



1A



1B

Diagram 1. (1A) showing the mechanism of unidirectional incompatibilities between infected and uninfected insects (after Rousset & Raymond, 1991); (1B) showing the expression of uni-(a) and bidirectional (b) incompatibilities (after Beard *et al.*, 1993). The mechanism is elaborated in the text (p. 13).

1.5 Incompatibility relationships

Incompatibility relationships of a pest species is of considerable interest for two main reasons. Firstly, it provides an excellent scope for evolutionary studies of the species concerned and secondly, it helps identifying strains from different geographical regions that are refractory or fully susceptible to vectoring a particular disease. For example, in order to study the inheritance of a disease susceptibility, therefore, it is necessary to find geographical strains which can be crossed with each other successfully. The information from such studies may eventually be applied to control a pest species by replacement of susceptible populations with refractory populations. Thus, crossing experiments between different strains or groups of mosquitoes (Meek & Macdonald, 1984; Magnin *et al.*, 1987), flour beetles (Wade & Stevens, 1985; O'Neill, 1989), alfalfa weevil (Hsiao & Hsiao, 1985a,b) and a parasitoid wasp (Breeuwer & Werren, 1993) have been carried out in a number of laboratories. It appeared from the above studies that CI is very significant in the determination of incompatibility relationships within the strains or groups of a species. In the present study, laboratory hybridization experiments with the red flour beetle *T. castaneum* have been conducted to estimate incompatibility relationships among some selected indigenous and exotic strains of the insect.

1.6 Uses of CI

CI could be a genetic method for controlling insect pests. It could provide 'ready-made' sterile-like males for a control operation. If incompatibility persists in a natural population, the consequence for the host population is reproductive waste (Arunachalam & Curtis, 1987). Since CI produces inviable progeny, is widely exhibited by plants and animals involving self replicating extra-chromosomal factor of a variety of types and without any involvement of chromosomal genes, and results in reduced rate of non-hatching but mostly embryonated eggs by inseminated females (Yen & Barr, 1973; Clements, 1992), it has been used in the control of main vector of bancroftian filariasis, *Culex pipiens* in which crosses between many strains of different geographical origin are sterile in both directions (Krishnamurthy & Laven, 1976). It is also helpful for studying chromosome imprinting and chromosome condensation, as well as elucidating modes of speciation (Breeuwer & Werren, 1990). Successful use of CI, however, is dependent on the degree of linkage that can be obtained between *Wolbachia pipientis* and the genetically altered symbiont. CI has the advantage over other gene spreading mechanisms, such as P-element mediated hybrid dysgenesis, in that it may be possible to use it to spread genes into the same population more than once (Beard *et al.*, 1993). Recent studies have shown that CI could be a means to drive genetically modified symbionts into a population. Braig *et al.* (1994) were able to replace the natural infection of *D. simulans* by means of microinjecting purified *Wolbachia* from the Asian tiger mosquito, *Aedes albopictus*. Turelli & Hoffmann (1999) generated models

involving microbe-induced CI as a mechanism for introducing transgenes into arthropod populations. The successful transfer of *Wolbachia* between distantly related hosts suggests that it may be possible to introduce this symbiont experimentally into arthropod species of medical and agricultural importance in order to manipulate populations genetically.

1.7 Aposymbiosis of the incompatible strains

It is possible to produce an aposymbiotic (i.e. 'cured' or free of wolbachiae) strains of a species infected with RMO. Yen & Barr (1973) were able to eliminate the microorganism *W. pipientis* from *C. pipiens* by rearing larvae in tetracycline-treated medium. Aposymbiosis in *Aedes polynesiensis* was induced by treatment with tetracycline hydrochloride or by rearing larvae at temperatures of 32°-33° C for 5-7 days (Wright & Wang, 1980). The fact that CI is largely mediated by the infectious microorganisms in most of the insects studied so far, recent studies have shown that elimination of these cytoplasmically inherited bacteria by heat or antibiotic treatments restores compatibility in insect species like *Aedes*, *Tribolium*, *Nasonia* and *Drosophila* (Trpis et al., 1981; Stevens, 1989; O'Neill, 1989; Breeuwer & Werren, 1993; Hoffmann & Turelli, 1997). In the present study, the incompatible strains of *T. castaneum* were subjected to tetracycline and UV radiation treatments to make them free of any microorganisms that might have caused CI in this species.

1.8 UV radiation

The radiations that are important in mutagenesis fall into two categories, *viz.* ionizing and non-ionizing. The former include X-rays, γ -rays, α and β rays, electrons, neutrons, protons and other fast-moving particles. Non-ionizing radiations include ultraviolet (UV) and visible lights, of these two, only UV light is important in causing mutations. Studies by Swanson (1940) indicated that only chromatid deletions are produced after UV exposure. In maize, UV appears to give rise chiefly to terminal deficiencies (Stadler, 1941). In sufficiently high doses, UV rays may kill exposed organisms and induce mutations among the survivors (Levine, 1980).

When UV light is absorbed by nucleic acids, the absorbed energy can cause alterations in the bond characteristics of the purines and pyrimidines. Pyrimidines are found to be more liable to such changes than purines. One of the consequences of the altered bond characteristics is the formation of covalent bonds between adjacent pyrimidines of the same DNA polynucleotide strand. The linked pyrimidines are called dimers. Of the three possible types of pyrimidine dimers that can be found in DNA, the thymine dimer is the one that forms most readily. Dimerization interferes with the proper base pairing of thymine with adenine and may result in thymine's pairing with guanine. Other products such as cytosine dimers are formed to a lesser extent and cause mutations by becoming deaminated to uracil dimers, since uracil acts like thymine. Studies of the mutagenic effects of UV raised a question about spontaneous mutation rates that nagged

geneticists for sometimes. It is obvious from recent experiments that any post UV treatment that affects the relative rates of DNA replication and DNA repair will affect the number of mutations resulting from the radiation (Levine, 1980).

Photosensitization of cells refers to response of the cells to visible lights. Visible lights are one of the categories of non-ionizing radiations that, like ultraviolet (UV) light, may kill organisms if exposed in sufficiently high doses and may induce mutations among the survivors (Levine, 1980). Studies aimed at explaining the mechanisms of photosensitization reveal that when non-ionizing radiation is absorbed by nucleic acids in the cell, the absorbed energy cause alterations in the bond characteristics of the purines and pyrimidines of the DNA molecule. Nitrogen bases so altered are called photoproducts. In addition, experiments on the genetic effect of visible lights on a variety of organisms suggest that cells vary in their susceptibility to photosensitization (Dulbecco, 1955; Jagger, 1960). Moreover, recent reviews have shown that the magnitude of photosensitization varies on the type and metabolic state of the cells (Chatterjee & Singh, 1987; Gallagher & Markow, 1991). Kelner (1949a) first reported that the number of *Escherichia coli* surviving large doses of UV radiation could be increased by a factor of several hundred thousand if, subsequent to irradiation, the organisms were exposed to an intense source of visible light. This phenomenon is known as photoreactivation, photorepair or photorestoration. Further work has shown that visible light can recover colony forming capacity of the post-irradiated *Streptomyces griseus* (Kelner 1949b). Later it was demonstrated

that the effective wavelengths comprise the short-wave path of the visible spectrum, up to about 480 nm, the red and yellow lights in particular (Dulbecco, 1955; Jagger, 1960). Stored in the dark, however, UV-irradiated cells or viruses may retain their response to visible light for differing times. Bacteria in the stationary phase, for example, lose response to photorepair in a few hours, while bacteria kept at -10°C or extracellularly stored viruses retain it over days or weeks (Jagger, 1960). Recent studies on higher organisms have considered the possibility of using UV rays to control or suppress various species of insects like *Culex* (Islam *et al.*, 1992), *Cadra* (Faruki & Khan, 1993) and *Exorista* (Hasan *et al.*, 1998). Keeping these findings in view, the present study was conducted to evaluate if UV treatment to the infected *T. castaneum* has any impact on the expression of CI in the immediate progenies.

1.9 Frequency-dependent regulation in *T. castaneum*

Intraspecific competition accounts for population growth in many insect species which breed in small enclosed places (Mori & Wada, 1978) or in artificially maintained population in the laboratory (Chubachi, 1979). Many agriculturally and medically important pests colonize in larger but less stable habitat which makes the measurement of competition much more difficult. The flour beetles too have been the subject of many experiments on the growth and limitation of animal populations. When a small number of flour beetles are placed in a box of flours there is an initial rapid increase followed by a tailing off to a steady maximum population. This is known as a sigmoid growth curve and

the rate of increase and maximum numbers can be altered by varying temperature, humidity so on (Begon, 1982). Although a number of studies with mosquitoes and other dipterans have been carried out to demonstrate that mortality and size variation operate in a density-dependent manner (Hawley, 1985; Renshaw *et al.*, 1993), no such data are available for different strains of *T. castaneum* explaining the phenomenon of its frequency-dependence. This led to design the present experiments where only the incompatible crosses involving indigenous *versus* exotic strains have been subjected to a frequency-dependent competition at various ratios from 1:1:1 to 1:1:3 to evaluate whether CI could account for the regulation of this pest species.

1.10 Mating competitiveness of the incompatible males

Mating competitiveness refers to the mating success of a treated or sterilized male when the latter is allowed to compete with untreated males for untreated females of the same species (Islam & Port, 1992). In control or the eradication programmes involving the sterile insect techniques (SIT), released sterile males must compete successfully with wild males for wild females. Mating competitiveness, therefore, is the most important propensity of cytoplasmically incompatible, chemosterilized or radiosterilized males, and should be maintained as naturally as possible. Experiments with *Culex* (El-Gazzar & Dame, 1983), *Callosobruchus* (Hussain & Imura, 1989) and *Tribolium* (Hasan, 1998) have shown that the sterilized males are fully competitive under laboratory conditions.

As Fried (1971), Hooper & Katiyar (1971) and Hooper & Horton (1981) showed, the use of egg-hatch and egg-infertility data for the determination of competitiveness of any sterilized insect has a major advantage in that several components of competitiveness are incorporated in one value. Thus egg-hatch data could be a realistic assessment of incompatible insects' competitiveness under laboratory conditions. Determination of the mating competitiveness values (CVs) of sterilized or otherwise incompatible males may be made either by direct observations or by the ratio test method (Islam & Port, 1992). In direct observations, mating competitiveness is estimated by assessing performance of the 'treated' males when they are allowed to compete with the untreated males for untreated females under caged conditions. In the ratio test method, which has widely been used in estimating total competitiveness of sterile males (Brower, 1982; Hasan, 1998), a known proportion of treated to untreated males are allowed to mate with a set number of untreated females. Perhaps this gives the best laboratory-based measure of CVs of the competing males in question because the resulting egg-hatch data reflect the interaction of a number of factors such as mating ability, sperm competition and the impact of multiple matings. Till now, however, no attempts have been made to estimate the mating competitiveness of the incompatible males of the red flour beetle *T. castaneum*, and this led to design a major part of the present investigation.

1.11 Aims of the study

With a view to investigate the effects of cross-matings among the members of the indigenous and some selected exotic strains of *T. castaneum* on such reproductive potential as fecundity, percentage of egg-hatch or hatchability and number of adult progenies, the present study was aimed at:

1. To determine if there exists any CI between the indigenous and exotic strains of the red flour beetle, *T. castaneum*;

2. To investigate the effects of backcrosses on the expression of CI in the insects;

3. To estimate incompatibility relationships of the strains under study; the relationships, in terms of the complete, partial or no CI that exist among the strains, would reflect if a control strategy might be feasible for the species;

4. To produce aposymbiotic strains by treating the beetles with chemical (antibiotic) and physical (UV radiation) agents;

5. To determine the ratio of incompatible males required for an effective regulation of the pest species, a frequency-dependent experiment is designed under laboratory conditions; and finally

6. To estimate the total mating competitiveness values (CVs) of the incompatible exotic males, if any, by introducing them into samples containing the indigenous female and male strains at varying ratios.

Findings of the present investigation, hopefully, might be of immense help in designing an effective genetic or integrated control strategy for an important and widespread pest species like *T. castaneum*.

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Indigenous and exotic strains of *T. castaneum*

Adults of the indigenous *T. castaneum* were collected from stored-grain shops of four main metropolitan cities viz. Chittagong, Dhaka, Khulna and Rajshahi of the country. The strains, designated by the first three letters such as CHI for Chittagong, DHA for Dhaka, KHU for Khulna and RAJ for Rajshahi, were inbred in the laboratory for two successive generations to eliminate natural and/or deleterious mutations, if any, that might have been incorporated in their genome over time.

In addition to the indigenous strains, four exotic strains viz. *Black* (BLA), *CTC-12* (CTC), *FSS-II* (FSS) and *Kano* (KAN), procured from the Central Science Laboratory, Slough, UK, which had been maintained in the Department of Zoology, University of Rajshahi, for the last three years, were used in the present investigation.

2.2 Maintenance of *T. castaneum*

T. castaneum is one of the major stored-grain coleopteran pests. It is commonly known as the 'red flour beetle'. This beetle has become cosmopolitan in distribution due to development of world trades (Cotton, 1947; Pruthi & Singh, 1950; Dyte & Rowlands, 1967; Sokoloff, 1974). It is more frequently kept as

laboratory insects because it spends its entire life-cycle in a single medium. The cultures of *T. castaneum* used in the present experiments were maintained as described below.

2.2.1 Mass culturing of *T. castaneum*

For maintaining the cultures of both strains of *T. castaneum*, the following techniques were used. The cultures were maintained in 500 ml beakers containing food medium (see below). Pieces of folded filter papers were placed inside each beaker for easy movements of the insects. The beakers were covered with pieces of cloth tied by rubber bands and kept in an incubator (Plate 1) at temperatures ranging from 29° to 31°C and at an uncontrolled relative humidity (RH).

2.2.2 Food medium

A standard mixture of whole wheat flour with dry brewer's yeast was used as a food medium. In this medium, the proportion of wheat flour and yeast was 950: 50 gm, in a ratio of 19: 1 (Park & Frank, 1948). The flour was sterilized at 120°C for 4 hours in an oven. After cooling for 48 hours, the flour was mixed with yeast dust and kept in an air-tight jars (Plate 2). The food was not used until at least 24 hours after sterilization to allow its moisture content to equilibrate with the laboratory environment. Each beaker was sub-cultured every three months to remove dead insects, exoskeleton and exhausted medium.

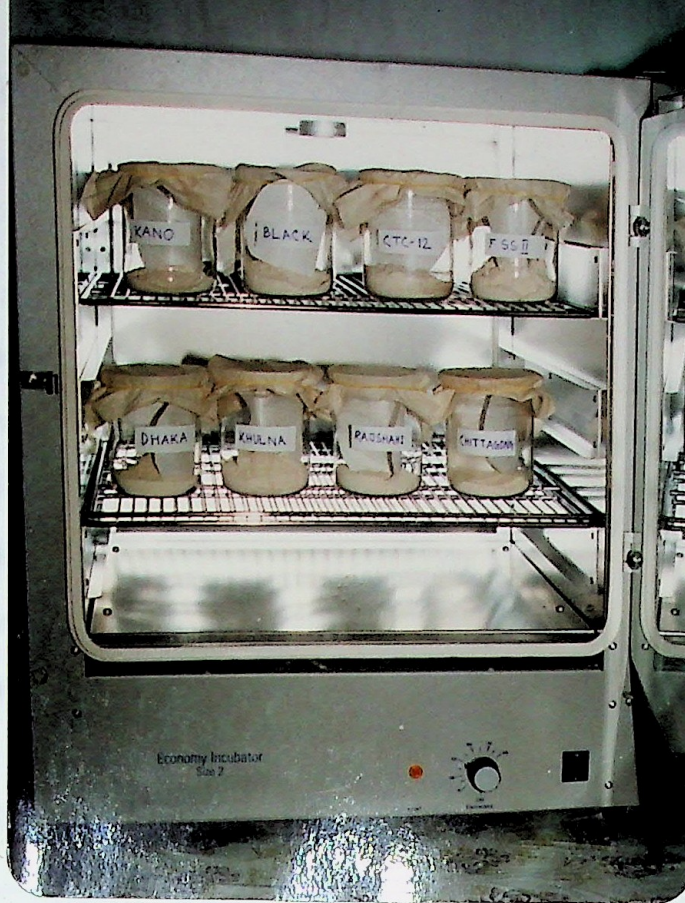


Plate 1. Mass cultures of the indigenous (bottom rack) and exotic (top rack) strains of *T. castaneum* maintained in an incubator.



Plate 2. Food medium kept in air-tight jars.

2.2.3 Collection of eggs

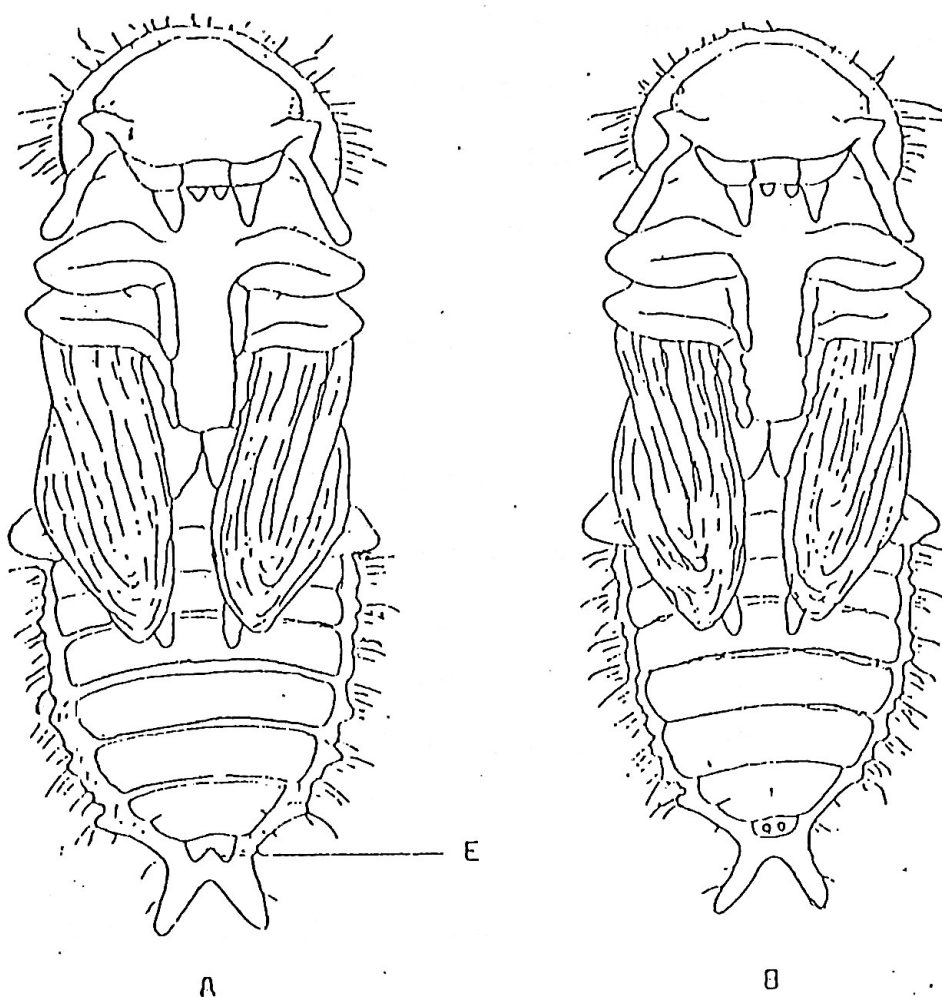
The experimental eggs from the mated females were collected as follows. At the time of setting single-pair matings for the purebred and hybrid crosses (mentioned below), the food medium was sieved consecutively five times through a fine sieve (holes 676 sq. cm^{-1}) and the females were allowed to oviposit in such medium. This ensured sieving out of any eggs that were laid by the females in the medium, while the medium alone passed through the sieve.

2.2.4 Collection of adults and their sexing

As it is not possible to differentiate *T. castaneum* males from females in adult and larval stages, pupae (Diagram 2) were sexed by the microscopic examination of the exogenital process, which in females is different from that in males (Halstead, 1963). The pupae were collected from the culture by sieving (coarse sieve, $225 \text{ holes sq. cm}^{-1}$) the food medium and separated by the help of a paint-brush and by examining them under a binocular microscope. The female and male pupae were kept in separate Petri dishes for the emergence of adults.

2.3 Measurements of reproductive potential

For measuring the reproductive potential, and subsequently for estimating cytoplasmic incompatibility, of the strains of *T. castaneum* under study, the following reproductive potential of the insects were measured.



E = Exogenital process

A = Female pupa

B = Male pupa

Diagram 2. Pupae of *T. castaneum* showing sexual dimorphism.

2.3.1 Fecundity

The total number of eggs laid by a female during a period of 72 hours after pairing with a male partner constituted its fecundity. The eggs were collected on food medium in small Petri dishes and counted under a binocular microscope before hatching.

2.3.2 Percentage of egg-hatch

Percentage of egg-hatch constitutes the hatchability of an insect. It is calculated by using the formula: % egg-hatch (hatchability) = number of eggs hatched ÷ total number of eggs laid x 100. For each experiment, the parameter was recorded for about 9 to 10 days after laying during which all the viable larvae hatched out and started feeding.

2.3.3 Number of adult progenies

The number of adult progenies emerged per replication in one of the observations, viz. the frequency-dependent regulation experiment (Section 2.7), was counted after about a month of pairing of the parents. Egg-to-adult developmental stages of *T. castaneum* are shown in Plate 3.

2.4 Estimation of CI and incompatibility relationships

The cytoplasmic incompatibility (inviability crosses) of the experimental strains of *T. castaneum*, if any, was determined by using the percentage of egg-hatch data of the strain(s) in

question. Finally, by compiling the results of all the purebred and hybrid crosses, the incompatibility relationships exhibited by the indigenous and exotic strains under study were established.

2.5 Crossing schedules

Apart from matings amongst the strains themselves (purebreds), each strain of *T. castaneum* was allowed to mate with all other strains reciprocally to produce hybrid progenies. Five replications per cross were maintained, and each of the single-pair crosses was performed in small Petri dishes provided with sieved food medium and covered with lids (Plate 4). All matings were carried out using the adults after 5 days of emergence. The following crossing schedules were carried out for estimating cytoplasmic incompatibility, if any, in the mating beetles.

2.5.1 Purebred crosses

No.	female	male
-----	--------	------

A. Indigenous strains

- | | | |
|----|-------|-----|
| 1. | CHI x | CHI |
| 2. | DHA x | DHA |
| 3. | KHU x | KHU |
| 4. | RAJ x | RAJ |

B. Exotic strains

- | | | |
|----|-------|-----|
| 5. | BLA x | BLA |
| 6. | CTC x | CTC |
| 7. | FSS x | FSS |
| 8. | KAN x | KAN |

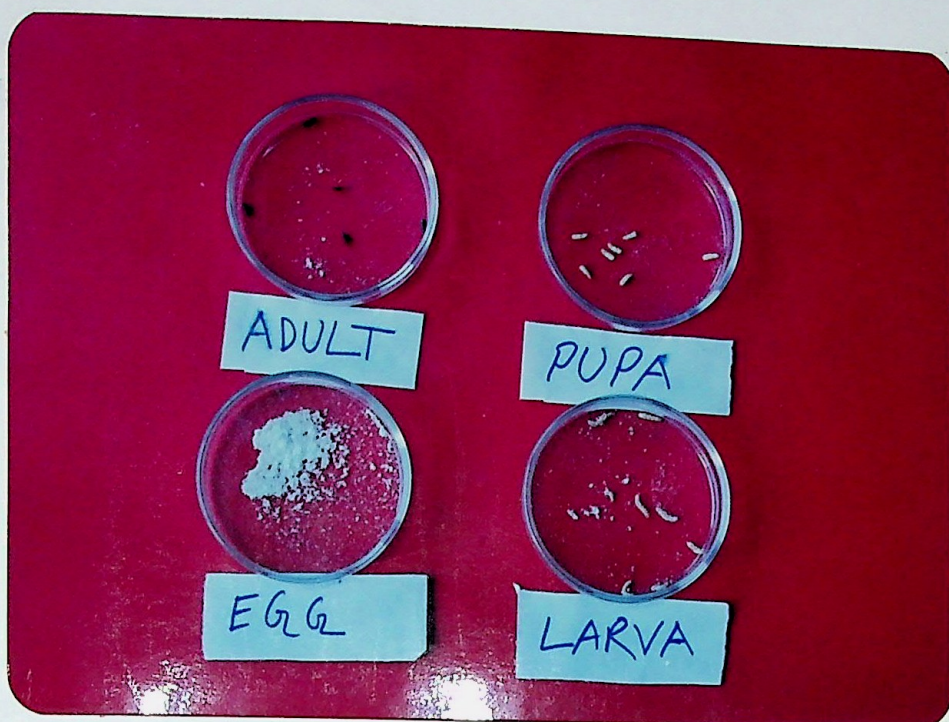


Plate 3. Egg-to-adult developmental stages of *T. castaneum*.



Plate 4. A few hybrid crosses (indigenous x indigenous strains) of *T. castaneum*.

2.5.2 Hybrid crosses

No. female male

A. Indigenous x indigenous strains

- | | | | |
|-----|-----|---|-----|
| 1. | CHI | x | DHA |
| 2. | CHI | x | KHU |
| 3. | CHI | x | RAJ |
| 4. | DHA | x | CHI |
| 5. | DHA | x | KHU |
| 6. | DHA | x | RAJ |
| 7. | KHU | x | CHI |
| 8. | KHU | x | DHA |
| 9. | KHU | x | RAJ |
| 10. | RAJ | x | CHI |
| 11. | RAJ | x | DHA |
| 12. | RAJ | x | KHU |

B. Indigenous x exotic strains

- | | | | |
|-----|-----|---|-----|
| 1. | CHI | x | BLA |
| 2. | CHI | x | CTC |
| 3. | CHI | x | FSS |
| 4. | CHI | x | KAN |
| 5. | DHA | x | BLA |
| 6. | DHA | x | CTC |
| 7. | DHA | x | FSS |
| 8. | DHA | x | KAN |
| 9. | KHU | x | BLA |
| 10. | KHU | x | CTC |
| 11. | KHU | x | FSS |
| 12. | KHU | x | KAN |

(contd.)

No. female male

B. Indigenous x exotic strains

(contd.)

13. RAJ x BLA

14. RAJ x CTC

15. RAJ x FSS

16. RAJ x KAN

C. Exotic x indigenous strains

1. BLA x CHI

2. BLA x DHA

3. BLA x KHU

4. BLA x RAJ

5. CTC x CHI

6. CTC x DHA

7. CTC x KHU

8. CTC x RAJ

9. FSS x CHI

10. FSS x DHA

11. FSS x KHU

12. FSS x RAJ

13. KAN x CHI

14. KAN x DHA

15. KAN x KHU

16. KAN x RAJ

It is to be mentioned that out of 64 crosses in an 8 x 8 factorial design, a total of 52 crosses were performed and 12 crosses in between the exotic strains themselves were excluded.



Plate 5. A few hybrid crosses (indigenous x exotic strains) of *T. castaneum*.



Plate 6. A few backcrosses (indigenous/exotic x F_1 progenies) of *T. castaneum*.

2.5.3 Backcrosses

Seven backcrosses between apparently uninfected exotic and indigenous females and infected F₁ males (derived from infected females x uninfected males) were performed. These were as follows:

<u>No.</u>	<u>female</u>	<u>male</u>
1.	CHI x	F ₁ (KHU x CHI)
2.	CHI x	F ₁ (RAJ x CHI)
3.	DHA x	F ₁ (KHU x DHA)
4.	DHA x	F ₁ (RAJ x DHA)
5.	BLA x	F ₁ (CTC x BLA)
6.	BLA x	F ₁ (FSS x BLA)
7.	BLA x	F ₁ (KAN x BLA)

2.6 Production of aposymbiotic strains

Incompatible strains of *T. castaneum* were treated with both an antibiotic and UV radiation separately to make them aposymbiotic or 'cured' (*i.e.* free of any RMO). The following procedures were maintained.

2.6.1 Antibiotic treatment

The incompatible indigenous and exotic strains *viz.* KHU, RAJ, CTC, FSS and KAN, determined from purebred and hybrid cross-matings described earlier (Section 2.5), were reared on food medium supplemented with 0.125% tetracycline (125 mg tetracycline in 100 g food medium). Tetracycline (C₁₂H₂₄N₂O₈;



Plate 7. A few cross-matings reared on antibiotic-treated food medium.



Plate 8. Pupae of *T. castaneum* subjected to UV radiation treatment.

mol. wt. 444.4) was procured from Sigma Co. (USA) as free base crystal and preserved in a freezer at -4°C until used. Pupae were sexed and single-pair matings were set-up in plastic Petri dishes containing either tetracycline-treated or normal food medium (Plate 7). After 5 days, each Petri dish was sifted through a fine sieve and the presence or absence of eggs (*i.e.* fecundity) was scored and the percentage of egg-hatch, where available, were recorded. A total of 100 cross-matings, 3 replications each, were used for this experiment (Tables 8-10).

2.6.2 UV radiation treatment

Three to four day-old pupae of the incompatible strains were subjected to UV radiation doses of 254 nm and 366 nm for 30 sec each. For the treatments, 10 male pupae and an equal number of female pupae of each strain were taken in separate Petri dishes. They were then exposed to UV radiation source for desired duration (Plate 8). After about two hours following UV treatments, the pupae were paired in separate Petri dishes provided with normal food medium. Fecundity and percentage of egg-hatch were estimated for each dose. Three replicates per dose per cross were maintained for which a total of 600 crosses were performed (Tables 11-15).

2.7 Frequency-dependent regulation in *T. castaneum*

To evaluate the frequency-dependent regulation in the experimental insects, an experiment was conducted between the incompatible indigenous (CHI and DHA) and exotic (CTC, FSS and KAN) strains of *T. castaneum* at three different ratios of 1:1:1, 1:1:2 and 1:1:3. For this experiment, 5 three day-old females and 5 males of the same age from each of the indigenous strains and 5 males of the incompatible exotic strain (*i.e.* in the ratio, 1: 1: 1) were placed in a beaker (500 ml) provided with normal diet. The ratio was increased to 1: 1: 2 and 1: 1: 3 by putting 10 and 15 exotic males respectively with the same number of indigenous females and males mentioned above. Indigenous purebreds (1: 1: 0) and indigenous female x exotic male crossbreds (1: 0: 1) were set for each set of cross as controls (see the crossing schedule). Three replications per cross and a total of (30 x 3) 90 crosses were maintained. Data on fecundity, percentage of egg-hatch and the number of adult progenies in each cross were recorded.

2.8 Estimation of mating competitiveness in *T. castaneum*

To determine the mating competitiveness values (CVs) of the incompatible exotic male strains of *T. castaneum*, egg-hatch data from the frequency-dependent regulation experiment (Section 2.7) were used. The percentage of egg-hatch expected on the assumption of unimpaired mating competitiveness of the incompatible males was calculated by using Hooper & Horton's (1981) formula while for estimating CVs, the ratio test method (Islam & Port, 1992) was employed.

For evaluating frequency-dependent regulation and estimating mating competitiveness values in *T. castaneum*, the following crossing schedules were maintained where each cross had 3 replications.

Number of mating insects (female: male: male)			Ratios
5 CHI:	5 CHI	0	1:1:0
5 CHI:	0	5 CTC	1:0:1
5 CHI:	5 CHI:	5 CTC	1:1:1
5 CHI:	5 CHI:	10 CTC	1:1:2
5 CHI:	5 CHI:	15 CTC	1:1:3
5 CHI:	5 CHI	0	1:1:0
5 CHI:	0	5 FSS	1:0:1
5 CHI:	5 CHI:	5 FSS	1:1:1
5 CHI:	5 CHI:	10 FSS	1:1:2
5 CHI:	5 CHI:	15 FSS	1:1:3
5 CHI:	5 CHI	0	1:1:0
5 CHI:	0	5 KAN	1:0:1
5 CHI:	5 CHI:	5 KAN	1:1:1
5 CHI:	5 CHI:	10 KAN	1:1:2
5 CHI:	5 CHI:	15 KAN	1:1:3
5 DHA:	5 DHA	0	1:1:0
5 DHA:	0	5 CTC	1:0:1
5 DHA:	5 DHA:	5 CTC	1:1:1
5 DHA:	5 DHA:	10 CTC	1:1:2
5 DHA:	5 DHA:	15 CTC	1:1:3
5 DHA:	5 DHA	0	1:1:0
5 DHA:	0	5 FSS	1:0:1
5 DHA:	5 DHA:	5 FSS	1:1:1
5 DHA:	5 DHA:	10 FSS	1:1:2
5 DHA:	5 DHA:	15 FSS	1:1:3
5 DHA:	5 DHA	0	1:1:0
5 DHA:	0	5 KAN	1:0:1
5 DHA:	5 DHA:	5 KAN	1:1:1
5 DHA:	5 DHA:	10 KAN	1:1:2
5 DHA:	5 DHA:	15 KAN	1:1:3

2.9 Analyses of the data

Data collected from different protocols mentioned above were processed, tabulated and analysed in accordance with the objective of the study. Percentages, means and standard errors of the data were calculated. Student's t-tests were used for comparisons between treatments. Expected percentage of egg-hatch and the CVs were calculated as follows:

Expected % egg-hatch at a given ratio, $H_e = \frac{I \times H_p + E \times H_h}{I + E}$

where, I = number of indigenous males
 E = number of exotic males
 H_p = mean % egg-hatch from the purebred cross (indigenous female x indigenous male)
 H_h = mean % egg-hatch from hybrid cross (indigenous female x exotic male).

Mating competitiveness value, $CV = \frac{I \times H_p - H_o}{E \times H_o - H_h}$

where, I = number of indigenous males
 E = number of exotic males
 H_p = mean % egg-hatch from the purebred cross (indigenous female x indigenous male)
 H_o = mean % egg-hatch observed at a given ratio
 H_h = mean % egg-hatch from hybrid cross (indigenous female x exotic male).

Some of the raw data as well as the methods of calculating t-values and CVs are presented in Appendices 1-11 (Chapter six; pp. 98-108). Figures (Figs. 1-15) are also appended at the end of the Chapter (pp.109-116).

CHAPTER THREE

RESULTS

RESULTS

3.1 Purebred and hybrid crosses

Data on the purebred crosses involving indigenous and exotic strains and their consequences on the reproductive potential in *T. castaneum* have been presented in Table 1. Mean $\pm$ SE fecundity in the indigenous strains was found to be 61.0 $\pm$ 9.9, 64.0 $\pm$ 4.1, 28.6 $\pm$ 2.1 and 47.2 $\pm$ 4.1 respectively in CHI, DHA, KHU and RAJ (Appendix 1), where DHA strain showed the highest and RAJ the lowest values. The corresponding data on the percentage of egg-hatch for the indigenous strains were 72.1 $\pm$ 5.6, 80.4 $\pm$ 2.3, 62.2 $\pm$ 3.0 and 82.4 $\pm$ 3.5 respectively, indicating the highest value in RAJ and the lowest in KHU strain. The exotic strains, on the other hand, showed an overall fewer number of eggs laid. Thus mean $\pm$ SE data for the BLA, CTC, FSS and KAN strains were 31.2 $\pm$ 3.1, 14.6 $\pm$ 2.9, 42.4 $\pm$ 3.2 and 15.8 $\pm$ 1.4 respectively (Appendix 2), showing the highest value in FSS and the lowest in CTC. The percentage of egg-hatch values were recorded to be 83.8 $\pm$ 3.3, 54.7 $\pm$ 8.9, 64.4 $\pm$ 1.4 and 69.0 $\pm$ 8.8 in the four exotic strains respectively. Thus all the purebred crosses were compatible, indicated by their $\geq$ 50% egg-hatch data.

Twelve hybrid crosses involving indigenous x indigenous strains (Table 2; Appendix 3), sixteen crosses involving indigenous x exotic strains (Table 3; Appendix 4) and sixteen crosses involving exotic x indigenous strains (Table 4; Appendix 4) showed variable figures for the reproductive potential.

Table 1. Effect of purebred crosses on the reproductive potential in the indigenous and exotic strains of *T. castaneum*

No.	Crossing schedules (female x male)	Fecundity* (mean±SE)	% egg-hatch* (mean±SE)
<i>a. Indigenous strains</i>			
1.	CHI x CHI	61.0±9.9	72.1±5.6
2.	DHA x DHA	64.0±4.1	80.4±2.3
3.	KHU x KHU	28.6±2.1	62.2±3.0
4.	RAJ x RAJ	47.2±4.1	82.4±3.5
<i>b. Exotic strains</i>			
5.	BLA x BLA	31.2±3.1	83.8±3.3
6.	CTC x CTC	14.6±2.9	54.7±8.9
7.	FSS x FSS	42.4±3.2	64.4±1.4
8.	KAN x KAN	15.8±1.4	69.0±8.8

*Mean of 5 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; BLA= Black; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Table 2. Effect of hybrid crosses (Indigenous x indigenous strains) on the reproductive potential in *T. castaneum*

No.	Crossing schedules (female x male)	Fecundity* (mean±SE)	% egg-hatch* (mean±SE)
<i>a. Indigenous x indigenous strains</i>			
1.	CHI x DHA	16.0±3.6	72.4±6.6
2.	CHI x KHU	45.4±2.0	42.6±1.3
3.	CHI x RAJ	31.0±4.5	40.8±0.4
4.	DHA x CHI	28.6±1.0	63.9±2.9
5.	DHA x KHU	36.4±3.6	47.2±1.2
6.	DHA x RAJ	37.5±2.2	49.6±3.4
7.	KHU x CHI	28.2±1.6	63.2±1.3
8.	KHU x DHA	46.0±2.6	71.0±0.7
9.	KHU x RAJ	24.2±1.2	66.2±1.7
10.	RAJ x CHI	14.6±2.3	57.8±3.0
11.	RAJ x DHA	29.2±1.2	62.3±3.4
12.	RAJ x KHU	17.6±0.9	64.3±2.1

*Mean of 5 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; BLA= Black; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Table 3. Effect of indigenous x exotic hybrid crosses on the reproductive potential in *T. castaneum*

No.	Crossing schedules (female x male)	Fecundity* (mean±SE)	% egg-hatch* (mean±SE)
<i>Indigenous x exotic strains</i>			
1.	CHI x BLA	30.8±4.6	72.9±4.9
2.	CHI x CTC	24.6±2.2	0.0±0.0
3.	CHI x FSS	18.2±2.4	0.0±0.0
4.	CHI x KAN	41.6±3.8	0.0±0.0
5.	DHA x BLA	49.2±2.1	80.2±2.1
6.	DHA x CTC	15.6±1.4	0.0±0.0
7.	DHA x FSS	14.2±1.6	0.0±0.0
8.	DHA x KAN	31.6±2.9	0.0±0.0
9.	KHU x BLA	29.6±3.5	58.4±2.2
10.	KHU x CTC	23.2±2.3	60.1±2.4
11.	KHU x FSS	19.2±1.4	71.5±1.8
12.	KHU x KAN	44.2±3.6	67.1±7.8
13.	RAJ x BLA	37.4±4.6	72.4±3.2
14.	RAJ x CTC	47.4±2.7	66.4±2.1
15.	RAJ x FSS	14.2±2.0	54.7±3.4
16.	RAJ x KAN	45.6±2.7	79.2±3.2

*Mean of 5 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; BLA= Black; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Of 12 indigenous x indigenous hybrid crosses (Table 2), 4 (CHI x KHU, CHI x RAJ, DHA x KHU and DHA x RAJ) were partially incompatible and the remaining 8 were compatible crosses. Whereas out of 16 indigenous x exotic crosses (Table 3), 6 (CHI x CTC, CHI x FSS, CHI x KAN; DHA x CTC, DHA x FSS and DHA x KAN) were incompatible and the rest 10 were fully compatible ones. Sixteen exotic x indigenous hybrids (Table 4), on the other hand, showed one-half fully compatible and the remaining one-half partially compatible crosses.

Table 4. Effect of exotic x indigenous hybrid crosses on the reproductive potential in *T. castaneum*

No.	Crossing schedules (female x male)	Fecundity* (mean±SE)	% egg-hatch* (mean±SE)
<i>Exotic x indigenous strains</i>			
1.	BLA x CHI	39.8±3.8	65.7±2.7
2.	BLA x DHA	29.4±3.1	70.2±3.0
3.	BLA x KHU	19.6±4.2	38.3±3.4
4.	BLA x RAJ	37.2±3.7	47.3±4.2
5.	CTC x CHI	24.8±2.3	72.8±1.7
6.	CTC x DHA	32.6±1.5	68.2±0.9
7.	CTC x KHU	22.6±3.1	42.1±7.1
8.	CTC x RAJ	34.8±1.7	32.4±1.6
9.	FSS x CHI	29.4±3.1	66.6±3.6
10.	FSS x DHA	14.2±1.6	60.8±2.7
11.	FSS x KHU	24.8±0.8	33.8±3.7
12.	FSS x RAJ	17.4±3.1	44.1±4.3
13.	KAN x CHI	44.6±2.6	74.3±1.0
14.	KAN x DHA	17.6±1.4	59.7±5.1
15.	KAN x KHU	17.2±1.8	15.5±5.6
16.	KAN x RAJ	29.8±2.9	42.8±5.4

*Mean of 5 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; BLA= Black; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

The above data on the percentage of egg-hatch were used to construct an incompatibility relationship chart for the eight strains of *T. castaneum* under study (Table 5). An analysis of the observed incompatibility relationships between the indigenous and exotic strains demonstrate that of 52 cross-matings, 34 (65.38%) were compatible, 12 (23.08%) were partially incompatible and 6 (11.54%) were incompatible crosses. The later ones, *i.e.* the incompatible crosses are of immense importance from genetic point of view (Section 1.6).

Table 5. Incompatibility relationships observed between the indigenous and exotic strains of *T. castaneum*

Female \ Male								
	CHI	DHA	KHU	RAJ	BLA	CTC	FSS	KAN
CHI	+	+	±	±	+	-	-	-
DHA	+	+	±	±	+	-	-	-
KHU	+	+	+	+	+	+	+	+
RAJ	+	+	+	+	+	+	+	+
BLA	+	+	±	±	+	0	0	0
CTC	+	+	±	±	0	+	0	0
FSS	+	+	±	±	0	0	+	0
KAN	+	+	±	±	0	0	0	+

CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano; (+) designates compatible crosses ($\geq 50\%$ egg-hatch), (±) partially incompatible crosses ($\leq 50\%$ egg-hatch) and (-) incompatible crosses ($\leq 1\%$ egg-hatch); 0= crosses not performed.

It is suggestive from Table 5 that two of the indigenous strains viz. KHU and RAJ and three of the exotic strains viz. CTC, FSS and KAN might be infected with RMO (rickettsia-like microorganisms).

3.2 Statistical analyses of the purebred and hybrid crosses

Egg-hatch data from crosses involving CHI and CTC (Fig. 1; CHI x CHI, CTC x CTC, CHI x CTC and CTC x CHI) CHI and FSS (Fig. 2; CHI x CHI, FSS x FSS, CHI x FSS and FSS x CHI) and CHI and KAN (Fig. 3; CHI x CHI, KAN x KAN, CHI x KAN and KAN x CHI) were subjected to t-tests (Table 6). Differences between the purebred crosses were statistically insignificant while those between each of the reciprocal crosses were highly significant, indicating that the exotic males (CTC, FSS and KAN) bring about a breakdown

of the gamete formation where neither the eggs hatch nor the progenies develop when they mate with the indigenous strain, CHI. Almost similar results (Figs. 4-6) were observed when the same three exotic males were mated each with DHA females.

Table 6. Comparisons among the purebred, hybrid and their reciprocal crosses showing either complete or partial cytoplasmic incompatibility in *T. castaneum*

No.	Comparisons between	t-values*	Probability
1.	CHI x CHI vs. CTC x CTC	1.66	ns
2.	CHI x CTC vs. CTC x CHI	84.63	<0.001
3.	CHI x CHI vs. FSS x FSS	1.34	ns
4.	CHI x FSS vs. FSS x CHI	19.76	<0.001
5.	CHI x CHI vs. KAN x KAN	0.30	ns
6.	CHI x KAN vs. KAN x CHI	34.31	<0.001
7.	DHA x DHA vs. CTC x CTC	2.80	<0.05
8.	DHA x CTC vs. CTC x DHA	53.26	<0.001
9.	DHA x DHA vs. FSS x FSS	6.23	<0.001
10.	DHA x FSS vs. FSS x DHA	37.42	<0.001
11.	DHA x DHA vs. KAN x KAN	1.23	ns
12.	DHA x KAN vs. KAN x DHA	36.40	<0.001
13.	KHU x KHU vs. BLA x BLA	5.60	<0.001
14.	KHU x BLA vs. BLA x KHU	8.17	<0.001
15.	RAJ x RAJ vs. BLA x BLA	0.33	ns
16.	RAJ x BLA vs. BLA x RAJ	7.55	<0.001

*All t-values are at 8 df; vs. = versus; ns = not significant.

Unlike CHI and DHA strains, KHU and RAJ females responded differently when both of them mated separately to each of the CTC, FSS and KAN males (Table 5; Appendix 5). These two strains, therefore, were compared with an apparently uninfected exotic strain, BLA (Figs. 7 & 8; Table 6). Differences in egg-hatch data between purebred crosses (KHU x KHU vs. BLA x BLA) as well as reciprocals (KHU x BLA vs. BLA x KHU) were both significant.

Although difference between RAJ x RAJ vs. BLA x BLA purebred cross was not significant ($t=0.33$), the reciprocal cross, RAJ x BLA vs. BLA x RAJ yielded a highly significant difference ($t= 7.55$; $P<0.001$). These results suggest that the indigenous KHU and RAJ and the exotic BLA are perhaps not infected with RMO.

3.3 Effect of backcrosses on the expression of CI

Table 7 (Appendix 6) shows the results of backcrosses between each of the uninfected indigenous and exotic females (CHI, DHA and BLA) with F_1 males derived from infected females (KHU, RAJ, CTC, FSS and KAN) mated with uninfected males (CHI, DHA and BLA) determined from incompatibility relationships data (Table 5). Though fecundity of these seven crosses varied between 16.8 ± 1.1 (BLA x F_1 (CTC x BLA) and 38.8 ± 1.2 (CHI x F_1 (RAJ x CHI), the percentage of egg-hatch was 0.0 ± 0.0 for each cross, suggesting that each of the F_1 males was infested with RMO when infected females were crossed with uninfected males in the parental generation.

Table 7. Effect of backcrosses on the reproductive parameters in *T. castaneum*

No.	Crossing schedules (female x F_1 male)	Fecundity* (mean $\pm$ SE)	% egg-hatch* (mean $\pm$ SE)
1.	CHI x F_1 (KHU x CHI)	33.2 $\pm$ 2.2	0.0 $\pm$ 0.0
2.	CHI x F_1 (RAJ x CHI)	38.8 $\pm$ 1.2	0.0 $\pm$ 0.0
3.	DHA x F_1 (KHU x DHA)	32.2 $\pm$ 1.5	0.0 $\pm$ 0.0
4.	DHA x F_1 (RAJ x DHA)	36.6 $\pm$ 1.7	0.0 $\pm$ 0.0
5.	BLA x F_1 (CTC x BLA)	16.8 $\pm$ 1.1	0.0 $\pm$ 0.0
6.	BLA x F_1 (FSS x BLA)	17.4 $\pm$ 0.9	0.0 $\pm$ 0.0
7.	BLA x F_1 (KAN x BLA)	30.8 $\pm$ 2.6	0.0 $\pm$ 0.0

*Mean of 5 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

3.4 Effect of tetracycline treatment on the expression of CI in *T. castaneum*

Effect of tetracycline treatment in the food medium and its consequence on the reproductive potential in different strains of *T. castaneum* have been presented in Tables 8 and 9 (Appendix 7). Results demonstrate that when males reared on normal (untreated) food medium are mated with the females reared on a similar medium (Table 8), all the infected strains show compatibility, i.e. having $\geq 50\%$ egg-hatch. On the other hand, when untreated males are allowed to mate with females reared on tetracycline-treated food, all the crosses resulted in incompatibility i.e. having $\leq 1\%$ egg-hatch. Apparently fecundity of the experimental insects was not affected by the tetracycline treatment.

Data on the tetracycline-treated food medium also show that when males reared on treated medium are crossed to females reared either on untreated or treated medium (Table 9), all the cross-matings become compatible. This indicated that the tetracycline treatment might have 'cured' the infected males from the RMO, if any. The results of the experiment, in terms of the expression of CI in *T. castaneum*, are summarized in Table 10.

Table 8. Effect of food medium (males untreated) on the reproductive potential in *T. castaneum*

Females	Males		Fecundity (% egg-hatch)*		
	KHU	RAJ	CTC	FSS	KAN
<u>Normal food</u>					
KHU	32.0±3.4 (68.4±5.3)	21.3±2.1 (58.0±3.8)	25.3±4.7 (83.8±4.4)	18.0±3.2 (78.8±6.1)	22.7±2.9 (64.0±3.2)
RAJ	21.7±1.8 (72.0±1.8)	52.3±4.2 (85.2±3.1)	36.0±5.1 (62.4±5.2)	19.3±2.8 (68.6±3.8)	38.7±6.3 (69.0±4.8)
CTC	19.7±3.4 (51.0±4.2)	28.3±2.8 (54.2±6.3)	30.7±5.1 (72.8±7.0)	23.7±3.4 (62.4±2.9)	18.3±2.1 (58.2±1.9)
FSS	22.0±4.1 (52.6±5.8)	26.3±2.8 (53.2±6.3)	34.7±5.2 (67.4±2.8)	38.0±3.5 (74.6±3.5)	24.3±5.1 (60.8±3.7)
KAN	26.7±4.2 (58.4±2.9)	34.0±5.6 (53.6±5.8)	28.7±3.7 (61.4±4.7)	19.0±1.8 (58.8±2.5)	23.3±4.2 (78.6±4.8)
<u>Tetracycline-treated food</u>					
KHU	22.3±4.2 (0.0±0.0)	31.7±2.9 (0.0±0.0)	23.0±5.8 (0.0±0.0)	28.0±6.2 (0.0±0.0)	23.7±2.7 (0.0±0.0)
RAJ	27.3±5.8 (0.0±0.0)	41.0±4.8 (0.0±0.0)	25.7±5.9 (0.0±0.0)	29.3±7.8 (0.0±0.0)	27.7±7.4 (0.0±0.0)
CTC	24.7±7.1 (0.0±0.0)	19.0±3.8 (0.0±0.0)	27.0±5.8 (0.0±0.0)	34.3±8.4 (0.0±0.0)	29.3±2.9 (0.0±0.0)
FSS	32.0±4.6 (0.0±0.0)	31.3±2.7 (0.0±0.0)	25.7±6.2 (0.0±0.0)	28.0±5.5 (0.0±0.0)	33.7±6.8 (0.0±0.0)
KAN	19.7±7.1 (0.0±0.0)	26.3±5.2 (0.0±0.0)	21.7±6.1 (0.0±0.0)	29.3±3.5 (0.0±0.0)	27.7±4.9 (0.0±0.0)

*Males reared on normal food; mean of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Table 9. Effect of food medium (males treated) on the reproductive potential in *T. castaneum*

Females	Males*				
	KHU	RAJ	Fecundity (% egg-hatch)		
			CTC	FSS	KAN
<u>Normal food</u>					
KHU	35.0±3.6 (72.3±3.9)	19.7±2.2 (69.0±4.8)	26.7±6.4 (73.2±5.5)	28.3±1.9 (69.8±7.4)	23.3±3.8 (73.0±6.4)
RAJ	27.3±6.8 (68.4±6.8)	32.7±4.6 (75.0±7.5)	26.0±5.8 (77.9±5.9)	29.7±2.7 (73.2±7.1)	24.3±6.7 (66.0±6.3)
CTC	25.7±6.2 (59.6±4.9)	31.3±6.4 (74.0±8.4)	36.0±5.6 (61.7±7.8)	29.7±3.8 (81.7±5.6)	34.7±8.3 (68.0±7.2)
FSS	29.3±4.8 (64.4±7.8)	32.7±7.8 (73.4±6.8)	24.7±5.8 (57.8±4.9)	27.7±6.3 (64.7±6.5)	29.7±9.1 (81.8±9.2)
KAN	31.7±4.7 (72.0±5.6)	24.3±6.2 (83.4±5.2)	29.3±6.2 (71.8±7.5)	39.0±8.8 (68.6±8.1)	27.7±5.23 (88.2±5.6)
<u>Tetracycline-treated food</u>					
KHU	27.3±7.1 (57.6±5.8)	26.0±6.2 (68.4±8.4)	21.3±3.6 (74.6±4.7)	28.0±7.3 (66.6±6.6)	29.7±6.6 (67.0±7.4)
RAJ	34.0±8.6 (61.4±7.3)	27.3±7.2 (74.2±8.1)	31.7±5.9 (69.4±7.9)	28.3±6.8 (60.8±4.6)	30.0±7.3 (71.0±6.3)
CTC	26.3±8.4 (62.0±5.2)	18.7±3.1 (74.4±8.6)	24.0±5.8 (62.4±7.8)	27.3±7.4 (71.8±12.3)	28.7±8.2 (67.8±8.1)
FSS	33.7±4.6 (71.6±5.2)	31.7±6.8 (63.2±6.5)	24.3±5.8 (65.6±7.8)	28.7±7.5 (64.2±5.5)	34.0±5.9 (69.8±7.8)
KAN	16.7±8.4 (64.4±6.1)	29.0±4.6 (63.3±6.8)	18.3±6.8 (59.7±6.9)	29.3±9.8 (73.6±8.5)	33.0±6.3 (58.6±8.8)

*Males reared on tetracycline-treated food; mean of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Table 10. Effects of tetracycline treatment on the expression of cytoplasmic incompatibility (CI) in *T. castaneum*

Females	Males									
	KHU	RAJ	Normal food		KAN	KHU	Tetracycline-treated food			
			CTC	FSS			RAJ	CTC	FSS	KAN
<u>Normal food</u>										
KHU	+	+	+	+	+	+	+	+	+	+
RAJ	+	+	+	+	+	+	+	+	+	+
CTC	+	+	+	+	+	+	+	+	+	+
FSS	+	+	+	+	+	+	+	+	+	+
KAN	+	+	+	+	+	+	+	+	+	+
<u>Tetracycline-treated food</u>										
KHU	-	-	-	-	-	+	+	+	+	+
RAJ	-	-	-	-	-	+	+	+	+	+
CTC	-	-	-	-	-	+	+	+	+	+
FSS	-	-	-	-	-	+	+	+	+	+
KAN	-	-	-	-	-	+	+	+	+	+

CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano; + = $\geq 50\%$ egg-hatch; - = $\leq 1\%$ egg-hatch.

3.5 Effect of UV radiation treatment on the expression of CI in *T. castaneum*

Results of the experiment on the effect of 254 nm UV radiation on the reproductive potential in *T. castaneum* have been presented in Tables 11 (males untreated) and 12 (males treated). As an example, raw data are shown in Appendix 8. When both parents are untreated, percentages of egg-hatch in 19 crosses are $\geq 50\%$, indicating that the crosses are fully compatible, while 6 crosses (*viz.* CTC, FSS and KAN females each paired either with KHU or RAJ males) are partially incompatible. When untreated males are crossed with treated females, however, dramatic

changes occur in the expression of CI. Crosses involving KHU and RAJ females and CTC, FSS and KAN males; and CTC, FSS and KAN females and KHU and RAJ males (12 crosses) were incompatible ($0.0 \pm 0.0\%$ egg-hatch) whereas the rest 13 crosses were partially incompatible ($\leq 50\%$ egg-hatch).

Table 11. Effect of UV radiation treatment (254 nm) on the reproductive potential in *T. castaneum* (males untreated)

Females \ Males*	Fecundity (% egg-hatch)				
	KHU	RAJ	CTC	FSS	KAN
<u>Untreated</u>					
KHU	18.0 $\pm$ 1.5 (51.9 $\pm$ 2.3)	13.7 $\pm$ 2.2 (53.5 $\pm$ 1.8)	14.3 $\pm$ 0.9 (55.9 $\pm$ 3.3)	20.0 $\pm$ 1.8 (50.0 $\pm$ 3.6)	17.0 $\pm$ 0.9 (53.8 $\pm$ 3.6)
RAJ	14.3 $\pm$ 0.7 (51.5 $\pm$ 2.5)	20.3 $\pm$ 1.2 (57.7 $\pm$ 1.3)	22.7 $\pm$ 4.8 (63.6 $\pm$ 9.6)	22.7 $\pm$ 2.8 (61.4 $\pm$ 4.6)	18.3 $\pm$ 1.9 (59.7 $\pm$ 5.2)
CTC	18.3 $\pm$ 2.7 (48.2 $\pm$ 5.3)	23.7 $\pm$ 3.6 (39.4 $\pm$ 5.5)	26.0 $\pm$ 4.8 (61.8 $\pm$ 7.3)	19.3 $\pm$ 2.8 (57.3 $\pm$ 4.2)	21.7 $\pm$ 3.4 (58.8 $\pm$ 3.5)
FSS	21.7 $\pm$ 2.1 (47.8 $\pm$ 2.9)	32.0 $\pm$ 5.7 (41.5 $\pm$ 3.8)	19.7 $\pm$ 3.1 (62.4 $\pm$ 6.2)	24.3 $\pm$ 4.8 (69.7 $\pm$ 4.8)	29.3 $\pm$ 5.1 (64.4 $\pm$ 7.1)
KAN	19.3 $\pm$ 2.7 (40.8 $\pm$ 3.8)	27.7 $\pm$ 3.2 (46.4 $\pm$ 4.8)	26.7 $\pm$ 4.1 (58.7 $\pm$ 5.1)	29.0 $\pm$ 3.6 (62.8 $\pm$ 6.4)	34.3 $\pm$ 5.2 (73.4 $\pm$ 7.2)
<u>Treated</u>					
KHU	17.7 $\pm$ 1.5 (41.9 $\pm$ 2.4)	21.7 $\pm$ 0.9 (44.6 $\pm$ 0.5)	14.3 $\pm$ 0.7 (0.0 $\pm$ 0.0)	18.0 $\pm$ 2.1 (0.0 $\pm$ 0.0)	12.0 $\pm$ 1.2 (0.0 $\pm$ 0.0)
RAJ	16.3 $\pm$ 0.9 (42.7 $\pm$ 0.8)	15.3 $\pm$ 0.9 (43.6 $\pm$ 1.6)	20.7 $\pm$ 1.2 (0.0 $\pm$ 0.0)	18.7 $\pm$ 2.1 (0.0 $\pm$ 0.0)	23.0 $\pm$ 3.4 (0.0 $\pm$ 0.0)
CTC	14.7 $\pm$ 2.1 (0.0 $\pm$ 0.0)	19.3 $\pm$ 1.9 (0.0 $\pm$ 0.0)	15.3 $\pm$ 0.9 (39.6 $\pm$ 4.1)	21.7 $\pm$ 2.6 (43.4 $\pm$ 5.2)	17.0 $\pm$ 3.9 (37.6 $\pm$ 3.9)
FSS	15.3 $\pm$ 0.7 (0.0 $\pm$ 0.0)	21.3 $\pm$ 2.1 (0.0 $\pm$ 0.0)	17.3 $\pm$ 1.2 (41.7 $\pm$ 3.2)	23.7 $\pm$ 2.8 (38.6 $\pm$ 4.2)	19.3 $\pm$ 1.8 (39.9 $\pm$ 3.7)
KAN	18.7 $\pm$ 1.5 (0.0 $\pm$ 0.0)	22.7 $\pm$ 0.9 (0.0 $\pm$ 0.0)	15.7 $\pm$ 3.1 (47.5 $\pm$ 3.1)	19.0 $\pm$ 2.1 (37.4 $\pm$ 2.8)	14.7 $\pm$ 1.2 (33.9 $\pm$ 4.8)

*Males untreated; figures are mean $\pm$ SE of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Contrary to the results on untreated males, when treated males are paired either with untreated or treated females (Table 12), all the $25 \times 2 = 50$ crosses become compatible (i.e. $\geq 50\%$ egg-hatch), suggesting that 254 nm UV treatment on males resulted the strains free of any RMO. Similar to tetracycline treatment, however, fecundity of the females was apparently unaffected by UV treatment.

Table 12. Effect of UV radiation treatment (254 nm) on the reproductive potential in *T. castaneum* (males treated).

Females \ Males*	Fecundity (% egg-hatch)				
	KHU	RAJ	CTC	FSS	KAN
<u>Untreated</u>					
KHU	12.7 $\pm$ 3.9 (67.2 $\pm$ 5.2)	15.3 $\pm$ 2.1 (59.9 $\pm$ 3.2)	16.7 $\pm$ 3.8 (53.3 $\pm$ 4.5)	22.0 $\pm$ 4.1 (73.0 $\pm$ 4.9)	18.3 $\pm$ 3.1 (61.9 $\pm$ 4.5)
RAJ	22.7 $\pm$ 3.6 (57.4 $\pm$ 5.6)	30.7 $\pm$ 5.3 (69.3 $\pm$ 7.2)	28.7 $\pm$ 4.2 (64.8 $\pm$ 6.1)	18.7 $\pm$ 4.3 (55.3 $\pm$ 5.2)	28.0 $\pm$ 5.6 (72.0 $\pm$ 8.1)
CTC	14.3 $\pm$ 2.2 (68.2 $\pm$ 7.2)	29.7 $\pm$ 5.1 (55.8 $\pm$ 5.1)	24.7 $\pm$ 3.2 (63.2 $\pm$ 4.6)	17.7 $\pm$ 3.8 (75.3 $\pm$ 4.2)	25.0 $\pm$ 1.2 (62.6 $\pm$ 2.5)
FSS	19.3 $\pm$ 2.5 (61.4 $\pm$ 5.6)	17.7 $\pm$ 4.1 (79.4 $\pm$ 8.2)	25.3 $\pm$ 3.1 (73.1 $\pm$ 6.1)	21.7 $\pm$ 4.3 (63.0 $\pm$ 6.1)	17.0 $\pm$ 2.1 (61.0 $\pm$ 7.2)
KAN	28.7 $\pm$ 5.1 (57.2 $\pm$ 5.1)	23.7 $\pm$ 2.3 (53.9 $\pm$ 2.1)	20.0 $\pm$ 1.9 (63.8 $\pm$ 4.2)	13.7 $\pm$ 2.1 (83.2 $\pm$ 9.9)	25.0 $\pm$ 6.3 (71.8 $\pm$ 7.5)
<u>Treated</u>					
KHU	17.3 $\pm$ 0.9 (62.8 $\pm$ 6.1)	19.3 $\pm$ 4.2 (64.8 $\pm$ 7.2)	16.3 $\pm$ 1.2 (55.2 $\pm$ 1.2)	15.0 $\pm$ 2.1 (64.0 $\pm$ 4.3)	18.0 $\pm$ 3.3 (67.3 $\pm$ 4.5)
RAJ	19.7 $\pm$ 3.5 (57.8 $\pm$ 8.1)	23.3 $\pm$ 3.1 (69.6 $\pm$ 6.2)	24.7 $\pm$ 6.2 (73.3 $\pm$ 5.2)	34.3 $\pm$ 7.2 (53.0 $\pm$ 0.9)	26.7 $\pm$ 3.4 (71.9 $\pm$ 8.5)
CTC	31.8.2 (61.8 $\pm$ 5.2)	25.7 $\pm$ 2.1 (59.0 $\pm$ 7.2)	18.3 $\pm$ 0.9 (61.8 $\pm$ 4.6)	27.7 $\pm$ 4.5 (63.1 $\pm$ 6.0)	19.7 $\pm$ 1.2 (62.3 $\pm$ 5.6)
FSS	18.3 $\pm$ 1.5 (81.2 $\pm$ 9.2)	22.0 $\pm$ 0.9 (63.9 $\pm$ 7.2)	15.7 $\pm$ 2.0 (55.8 $\pm$ 5.1)	19.7 $\pm$ 2.1 (74.0 $\pm$ 8.9)	14.0 $\pm$ 1.1 (81.9 $\pm$ 4.9)
KAN	27.3 $\pm$ 6.2 (57.2 $\pm$ 4.8)	21.3 $\pm$ 4.2 (67.2 $\pm$ 8.1)	29.0 $\pm$ 4.8 (73.0 $\pm$ 3.6)	14.7 $\pm$ 2.0 (63.0 $\pm$ 6.1)	15.3 $\pm$ 0.9 (67.2 $\pm$ 8.1)

*Males treated; figures are mean $\pm$ SE of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Results of the effect of 366 nm UV radiation on the reproductive potential of the experimental insects are shown in Table 13 (males untreated) and Table 14 (males treated). Some of the raw data are presented in Appendix 9. Untreated males mated to untreated females mostly were compatible except six crosses involving either KHU or RAJ males and CTC, FSS and KAN females where they yielded partial incompatibility.

Table 13. Effect of UV radiation treatment (366 nm) on the reproductive potential in *T. castaneum* (males untreated)

Females	Males*		Fecundity (% egg-hatch)		
	KHU	RAJ	CTC	FSS	KAN
<u>Untreated</u>					
KHU	31.3±5.7 (58.2±5.1)	24.7±3.8 (61.4±5.1)	29.7±3.6 (58.6±1.0)	30.7±0.9 (71.2±7.8)	25.7±4.3 (56.4±4.8)
RAJ	21.3±4.8 (60.2±6.1)	14.3±2.2 (63.0±4.8)	21.7±7.1 (57.2±6.0)	20.0±1.9 (61.7±5.3)	22.0±6.3 (66.6±6.6)
CTC	23.7±6.1 (38.1±4.4)	27.7±4.5 (41.2±5.8)	23.3±7.1 (67.8±7.9)	32.3±2.1 (56.2±3.8)	24.3±5.1 (56.8±2.8)
FSS	30.0±2.9 (29.2±0.9)	26.3±5.2 (31.4±5.1)	25.0±6.4 (57.8±2.1)	23.7±3.4 (73.8±0.8)	26.7±7.0 (76.4±8.8)
KAN	19.3±3.7 (37.7±4.1)	29.7±3.8 (44.0±2.1)	27.3±5.1 (69.4±5.1)	24.0±0.9 (70.8±3.5)	28.3±1.5 (63.5±6.2)
<u>Treated</u>					
KHU	29.3±2.9 (43.3±5.1)	23.3±2.2 (34.0±0.9)	20.7±1.2 (0.0±0.0)	32.7±1.5 (0.0±0.0)	16.7±0.9 (0.0±0.0)
RAJ	21.3±0.9 (37.3±4.1)	24.0±8.1 (44.2±1.2)	31.3±1.2 (0.0±0.0)	22.0±5.2 (0.0±0.0)	12.3±2.8 (0.0±0.0)
CTC	27.7±2.7 (31.3±5.1)	25.7±6.0 (38.0±1.9)	30.3±2.8 (40.0±2.8)	29.3±3.5 (45.3±2.0)	36.0±3.1 (39.1±0.8)
FSS	28.0±3.1 (41.3±6.1)	26.3±4.2 (35.1±3.9)	25.7±2.4 (36.0±2.3)	17.7±4.4 (32.1±8.2)	26.7±0.9 (38.2±2.5)
KAN	24.3±4.9 (33.0±3.2)	27.7±6.2 (44.6±2.6)	24.0±2.0 (41.7±1.3)	19.3±3.1 (30.0±2.1)	22.7±5.2 (46.9±3.1)

*Males untreated; figures are mean±SE of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

When untreated males were paired with treated females, the results were quite different. Six crosses (involving each of the KHU and RAJ females and CTC, FSS and KAN males) were incompatible and the rest 19 crosses were partially incompatible. But unlike 254 nm treatment, 366 nm-treated males paired with untreated females gave rise to 6 incompatible crosses, 6 partially incompatible crosses and 13 compatible crosses (Table 14).

Table 14. Effect of UV radiation treatment (366 nm) on the reproductive potential in *T. castaneum* (males treated)

Females \ Males*	Fecundity (% egg-hatch)				
	KHU	RAJ	CTC	FSS	KAN
<u>Untreated</u>					
KHU	35.3±3.7 (71.8±5.2)	33.7±5.1 (64.2±4.2)	37.7±4.6 (0.0±0.0)	36.3±8.1 (0.0±0.0)	29.0±1.7 (0.0±0.0)
RAJ	31.3±5.2 (59.7±4.1)	23.7±6.8 (67.4±6.3)	27.7±6.1 (0.0±0.0)	16.3±0.9 (0.0±0.0)	19.3±3.8 (0.0±0.0)
CTC	25.7±7.3 (36.8±6.7)	31.0±4.4 (40.8±3.9)	17.7±8.1 (77.1±0.8)	25.7±2.8 (80.0±8.2)	22.7±2.6 (65.5±4.5)
FSS	15.7±8.2 (34.4±8.7)	26.0±5.3 (32.8±6.7)	29.7±5.0 (67.3±1.9)	26.3±8.1 (70.5±6.2)	21.7±3.4 (61.5±7.5)
KAN	32.3±9.0 (38.8±5.2)	23.3±7.2 (43.3±4.8)	34.7±3.2 (56.4±2.8)	36.7±7.2 (61.7±3.4)	20.0±0.9 (55.9±3.9)
<u>Treated</u>					
KHU	18.3±2.4 (67.4±5.1)	22.7±4.1 (69.3±5.8)	16.3±0.8 (73.5±2.1)	13.7±1.1 (60.9±6.3)	16.3±1.2 (71.3±6.9)
RAJ	27.3±2.4 (79.6±9.2)	31.0±4.8 (80.5±5.5)	25.7±0.8 (66.6±6.6)	24.3±3.2 (75.0±7.2)	25.7±2.8 (81.2±8.1)
CTC	16.7±2.4 (64.4±4.4)	32.3±3.4 (71.7±3.3)	26.7±0.8 (72.8±5.8)	33.3±4.1 (68.2±3.6)	26.7±7.2 (69.9±1.8)
FSS	38.3±2.4 (59.8±0.9)	19.7±4.6 (63.3±5.2)	29.3±0.8 (58.8±5.6)	23.0±1.5 (72.1±1.9)	27.3±1.9 (69.0±3.7)
KAN	28.7±2.4 (68.8±7.4)	23.0±0.9 (70.0±6.8)	36.3±0.8 (62.2±5.8)	23.7±0.8 (74.4±4.8)	19.3±0.3 (66.8±6.8)

*Males treated; figures are mean±SE of 3 replicates; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

Finally, as with 254 nm UV treatment, 366 nm radiation also yielded all compatible crosses when both parents were treated. Data on 254 nm and 366 nm UV radiation treatment on the expression of CI in *T. castaneum* have been summarized in Table 15.

Table 15. Effects of UV radiation treatment on the expression of cytoplasmic incompatibility (CI) in *T. castaneum*

Females \ Males	Untreated					Treated				
	KHU	RAJ	CTC	FSS	KAN	KHU	RAJ	CTC	FSS	KAN
254 nm										
<u>Untreated</u>										
KHU	+	+	+	+	+	+	+	+	+	+
RAJ	+	+	+	+	+	+	+	+	+	+
CTC	±	±	+	+	+	+	+	+	+	+
FSS	±	±	+	+	+	+	+	+	+	+
KAN	±	±	+	+	+	+	+	+	+	+
<u>Treated</u>										
KHU	±	±	-	-	-	+	+	+	+	+
RAJ	±	±	-	-	-	+	+	+	+	+
CTC	-	-	±	±	±	+	+	+	+	+
FSS	-	-	±	±	±	+	+	+	+	+
KAN	-	-	±	±	±	+	+	+	+	+
366 nm										
<u>Untreated</u>										
KHU	+	+	+	+	+	+	+	-	-	-
RAJ	+	+	+	+	+	+	+	-	-	-
CTC	±	±	+	+	+	±	±	+	+	+
FSS	±	±	+	+	+	±	±	+	+	+
KAN	±	±	+	+	+	±	±	+	+	+
<u>Treated</u>										
KHU	±	±	-	-	-	+	+	+	+	+
RAJ	±	±	-	-	-	+	+	+	+	+
CTC	±	±	±	±	±	+	+	+	+	+
FSS	±	±	±	±	±	+	+	+	+	+
KAN	±	±	±	±	±	+	+	+	+	+

*Insects were exposed for 30 sec in both 254 nm and 366 nm doses; CHI= Chittagong; DHA= Dhaka; KHU= Khulna; RAJ= Rajshahi; CTC= CTC-12; FSS= FSS-II; KAN= Kano; + = ≥50% egg-hatch; ± = ≤50% egg-hatch; - = ≤1% egg-hatch.

3.6 Frequency-dependent regulation in *T. castaneum*

Inferences drawn from the results obtained in the purebred and hybrid crosses, backcrosses and tetracycline and UV radiation treatments were applied to evaluate whether cytoplasmically incompatible exotic males (CTC, FSS and KAN) could be used to regulate the population of apparently uninfected and indigenous females of the strains (CHI and DHA) of *T. castaneum*. Keeping the ratios of indigenous females to males constant, the proportion of exotic males was gradually increased from 1 to 3 and the consequence was recorded in terms of fecundity, percentage of egg-hatch and the number of adult progenies. Results of such an experiment (Table 16; Appendix 10) demonstrate that for CHI x CTC crosses, both the percentage of egg-hatch and the number of adult progenies decrease from 77.3 ± 2.7 and 103.0 ± 7.0 in the control to 36.8 ± 1.3 and 29.7 ± 1.1 respectively for the two traits at the 1:1:3 ratio (Fig. 9). The values for the two parameters were 39.1 ± 3.0 and 58.0 ± 3.2 (Fig. 10) and 34.3 ± 3.2 and 52.7 ± 3.8 (Fig. 11) respectively CHI females were mated each with FSS and KAN males at the ratio of 1:1:3. When DHA females were paired with each of the CTC, FSS and KAN males at three different ratios, on the other hand, the percentage of egg-hatch as well as the number of adult progenies were found to decrease to 48.7 ± 1.3 and 85.3 ± 2.9 (Fig. 12), 48.2 ± 1.2 and 58.0 ± 2.4 (Fig. 13) and 47.3 ± 1.4 and 67.0 ± 8.5 (Fig. 14) compared to the respective controls, but the extent was slightly lower than that recorded for CHI females mentioned earlier. These findings indicate that the indigenous strain CHI could be effectively regulated by the exotic male CTC provided that sufficient males of the latter strain are released.

Table 16. Effect of matings between indigenous strains (CHI and DHA) and cytoplasmically incompatible exotic male strains (CTC, FSS and KAN) on the reproductive potential and progeny production in *T. castaneum*

(female:	Ratios of mating insects male:	male)	Fecundity* (mean±SE)	% egg-hatch* (mean±SE)	Adult progenies* (mean±SE)
1CHI:	1CHI	—	145.7±3.5	77.3±2.7	103.0±7.0
1CHI:	—	1CTC	113.3±6.0	0.0±0.0	0.0±0.0
1CHI:	1CHI:	1CTC	118.3±6.9	48.3±1.1	49.0±5.8
1CHI:	1CHI:	2CTC	115.7±2.3	40.1±1.7	37.3±1.5
1CHI:	1CHI:	3CTC	102.3±2.6	36.8±1.3	29.7±1.1
1CHI:	1CHI	—	188.7±5.1	73.3±1.9	127.7±5.4
1CHI:	—	1FSS	179.3±2.3	0.0±0.0	0.0±0.0
1CHI:	1CHI:	1FSS	218.7±5.3	53.7±5.3	96.0±3.7
1CHI:	1CHI:	2FSS	198.3±6.1	46.3±2.7	80.3±5.0
1CHI:	1CHI:	3FSS	203.3±2.9	39.1±3.0	58.0±3.2
1CHI:	1CHI	—	159.7±6.1	78.7±6.6	118.7±2.2
1CHI:	—	1KAN	183.3±7.2	0.0±0.0	0.0±0.0
1CHI:	1CHI:	1KAN	224.3±5.8	68.3±2.6	129.3±6.8
1CHI:	1CHI:	2KAN	168.7±9.2	46.7±2.8	67.0±5.3
1CHI:	1CHI:	3KAN	193.3±2.6	34.3±3.2	52.7±3.8
1DHA:	1DHA	—	288.3±4.4	75.5±1.4	205.3±5.0
1DHA:	—	1CTC	203.3±4.5	0.0±0.0	0.0±0.0
1DHA:	1DHA:	1CTC	199.3±2.9	62.4±3.2	115.0±4.3
1DHA:	1DHA:	2CTC	202.7±5.8	55.6±3.0	108.7±2.2
1DHA:	1DHA:	3CTC	197.3±5.7	48.7±1.3	85.3±2.9
1DHA:	1DHA	—	225.0±3.5	79.2±2.6	168.7±5.0
1DHA:	—	1FSS	218.7±5.8	0.0±0.0	0.0±0.0
1DHA:	1DHA:	1FSS	206.7±6.1	64.2±2.8	112.3±7.8
1DHA:	1DHA:	2FSS	202.3±3.5	58.1±1.7	87.7±3.2
1DHA:	1DHA:	3FSS	201.0±3.2	48.2±1.2	58.0±2.4
1DHA:	1DHA	—	297.7±1.4	79.5±0.8	202.7±4.6
1DHA:	—	1KAN	201.7±9.2	0.0±0.0	0.0±0.0
1DHA:	1DHA:	1KAN	192.0±6.2	59.5±0.5	109.7±10.8
1DHA:	1DHA:	2KAN	191.0±3.1	53.6±1.0	93.3±5.2
1DHA:	1DHA:	3KAN	180.0±2.9	47.3±1.4	67.0±8.5

*Mean of 3 replicates; CHI= Chittagong; DHA= Dhaka; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

3.7 Mating competitiveness of the incompatible males

Mating competitiveness values (CVs) of the three exotic males of *T. castaneum* viz. CTC, FSS and KAN were estimated from the percentage of egg-hatch data obtained in the experiment described in Section 3.6. The estimated CVs are summarized in Table 17 (Appendix 11) and plotted in Fig. 15. Results show that CVs of the CTC males competed with CHI females and males were 0.60, 0.46 and 0.37 at 1:1:1, 1:1:2 and 1:1:3 ratios respectively. Those of the FSS and KAN males competed each with CHI females and males were 0.36, 0.14 and 0.29 and 0.15, 0.34 and 0.43, respectively.

The estimated CVs of 0.60-0.37 in CHI x CHI x CTC crosses indicate that the population of CTC males are to be increased from 1.67 (i.e. $1 \div 0.60$) to 2.70 (i.e. $1 \div 0.37$) times to achieve the effect of reduction in percentage of egg-hatch that CHI female x CTC male mating would have given. Similarly, CVs of 0.36-0.14 in CHI x CHI x FSS crosses indicate that the number of FSS males are to be increased from 2.78 to 7.14 times to achieve an expected reduction in population numbers. Calculated CVs of 0.43-0.15 in CHI x CHI x KAN crosses, on the other hand, suggest that the number of KAN males are to be increased from 2.33 to 6.67 times to achieve an expected reduction in population number of the indigenous CHI strain.

Table 17. Estimated mating competitiveness values (CVs) of the three exotic male strains of *T. castaneum* (CTC, FSS and KAN) from percentage of egg-hatch data

Ratios of mating insects (female: male: male)			% egg-hatch*	Expected % egg-hatch	Estimated CVs
1CHI:	1CHI	—	77.3±2.7	—	—
1CHI:	—	1CTC	0.0±0.0	—	—
1CHI:	1CHI:	1CTC	48.3±1.1	38.7	0.60
1CHI:	1CHI:	2CTC	40.1±1.7	25.8	0.46
1CHI:	1CHI:	3CTC	36.8±1.3	19.3	0.37
1CHI:	1CHI	—	73.3±1.9	—	—
1CHI:	—	1FSS	0.0±0.0	—	—
1CHI:	1CHI:	1FSS	53.7±5.3	36.7	0.36
1CHI:	1CHI:	2FSS	46.3±2.7	24.4	0.14
1CHI:	1CHI:	3FSS	39.1±3.0	18.3	0.29
1CHI:	1CHI	—	78.7±6.6	—	—
1CHI:	—	1KAN	0.0±0.0	—	—
1CHI:	1CHI:	1KAN	68.3±2.6	39.4	0.15
1CHI:	1CHI:	2KAN	46.7±2.8	26.3	0.34
1CHI:	1CHI:	3KAN	34.3±3.2	19.7	0.43
1DHA:	1DHA	—	75.5±1.4	—	—
1DHA:	—	1CTC	0.0±0.0	—	—
1DHA:	1DHA:	1CTC	62.4±3.2	37.8	0.21
1DHA:	1DHA:	2CTC	55.6±3.0	25.2	0.18
1DHA:	1DHA:	3CTC	48.7±1.3	18.9	0.18
1DHA:	1DHA	—	79.2±2.6	—	—
1DHA:	—	1FSS	0.0±0.0	—	—
1DHA:	1DHA:	1FSS	64.2±2.8	39.6	0.23
1DHA:	1DHA:	2FSS	58.1±1.7	26.4	0.18
1DHA:	1DHA:	3FSS	48.2±1.2	19.8	0.21
1DHA:	1DHA	—	79.5±0.8	—	—
1DHA:	—	1KAN	0.0±0.0	—	—
1DHA:	1DHA:	1KAN	59.5±0.5	39.8	0.34
1DHA:	1DHA:	2KAN	53.6±1.0	26.5	0.24
1DHA:	1DHA:	3KAN	47.3±1.4	19.9	0.23

*Mean of 3 replicates; CHI= Chittagong; DHA= Dhaka; CTC= CTC-12; FSS= FSS-II; KAN= Kano.

The exotic males CTC, FSS or KAN, however, when competed with DHA females and males, gave slightly different CVs. These were 0.21, 0.18 and 0.18; 0.23, 0.18 and 0.21 and 0.34, 0.24 and 0.23, respectively at the three ratios, suggesting that the exotic males performed better with the indigenous CHI strain than did with DHA strain.

CHAPTER FOUR

DISCUSSION

DISCUSSION

A detailed account of the cross-matings between the purebred indigenous and exotic strains, their hybrid and back crosses amongst the strains has been presented to demonstrate the genetics of crossing relationships in *T. castaneum*. The pattern of unidirectional incompatibility is suggestive of a maternally inherited character. This was further investigated using antibiotic and UV radiation treatments to elucidate the nature of CI inheritance in the strains. Finally, the frequency-dependent and mating competitiveness experiments between the apparently uninfected indigenous and incompatible exotic male strains were carried out to evaluate if the growth of *T. castaneum* could be kept to a minimum by introducing incompatible exotic males into the uninfected indigenous strains of this species. For convenience, the topics of the present study are being discussed in the following heads.

4.1 Effect of hybrid crosses on the expression of CI

Hybrid crosses between indigenous CHI and DHA females mated each with KHU and RAJ males manifested in partial incompatibility (Table 5; p. 46), suggesting that the latter males are infected with RMO. The same females when mated each with the exotic CTC, FSS and KAN males did not produce any progeny, indicating that the exotic males carry RMO. Indigenous KHU and RAJ males, on the other hand, when paired each with BLA, CTC,

FSS and KAN females, showed partial incompatibility. The rest of the hybrid crosses were compatible.

CI in insects is an intra-populational sterility phenomenon. Although known for nearly half a century, it has only recently attracted the attention of evolutionary biologists, having been found in an increasing number of species belonging to Diptera, Coleoptera, Lepidoptera, Hymenoptera and Homoptera. It has also been reported in an isopod (Crustacea), showing that cytoplasmic incompatibility is not restricted to insects (Rousset & Raymond, 1991; O'Neill *et al.*, 1992; Beard *et al.*, 1993). Many studies have dealt with the mode of inheritance of cytoplasmic incompatibility, its physiological cause, its potential in eradication programme for pest insects, and its possible involvement in speciation.

Incompatible crosses are characterized by abortive karyogamy, which for most insect species leads to early embryonic death. Although the molecular basis of cytoplasmic incompatibility is unknown yet, it has been demonstrated that the genetic determinants are maternally inherited and correlate with the presence of rickettsia-like bacterial symbionts in the arthropod's gonad tissue (Yen & Barr, 1973; O'Neill, 1989; Beard *et al.*, 1993; Braig *et al.*, 1994). Since its first characterization in *Culex pipiens* by Laven (1967), experiments designed to demonstrate incompatibility in a number of such other insects as *Culex* (Barr, 1980; Wright & Wang, 1980; Magnin *et al.*, 1987), *Aedes* (Trpis *et al.*, 1981; Meek & Macdonald, 1984), *Hypera* (Hsiao & Hsiao, 1985a,b), *Nasonia* (Breeuwer & Werren, 1990, 1993),

Tribolium (Wade & Stevens, 1985; O'Neill, 1989; Fialho & Stevens, 1996) and *Drosophila* (Turelli & Hoffmann, 1991; Hoffmann et al., 1994, 1998; Clancy & Hoffmann, 1998) suggest that it is mediated by the same microorganism in different insect species.

Barr (1980) reported incompatibility in crosses of sympatric American strains of *C. pipiens* while Magnin et al. (1987) found both uni- and bidirectional incompatibility in the natural populations collected from southern France which demonstrated that incompatibility is related to the geographical distance separating the parental strains. Meek & Macdonald (1984) examined the possible crossing types among seven members of the group of *Aedes scutellaris* and found that, as in *Culex pipiens* rickettsia-like symbionts are responsible for cytoplasmic incompatibility in this species and that each crossing type was maternally inherited. It is not apparent from the present results, however, that whether the inherited incompatibility in the indigenous strains of *T. castaneum* was distance-dependent. Further work is needed to clarify this point.

A unidirectional incompatibility system in Californian *Drosophila simulans* has been described (Turelli & Hoffmann, 1991) where an inherited cytoplasmic incompatibility factor reduced egg-hatch when infected males mated with uninfected females. The infection was found to spread at a rate of more than 100 km per year, suggesting that the phenomenon of cytoplasmic incompatibility could play an important role as a post-mating isolating mechanism. A recent investigation (Braig et al., 1994) has shown that it is possible to transfer the symbiont

responsible for incompatibility between distantly related hosts like *Aedes albopictus* and *D. simulans*. This and subsequent findings opened a new way of manipulating natural populations genetically by experimental introduction of the incompatibility agent into the arthropod species of medical and agricultural importance (Turelli & Hoffmann, 1999).

The parasitic wasp *Nasonia* is one of the few hymenopteran species in which the cytogenetic mechanism of incompatibility is known (Breeuwer & Werren, 1990, 1993). Studies show that cytoplasmic microorganisms are associated with complete bidirectional incompatibility between *N. vitripennis* and *N. giraulti*. As a result, hybrid female offspring are not produced in interspecific crosses normally, but elimination of the cytoplasmically inherited bacteria by antibiotic treatments restores compatibility, when hybrid females are produced.

Hsiao & Hsiao (1985a) first reported the presence of a rickettsial symbiont in Coleoptera, in the alfalfa weevil, *Hypera postica* that was associated with unidirectional cytoplasmic incompatibility. A unique feature of such incompatibility was that only the crosses between western males and females of eastern or Egyptian weevils produce infertile eggs. The reciprocal crosses yield viable progeny with a distorted sex-ratio, predominantly female. No incompatibility was observed in crosses between eastern and Egyptian weevils. Of eight weevil populations surveyed, only the western weevil and a weevil population from the Netherlands were found to harbour the microorganism (Hsiao & Hsiao, 1985b).

The occurrence of cytoplasmic incompatibility in *Tribolium* was first reported in 1961 by Stanley (cited in Wade & Stevens, 1985), who found that females of the McGill black strain of *T. confusum* 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. Later on, Wade & Stevens (1985) were able to induce reproductive isolation by cytoplasmically mediated microorganisms in diverse geographic strains of *T. confusum*. They further demonstrated that the infected strains of these beetles can be cured by adding tetracycline or other antibiotics to the flour medium, and the treated (cured) strains become partially reproductively isolated from all other non-cured strains. Further studies by O'Neill (1989) revealed that in two laboratory strains of *T. confusum*, rickettsia-like microorganisms in the ovaries are responsible for cytoplasmic incompatibility. Using a multi-strain single-pair mating assay (8 x 8 factorial design), Fialho & Stevens (1996) provided evidence in favour of a common incompatibility type across strains of this species. However, three laboratory strains of *T. castaneum* were examined under the electron microscope, but no microorganisms were found (O'Neill 1989). This contradicts with a recent finding in that partial as well as complete cytoplasmic incompatibilities between some of the local and exotic strains of *T. castaneum* are possible (Islam et al., 1997). In agreement with the results of Islam et al. (1997), the present experiments show that three of the exotic strains viz. CTC, FSS and KAN were incompatible with the indigenous DHA

and CHI strains. This is further supported by the incompatibility relationships among the strains described below.

4.2 Incompatibility relationships in *T. castaneum*

The sterility that occurs when incompatible strains are crossed is expressed as the production of non-hatching but mostly embryonated eggs by inseminated females. Most authors have recognized three categories of cross, although their definitions have varied. These are, according to Clements (1992), as follows. (i) compatible crosses; (ii) partially incompatible crosses; and (iii) incompatible crosses. Further, incompatible crosses may be unidirectional, when infertile in one direction only involving either males or females, or else bidirectional, when infertile in both directions involving males and females.

The occurrence of unidirectional incompatibility prevailed in the mosquito, *A. cooki* (Macdonald, 1976). Bidirectional incompatibility in crosses between two species of *Aedes* has been reported by Meek & Macdonald (1984), while In *Culex pipiens*, compatible, partially incompatible and incompatible crosses between strains of the mosquito have been well-established by a number of works (Laven, 1967; Subbarao *et al.*, 1977a; Magnin *et al.*, 1987). It is to be noted that none of the mosquito strains displayed exactly the same incompatibility relationships with the others, indicating that each strain was of a unique crossing type. Moreover, incompatibility relationships in terms of the distance between the collection sites showed that strains collected in sites separated by 30 to 2000 m were bi- or

unidirectionally compatible, whereas strains separated by 2 to 200 km were uni- or bidirectionally incompatible (Magnin *et al.*, 1987). Of six cross-mating types in *Hypera postica*, Hsiao & Hsiao (1985b) found two unidirectional incompatibility crosses between the Egyptian x Western and Eastern x Western strains. In *Nasonia* spp. however, the incompatible relationships were found to be independent of the host genotype but were based on differences between cytoplasmic bacteria (Breeuwer & Werren, 1990, 1993). By examining incompatibility relationships between 12 strains of *T. confusum*, Wade & Stevens (1985) found only two strains, females of which always failed to produce progeny when crossed with males from any of the other ten strains, and remarkably no partial incompatibilities were detected. A recent study by Islam *et al.* (1997) have shown that out of 15 cross-matings between the local and exotic strains of *T. castaneum*, only one showed unidirectional incompatibility, while two others were partially incompatible. In the present results, 6 crosses were incompatible, 12 partially incompatible and the rest 34 crosses were compatible. Data reported here show for the first time a complete analysis of the incompatibility relationships in this important pest species.

4.3 Effect of backcrosses on the expression of CI

Backcrosses are designed for checking if an apparently compatible male offspring from a cross involving uninfected male x infected female, when mated back to uninfected females, give rise to incompatibility. This was first observed in *C. pipiens* by Laven (1956) where F₁ female offspring from a compatible cross

(*Ha* female x *Og* male), if mated to the male parent (*Og* male), produced infertile F₂ progenies. Similar results were reported by Wade & Stevens (1985) for *T. confusum*. Uninfected *bl* males mated to infected *Chicago* females resulted in compatibility, but when F₁ (infected) males were paired with *bl* females (uninfected), incompatibility was manifested. In the same way, uninfected male *McGill* strain of *T. confusum* mated with infected female *black* strain yielded in fertile offspring, but when the *McGill* females were backcrossed to the hybrid males, incompatibility was induced.

In alignment with the above findings, the present results of 7 backcrosses between each of the CHI, DHA and BLA females x F₁ males derived from compatible crosses determined earlier (Section 4. 1) demonstrate that all the backcrosses were incompatible as manifested by the presence of unhatched eggs laid by the females (Table 7; p. 48) .

4.4 Effect of tetracycline treatment on the expression of CI in *T. castaneum*

To determine whether or not RMO/*Wolbachia* causes incompatibility in *Culex*, it was necessary to render the mosquitoes free of the symbionts. Yen & Barr (1973, 1974) showed that *W. pipientis* is sensitive to antibiotics and thus *C. pipiens* larvae reared in tetracycline-treated water were free of the symbiont and remained free (aposymbiotic) in future generations. Similar results were also reported by Portaró & Barr (1975), Fine *et al.* (1977) and Wright & Wang (1980), although

tetracycline treatment of *Aedes scutellaris* complex was not successful for rendering aposymbiosis (Macdonald, 1976). Wright & Wang (1980) and Trpis et al. (1981) further demonstrated that aposymbiosis in *A. polynesiensis* and *A. scutellaris* could also be induced by rearing the mosquito larvae at temperatures of 32-33°C for 5-7 days. Of course, it should be borne in mind that any drug or heat treatment is not proof of the cause of incompatibility, because the alteration of physiological functions and structure of the cytoplasmic organelles like mitochondria and ribosomes of the eukaryotic cells with such treatment may be responsible for the alteration of incompatibility as well (Trpis et al. 1981).

Apart from mosquitoes, antibiotic treatment is also capable of inducing aposymbiosis in coleopterans like *Hypera postica* (Hsiao & Hsiao, 1985a), *T. confusum* (Wade & Stevens, 1985; O'Neill, 1989) and a hymenopteran, *Nasonia* (Breeuwer & Werren, 1990, 1993). Beetles reared on 0.125% crystalline tetracycline chloride medium was 'cured' of the microorganisms causing incompatibility in *T. confusum* and the 'cured' strains remained fertile in future generations. By rearing 'infected' strains like bSM, b-Circle A and McGill black on an antibiotic-treated food, reproductive compatibility in this species was regained. Partial cures were obtained with very low doses (e.g. 0.005%) of tetracycline, erythromycin and spectinomycin (Wade & Stevens, 1985). Two other strains of *T. confusum* reared on a diet supplemented with 0.125% tetracycline became aposymbiotic and the cure was found permanent (O'Neill, 1989).

In agreement with the results of Wade & Stevens (1985) and O'Neill (1989), the present data (Tables 8-10; pp. 50-52) clearly show that apparently 'infected' indigenous and exotic female strains of *T. castaneum* viz. KHU, RAJ, CTC, FSS and KAN become 'uninfected' as indicated by the incompatible crosses between the tetracycline-treated females and males reared on normal (untreated) food. Males reared either on normal or tetracycline-treated food, when mated with females reared either on normal or treated food respectively, always resulted in compatibility.

4.5 Effect of UV radiation treatment on the expression of CI in *T. castaneum*

The idea of treating 'infected' strains of *T. castaneum* with UV radiation to 'cure' them is a new one. It sprang from the findings that UV is capable of inducing mutations as well as chromosomal aberrations in eukaryotic cells (Swanson & Stadler, 1955; Kirby-Smith & Craig, 1955). Moreover, inhibition of spindle formation (Carlson & Hollaender, 1948), internal deficiencies and breakages (Slizynski, 1950), terminal deficiencies and interstitial losses of chromosomes (Barton, 1954) and inhibition of mitosis (Swanson, 1957) have been observed following UV treatments to various organisms. Recent investigations reveal that though UV rays do not possess sufficient energy to induce ionizations, they are readily absorbed by certain substances such as purines and pyrimidines, which then enter a more reactive or excited state (Gardner *et al.*, 1991). Because of their lower energy, UV penetrate tissues only slightly and usually the surface layer of cells in multicellular organisms are affected, but it is a

potent mutagen for unicellular organisms. The maximum absorption of UV rays by DNA is at a wavelength of 254 nm and the maximum mutagenicity also occurs at 254 nm, suggesting that the UV-induced mutation process is mediated directly by the absorption of UV by the nitrogen bases of the nucleic acids. Several lines of evidence indicate that thymine dimerization is probably the major mutagenic effect of UV (Witkin, 1969; Howard-Flanders, 1981). However, the relationship between mutation rate and UV dosage is highly variable; it could either be linear and wavelength-dependent for chromosomal aberrations (Swanson & Stadler, 1955), or non-linear for mutations (Kirby-Smith & Craig, 1955). All these vary depending on the type of mutation, the organism and the conditions employed (Gardner et al., 1991).

The present data with 254 nm UV treatment on the expression of CI in *T. castaneum* (Table 15; p. 57) show that both indigenous and exotic males, which were incompatible, become compatible with untreated females of the same strains. This clearly demonstrates that, like antibiotic treatment and heat-shock, a brief exposure (30 sec) of 254 nm UV dose could also be effective in 'curing' the strains of RMO, thus recovering the compatibility. Untreated males when mated to UV-treated females, on the other hand, resulted in partial incompatibility in 13 crosses and complete incompatibility in 12 crosses, while all the 25 crosses (5 x 5) were compatible when treated males were paired with treated females.

The effect of 366 nm UV treatment on the expression of CI in *T. castaneum* appears to be a bit erratic in a sense that no generalized comments could be made out of the present results. Treated males mated to untreated females manifested in 6 incompatible crosses, 6 partially incompatible crosses and 13 compatible crosses, whereas untreated males paired with UV-treated females resulted in 6 incompatible and the rest 19 partially incompatible crosses. Treated males x treated females had no effect on the manifestation of CI (Table 15; p. 57).

Unfortunately no comparisons could be made owing to lack of information about the effect of UV radiation on the expression of CI in *T. castaneum*. It could only be assumed that 254 nm of UV treatment on the pupae might have resulted in mutations coupled with chromosomal aberration(s) in the beetles. Simultaneously, genetic as well as somatic changes in the RMO/*Wolbachia*, if any, present in the host might be responsible for the apparent UV-induced compatibility phenomenon reported here for the first time.

4.6 Frequency-dependent regulation in *T. castaneum*

In spite of the significance of *T. castaneum* as an important insect pest of stored products, many aspects of its population dynamics are not yet understood. However, an understanding of the dynamics and of the processes that govern the dynamics may be helpful in controlling the flour beetle population densities and thus infestation of food grains. Obviously, any explanation of *T. castaneum* dynamics must include the mechanisms that form the

life-history traits such as fecundity and percentage of egg-hatch. Unfortunately, such studies are lacking for this species and there is little or perhaps no information on the density-dependent regulation in *T. castaneum*, although the beetle is one of the most prominent of the numerous species of stored product pests. Studies with *Anopheles cantans* revealed that under crowded conditions, density-dependent competition in this species led to reduction in the size of both larvae and adults, while cannibalism and contact inhibition leading to food shortage were recognized to be the causative factors by Renshaw *et al.* (1993), who found that as density increased, both larval and adult-sizes decreased along with the increase in larval mortality. Growth-rate and adult-size were found to be dependent on temperature and larval rearing densities in *A. gambiae* (Lyimo *et al.* 1992). They demonstrated that age at pupation increased as the larval rearing density decreased, but the adult-size decreased as the larval density increased. Similar effect was found for growth-rate which also decreased as the density increased. These findings fit with the present results in that fecundity, percentage of egg-hatch and the number of adult progenies in *T. castaneum* were adversely affected by increasing density of the incompatible exotic males in the population.

That rearing densities have pronounced effects on the life-history traits in mosquitoes are demonstrated by Istock (1985). Jahan (1997) reported that fecundity in *C. quinquefasciatus* females originating from a higher larval density cultures was significantly lower than that from a lower density culture. Khan (1998) has shown that though larval rearing density has no

significant effect on fecundity in *Musca domestica*, the hatchability, egg-volume and pupal weight were density-dependent traits. The present findings are in well agreement with these studies in that the adult rearing densities affect at least some of the reproductive parameters like fecundity, egg-hatch and adult emergence in *T. castaneum*. Environmental and genetic parameters like RMO and intraspecific competition might interact to influence these traits. It appeared, therefore, that much work is needed to untangle the complex relationships between the genotype-environment and the life-history parameters to understand the population dynamics and intra-strain competition in *T. castaneum*.

As for the incompatibility relationships, an attempt to explain the frequency-dependent regulation in *T. castaneum* under laboratory conditions has been presented here for the first time. By allowing both sexes of two indigenous strains (viz. CHI and DHA) to mate each with three exotic male strains (viz. CTC, FSS and KAN) which previously showed partial and/or complete incompatibilities, reproductive performance that reflected the growth of the beetle was examined at varying ratios from 1:1:1 to 1:1:3. Results of this experiment revealed that it is possible to suppress the indigenous pest species by selecting the appropriate mating types of the exotic male strains. It has clearly been demonstrated that each of the CTC, FSS and KAN males, when mated either to CHI or DHA females, produced no offspring due to unidirectional incompatibility (Table 16; p. 59). Whereas if the incompatible exotic males are introduced into the indigenous populations (CHI x CHI or DHA x DHA) at an increasing ratios, the

number of adult progenies decrease owing to the successful mating competitiveness of the exotic males for indigenous females. In this regard, compared to FSS and KAN, CTC males were found suitable for suppressing CHI population, while in comparison with CTC and KAN, FSS males reduced DHA population to a minimum. These findings are encouraging with respect to any strategy aimed at controlling *T. castaneum* under laboratory as well as field conditions. .

4.7 Mating competitiveness of the incompatible males

Assessment of mating competitiveness of incompatible exotic males of *T. castaneum* by ratio test method is being reported here for the first time. Results show that as the ratios of the exotic males (CTC, FSS and KAN) increased, percentage of egg-hatch in both of the indigenous females (CHI and DHA) declined, but the decline was not as great as that expected from 1:0:1 (*i.e.* indigenous female x exotic male) matings. The CVs of 0.37-0.60, 0.14-0.36 and 0.15-0.43 respectively for the CTC, FSS and KAN males paired to CHI females and those of 0.18-0.21, 0.18-0.23 and 0.23-0.34 respectively for the same males mated to DHA females indicated that CTC males performed well with CHI females while KAN males competed better with DHA females. But, it should be kept in mind that mating propensity of the incompatible exotic males in the wild *e.g.* field or ware house situations, might be quite different from that recorded in the laboratory conditions.

Mating competitiveness values of sterile male insects is frequently evaluated from competitive mating tests, and the ratio test method has been one of the widely used techniques. Brower (1972) assessed CVs in *Tenebrio molitor*, Ahmed & Abdel-Malek (1980) and Hussain & Imura (1989) estimated the values in *Callosobruchus maculatus* and *C. chinensis* respectively irradiated with 10-80 Gy and Abdel-Salam (1989) calculated them in *T. castaneum* treated with gamma radiation doses of 35-50 Gy. Recently Hasan (1998) reported CVs of gamma-sterilized males of five species of *Tribolium* including *T. castaneum*. By increasing the proportion of normal females: normal males: irradiated males from 1:1:1 to 1:1:25, Hasan (1998) found CVs ranging between 0.38 and 0.80 in *T. castaneum*. Previous reports on estimated mating competitiveness values in a variety of insect species reveal that CVs may be as small as 0.3-0.4 (Fried, 1971) or as great as 0.91-0.93 (Brower, 1982) and 0.83-0.96 (El-Gazzar & Dame, 1983). It is to be noted, however, that larger release ratios do not necessarily ensure higher CVs. For example, in *Ceratitis capitata*, CVs of irradiated males were found to be 0.38, 0.23 and 0.15 at 1:1:5, 1:1:9 and 1:1:19 ratios, respectively (Hooper & Katiyar, 1971), whereas in *Ephestia cautella* the values were estimated to be 0.93 and 0.91 at 1:1:4 and 1:1:9, respectively (Brower, 1982). These findings fit well with those of the present results. The CVs obtained in this study, therefore, imply that further trials in laboratory and then in nature with larger release ratios should be worthwhile.

4.8 Conclusions and recommendations for further work

A detailed analysis of the cytoplasmic incompatibility phenomenon in *T. castaneum* has been presented here. Apart from KHU and RAJ males mated each with CHI and DHA females, which showed partial incompatibility, all the possible crosses between the indigenous strains were compatible, indicating that there exists a little or no genetic diversity in these strains under study. Incompatibility relationships between four indigenous and four exotic strains, involving a total of 52 crosses, showed that there existed 6 incompatible and 12 partially incompatible mating types. The experiments on frequency-dependent regulation and mating competitiveness in *T. castaneum* demonstrated that cytoplasmic incompatibility could be a potential tool for suppressing this important pest species.

Keeping the present results and their implications in designing a control programme for *T. castaneum* in mind, it would be worth trying the ultra-microscopic examination as well as PCR test of the strains for the confirmation of the cytoplasmic endosymbionts such as RMO or *Wolbachia* responsible for inherited incompatibility in this species. In addition, field trials to determine the proportion of incompatible exotic males to be released for an effective control of the pest would be worth considering.

CHAPTER FIVE

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LITERATURE CITED

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CHAPTER SIX

APPENDICES

Appendix 1. Reproductive potential in *T. castaneum* resulted from indigenous purebred crosses

N	No. eggs laid (Fecundity)	No. eggshatched	% egg-hatch (Hatchability)
CHI x CHI			
1.	40	30	75.0
2.	70	54	77.1
3.	42	35	83.3
4.	94	70	74.5
5.	59	30	50.8
Mean±SE	61.0±9.9		72.1±5.6
DHA x DHA			
1.	67	50	74.6
2.	71	62	87.3
3.	50	42	84.0
4.	64	50	78.1
5.	68	15	77.9
Mean±SE	64.0±4.1		80.4±2.3
KHU x KHU			
1.	21	15	71.4
2.	30	20	66.7
3.	34	19	55.9
4.	28	16	57.1
5.	30	18	60.0
Mean±SE	28.6±2.1		62.2±3.0
CHI x CHI			
1.	40	32	80.0
2.	58	50	86.2
3.	44	31	70.5
4.	38	32	84.2
5.	56	51	91.0
Mean±SE	47.2±4.1		82.4±3.5

Appendix 2. Reproductive potential in *T. castaneum* resulted from exotic purebred crosses

N	No. eggs laid (Fecundity)	No. eggshatched	% egg-hatch (Hatchability)
BLA x BLA			
1.	37	32	86.0
2.	34	31	91.2
3.	38	30	78.9
4.	20	17	85.0
5.	27	21	77.7
Mean±SE	31.2±3.1		83.8±3.3
CTC x CTC			
1.	8	3	37.5
2.	12	4	33.3
3.	20	12	60.0
4.	10	6	60.0
5.	23	19	82.6
Mean±SE	14.6±2.9		54.7±8.9
FSS x FSS			
1.	44	27	61.4
2.	32	20	62.5
3.	52	35	67.3
4.	40	25	62.5
5.	44	30	68.2
Mean±SE	42.4±3.2		64.4±1.4
KAN x KAN			
1.	12	8	66.7
2.	15	6	62.5
3.	18	12	66.7
4.	20	18	90.0
5.	14	12	85.0
Mean±SE	15.8±1.4		59.0±8.8

Appendix 3. Reproductive potential in *T. castaneum* resulted from indigenous x indigenous hybrid crosses.

N	No. eggs laid (Fecundity)	No. eggshatched	% egg-hatch (Hatchability)
CHI x DHA			
1.	4	2	50.0
2.	19	14	73.9
3.	23	17	73.9
4.	12	11	91.6
5.	22	16	72.7
Mean±SE	16.0±3.6		72.4±6.6
DHA x CHI			
1.	25	14	56.0
2.	29	18	62.0
3.	28	18	64.3
4.	31	23	74.0
5.	30	19	63.6
Mean±SE	28.6±1.0		63.9±2.9
DHA x RAJ			
1.	42	20	47.6
2.	32	18	56.3
3.	42	22	52.4
4.	40	15	37.5
5.	33	18	54.5
Mean±SE	37.5±2.2		49.6±3.4
RAJ x DHA			
1.	25	14	56.0
2.	30	18	60.0
3.	32	20	62.5
4.	28	21	75.0
5.	31	18	58.0
Mean±SE	29.2±1.2		62.3±3.4

Appendix 4. Reproductive potential in *T. castaneum* resulted from indigenous x exotic and exotic x indigenous hybrid crosses.

N	No. eggs laid (Fecundity)	No. eggshatched	% egg-hatch (Hatchability)
DHA x CTC			
1.	20	0	0.0
2.	15	0	0.0
3.	17	0	0.0
4.	12	0	0.0
5.	14	0	0.0
Mean±SE	15.6±1.4		0.0±0.0
RAJ x KAN			
1.	50	35	70.0
2.	45	38	84.4
3.	42	31	73.8
4.	38	33	86.8
5.	53	43	81.1
Mean±SE	45.6±2.7		79.2±3.2
KAN x CHI			
1.	40	30	75.0
2.	48	36	75.0
3.	38	28	73.6
4.	45	32	71.1
5.	52	40	76.9
Mean±SE	44.6±2.6		74.3±1.0
KAN x KHU			
1.	19	0	0.0
2.	12	1	8.3
3.	20	0	0.0
4.	14	5	35.7
5.	21	7	33.3
Mean±SE	17.2±1.8		15.5±5.6

Appendix 5. Significance tests between purebred and hybrid crosses for percentage of egg-hatch data in *T. castaneum*

1. Comparisons between: CHI x CHI vs. CTC x CTC

No.	X ₁	(X ₁ - $\bar{X}_1$) ²	X ₂	(X ₂ - $\bar{X}_2$) ²
1.	75.0	8.41	37.5	295.84
2.	77.1	25.0	33.3	457.96
3.	83.3	125.44	60.0	28.09
4.	74.5	5.76	60.0	28.09
5.	50.8	453.69	82.6	778.41
Σ	360.7 $\bar{X}_1 = 72.1$	618.3	273.3 $\bar{X}_2 = 54.7$	1588.39

$$sd = \pm \sqrt{\frac{\Sigma((X_1 - \bar{X}_1)^2 + \Sigma(X_2 - \bar{X}_2)^2)}{5 + 5 - 2}}$$

$$= \pm \sqrt{\frac{618.3 + 1588.39}{8}}$$

$$= \pm 16.61$$

$$t = \frac{X_1 - \bar{X}_2}{sd \sqrt{1/5 + 1/5}} = \frac{72.1 - 54.7}{16.61 \times 0.6324} = \frac{17.4}{10.5} = 1.66$$

$$\sqrt{1/5 + 1/5} = 0.6324$$

2. Comparisons between: CHI x CHI vs. FSS x FSS

No.	X ₁	(X ₁ - $\bar{X}_1$) ²	X ₂	(X ₂ - $\bar{X}_2$) ²
1.	75.0	8.41	61.4	9.0
2.	77.1	25.0	62.5	3.61
3.	83.3	125.44	67.3	8.41
4.	74.5	5.76	62.5	3.61
5.	50.8	453.69	68.2	14.44
Σ	360.7 $X_1 = 72.1$	618.3	321.9 $X_2 = 64.4$	39.07

$$sd = \pm \sqrt{\frac{\Sigma((X_1 - \bar{X}_1)^2 + \Sigma(X_2 - \bar{X}_2)^2)}{5 + 5 - 2}}$$

$$= \pm \sqrt{\frac{618.3 + 39.07}{8}}$$

$$= \pm 9.06$$

$$t = \frac{X_1 - \bar{X}_2}{sd \sqrt{1/5 + 1/5}} = \frac{72.1 - 64.4}{9.06 \times 0.6324} = \frac{7.7}{5.73} = 1.34$$

$$\sqrt{1/5 + 1/5} = 0.6324$$

Appendix 6. Effect of backcrosses on the reproductive potential in *T. castaneum*

N	No. eggs laid (Fecundity)	No. eggshatched	% egg-hatch (Hatchability)
CHI x F ₁ (KHU x CHI)			
1.	26	0	0.0
2.	35	0	0.0
3.	38	0	0.0
4.	37	0	0.0
5.	30	0	0.0
Mean±SE	33.2±2.2		0.0±0.0
CHI x F ₁ (RAJ x CHI)			
1.	40	0	0.0
2.	42	0	0.0
3.	38	0	0.0
4.	39	0	0.0
5.	35	0	0.0
Mean±SE	38.8±1.2		0.0±0.0
DHA x F ₁ (KHU x DHA)			
1.	35	0	0.0
2.	30	0	0.0
3.	32	0	0.0
4.	28	0	0.0
5.	36	0	0.0
Mean±SE	32.2±1.5		0.0±0.0
DHA x F ₁ (RAJ x DHA)			
1.	39	0	0.0
2.	31	0	0.0
3.	35	0	0.0
4.	37	0	0.0
5.	41	0	0.0
Mean±SE	36.6±1.7		0.0±0.0

Appendix 7. Effect of tetracycline treatment in food medium on the reproductive potential in *T. castaneum*

No.	Females x males	No. eggs laid	No. eggshatched	% egg-hatch
<i>Normal food x normal food</i>				
1.	KHU x CTC	27	21	77.8
2.	KHU x CTC	27	21	77.8
3.	KHU x CTC	27	21	77.8
Mean±SE		25.3±4.7		83.8±4.4
<i>Tetracycline-treated food x normal food</i>				
1.	KHU x CTC	16	0	0.0
2.	KHU x CTC	32	0	0.0
3.	KHU x CTC	21	0	0.0
Mean±SE		23.0±5.8		0.0±0.0
<i>Normal food x tetracycline-treated food</i>				
1.	KHU x CTC	28	21	75.0
2.	KHU x CTC	17	11	64.7
3.	KHU x CTC	35	28	80.0
Mean±SE		26.7±6.4		73.2±5.5
<i>Tetracycline-treated food x tetracycline-treated food</i>				
1.	KHU x CTC	22	18	81.8
2.	KHU x CTC	16	11	68.8
3.	KHU x CTC	26	19	73.1
Mean±SE		21.3±3.6		74.6±4.7

Appendix 8. Effect of UV radiation treatment (254 nm) on the reproductive potential in *T. castaneum*

Females x males	No. eggs laid	No. eggshatched	% egg-hatch
Untreated x untreated			
KHU x CTC			
1.	16	9	56.3
2.	14	7	50.0
3.	13	8	61.5
Mean±SE	14.3±0.9		55.9±3.3

No.	No. eggs laid	No. eggshatched	% egg-hatch
Treated x untreated			
KHU x CTC			
1.	15	0	0.0
2.	13	0	0.0
3.	15	0	0.0
Mean±SE	14.3±0.7		0.0±0.0

No.	No. eggs laid	No. eggshatched	% egg-hatch
Untreated x treated			
KHU x CTC			
1.	12	7	58.3
2.	14	8	57.1
3.	9	4	44.4
Mean±SE	16.7±3.8		53.3±4.5

No.	No. eggs laid	No. eggshatched	% egg-hatch
Treated x treated			
KHU x CTC			
1.	17	9	52.9
2.	14	8	57.1
3.	18	10	55.6
Mean±SE	16.3±1.2		55.2±1.2

Appendix 9. Effect of UV radiation treatment (366 nm) on the reproductive potential in *T. castaneum*

Females x males	No. eggs laid	No. eggshatched	% egg-hatch
Untreated x untreated			
KHU x CTC			
1.	29	17	58.6
2.	25	15	60.0
3.	35	20	57.1
Mean±SE	29.7±3.6		58.6±1.0

No.	No. eggs laid	No. eggshatched	% egg-hatch
Treated x untreated			
KHU x CTC			
1.	20	0	0.0
2.	23	0	0.0
3.	19	0	0.0
Mean±SE	20.7±1.2		0.0±0.0

No.	No. eggs laid	No. eggshatched	% egg-hatch
Untreated x treated			
KHU x CTC			
1.	38	0	0.0
2.	31	0	0.0
3.	44	0	0.0
Mean±SE	37.7±4.6		0.0±0.0

No.	No. eggs laid	No. eggshatched	% egg-hatch
Treated x treated			
KHU x CTC			
1.	15	11	73.3
2.	17	12	70.6
3.	17	13	76.5
Mean±SE	16.3±0.8		73.5±2.1

Appendix 10. Effect of matings between indigenous strains (CHI and DHA) and cytoplasmically incompatible exotic male strains (CTC, FSS and KAN) on the reproductive potential and progeny production in *T. castaneum*

Ratios (♀♀:♂♂:♂♂)	No. eggs laid	No. eggs hatched	% egg-hatch	No. adult progenies
1CHI:1CHI: -	145	120	82.8	111
	140	105	75.0	92
	151	112	74.2	106
Mean±SE	145.7±3.5		77.3±2.7	103.0±7.0
1CHI: -:1CTC	110	0	0.0	0
	105	0	0.0	0
	125	0	0.0	0
Mean±SE	113.3±6.0		0.0±0.0	0.0±0.0
1CHI:1CHI:1CTC	105	53	50.5	47
	128	60	46.9	58
	122	58	47.5	42
Mean±SE	118.3±6.9		48.3±1.1	49.0±5.8
1CHI:1CHI:2CTC	112	47	42.0	38
	115	48	41.7	39
	120	44	36.7	35
Mean±SE	115.7±2.3		40.1±1.7	37.3±1.5
1CHI:1CHI:3CTC	102	40	39.2	31
	107	39	36.4	30
	98	34	34.7	28
Mean±SE	102.3±2.6		36.8±1.3	29.7±1.1

Appendix 11. Estimation of mating competitiveness values (CVs) of the exotic male strains of *T. castaneum* from percentage of egg-hatch data (Appendix 10)

Expected % egg-hatch at a given ratio, $H_e = \frac{I \times H_p + E \times H_h}{I+E}$

where, I = number of indigenous males

E = number of exotic males

H_p = mean % egg-hatch from the purebred cross (indigenous female x indigenous male)

H_h = mean % egg-hatch from hybrid cross (indigenous female x exotic male).

Mating competitiveness value, $CV = \frac{I \times H_p - H_o}{E \times H_o - H_h}$

where, I = number of indigenous males

E = number of exotic males

H_p = mean % egg-hatch from the purebred cross (indigenous female x indigenous male)

H_o = mean % egg-hatch observed at a given ratio

H_h = mean % egg-hatch from hybrid cross (indigenous female x exotic male).

Calculation of expected % egg-hatch

Number of mating insects
(ratios)

5CHI: 5CHI: 5CTC (1:1:1)	$He = \frac{5 \times 77.3 + 5 \times 0.0}{5+5}$	$= \frac{386.5}{10}$	= 38.7
5CHI: 5CHI: 10CTC (1:1:2)	$He = \frac{5 \times 77.3 + 10 \times 0.0}{5+10}$	$= \frac{386.5}{15}$	= 25.8
5CHI: 5CHI: 15CTC (1:1:3)	$He = \frac{5 \times 77.3 + 15 \times 0.0}{5+15}$	$= \frac{386.5}{20}$	= 19.3

Calculation of mating competitiveness values (CVs)

Number of mating insects
(ratios)

5CHI: 5CHI: 5CTC (1:1:1)	$CV = \frac{5 \times 77.3 - 48.3}{5 \times 48.3 - 0.0}$	$= \frac{145.0}{241.5}$	= 0.60
5CHI: 5CHI: 10CTC (1:1:2)	$CV = \frac{5 \times 77.3 - 40.1}{10 \times 40.1 - 0.0}$	$= \frac{186.0}{401.0}$	= 0.46
5CHI: 5CHI: 15CTC (1:1:3)	$CV = \frac{5 \times 77.3 - 36.8}{15 \times 36.8 - 0.0}$	$= \frac{202.5}{552}$	= 0.37

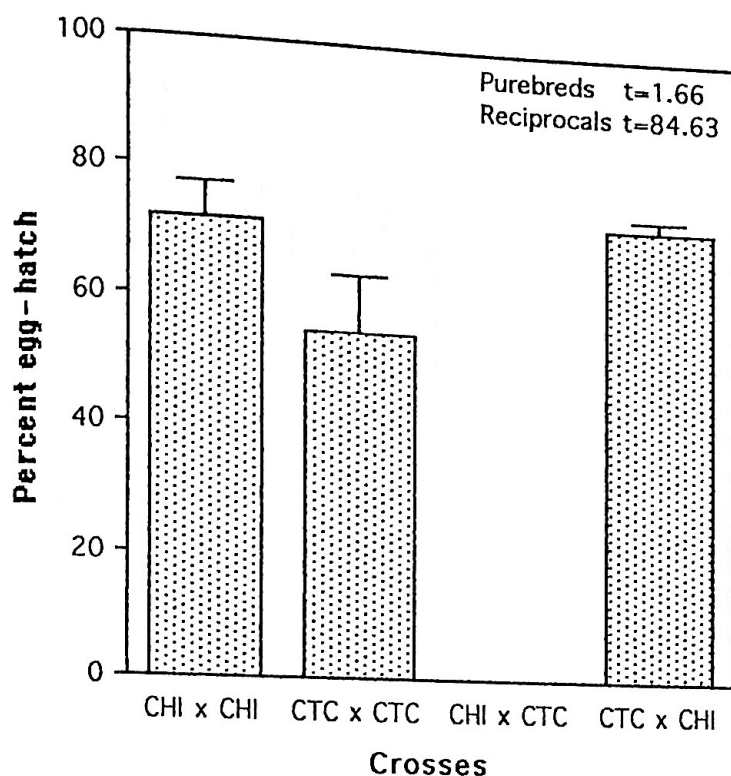


Fig. 1. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous CHI and exotic CTC of *T. castaneum*. Bars indicate $\pm$ SE.

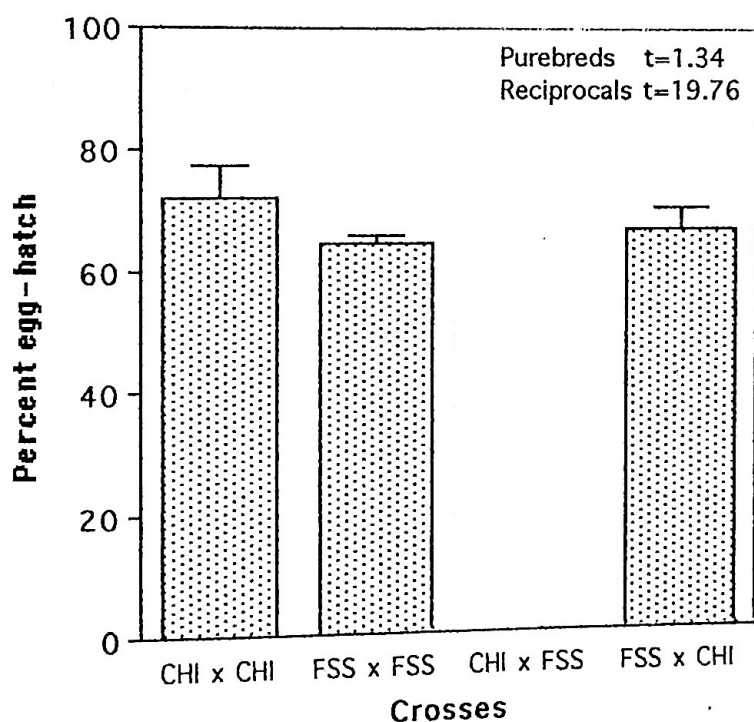


Fig. 2. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous CHI and exotic FSS of *T. castaneum*. Bars indicate $\pm$ SE.

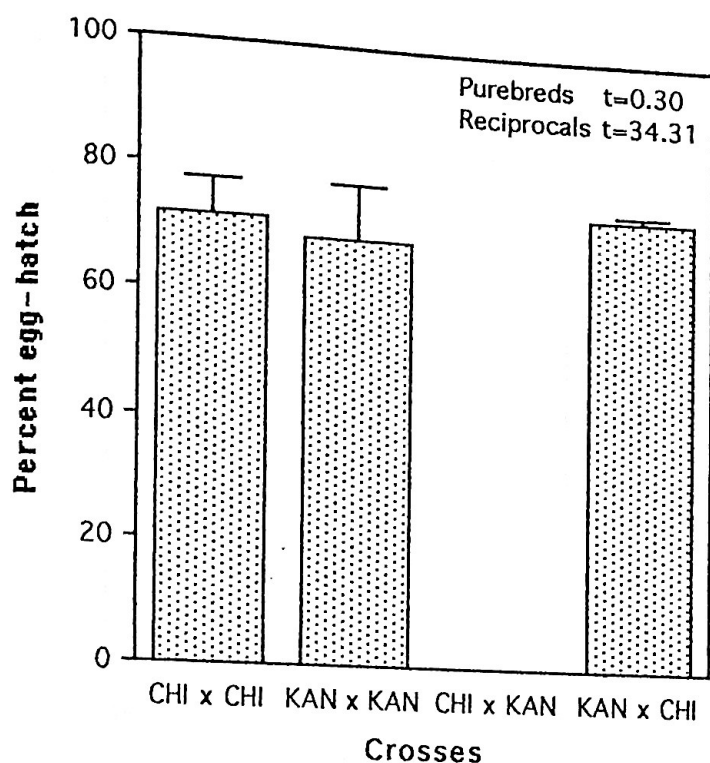


Fig. 3. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous CHI and exotic KAN of *T. castaneum*. Bars indicate $\pm$ SE.

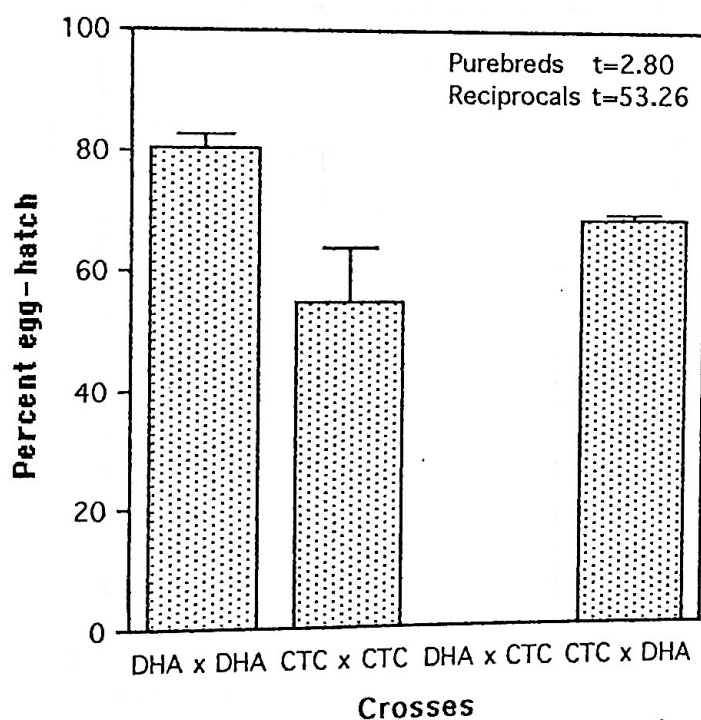


Fig. 4. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous DHA and exotic CTC of *T. castaneum*. Bars indicate $\pm$ SE.

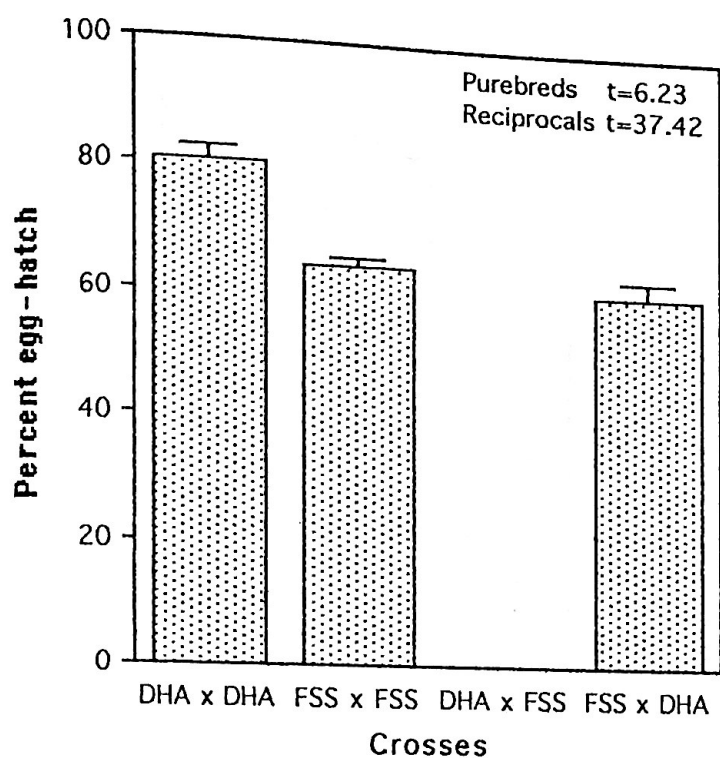


Fig. 5. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous DHA and exotic FSS of *T. castaneum*. Bars indicate $\pm$ SE.

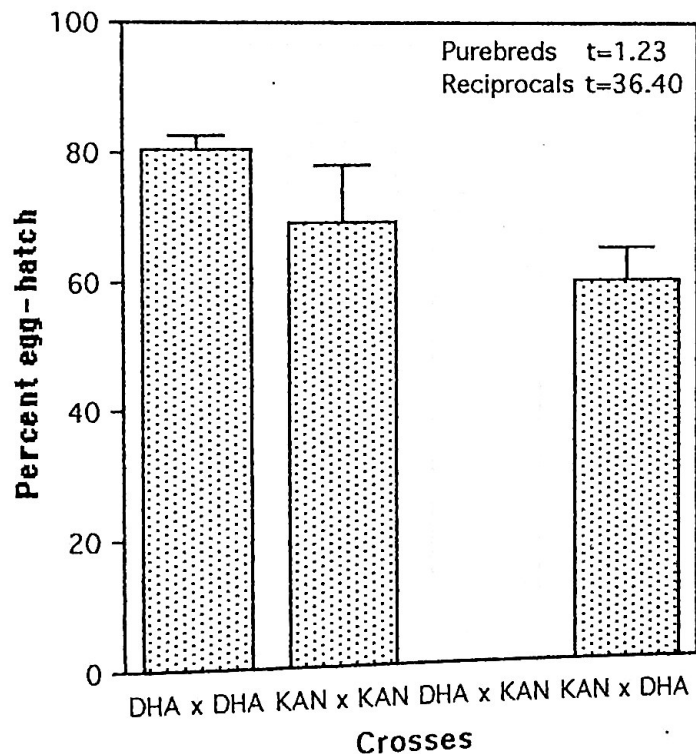


Fig. 6. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous DHA and exotic KAN of *T. castaneum*. Bars indicate $\pm$ SE.

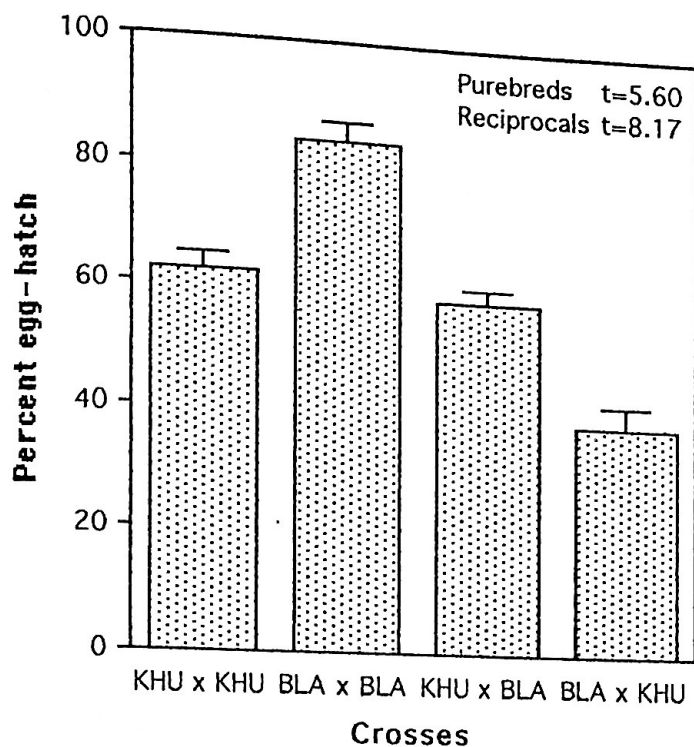


Fig. 7. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous KHU and exotic BLA of *T. castaneum*. Bars indicate $\pm$ SE.

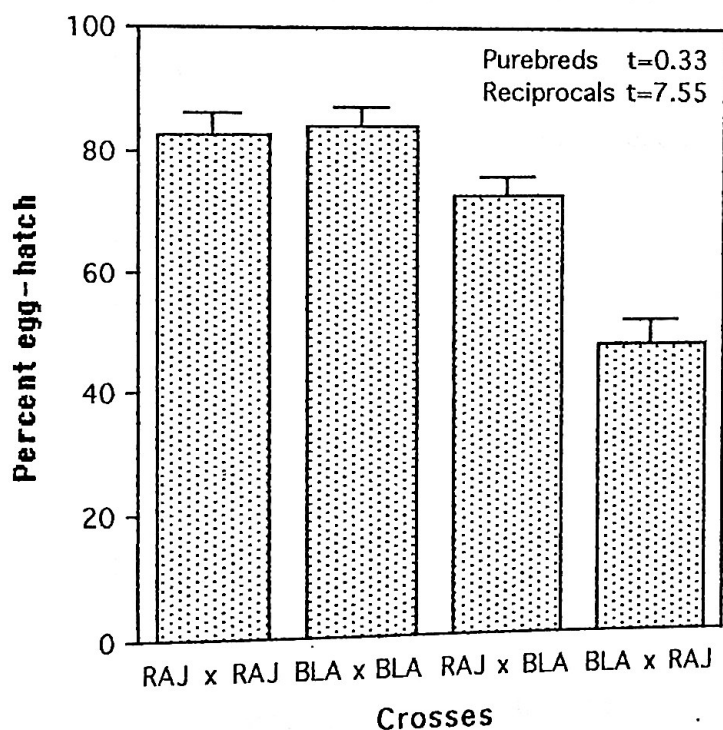


Fig. 8. Percentage of egg-hatch data showing differences between the purebred and reciprocal crosses involving indigenous RAJ and exotic BLA of *T. castaneum*. Bars indicate $\pm$ SE.

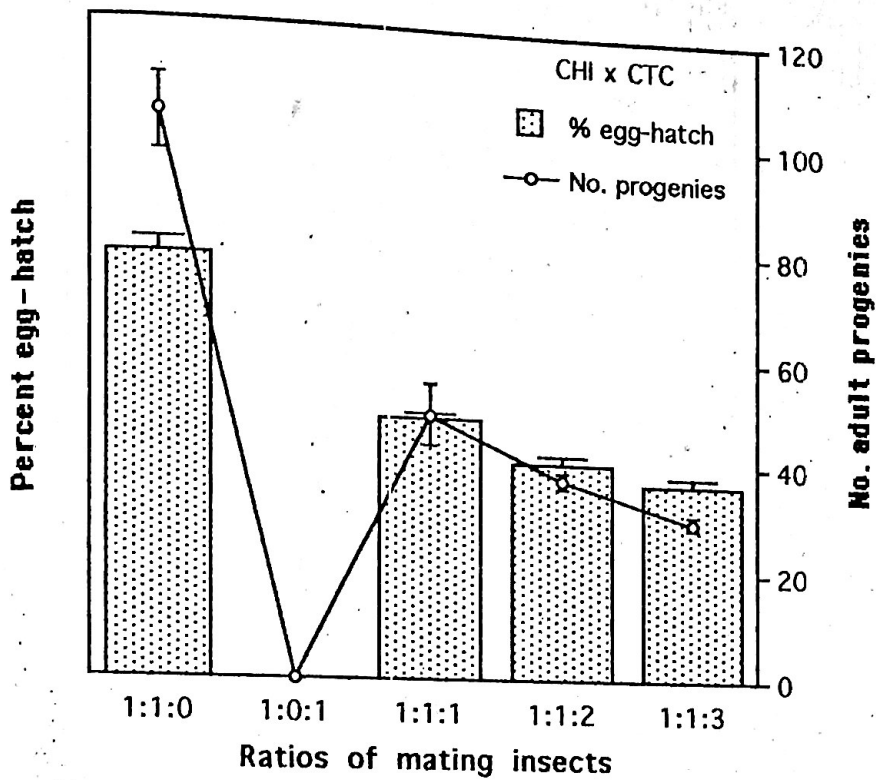


Fig. 9. Percentage of egg-hatch and number of adult progenies resulted from the cross CHI x CTC at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

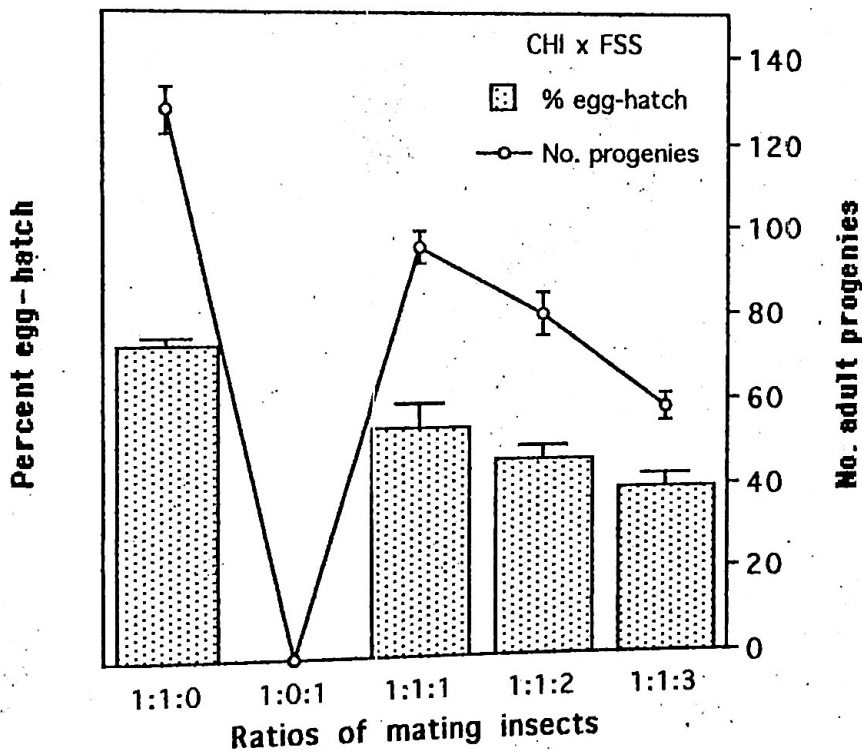


Fig. 10. Percentage of egg-hatch and number of adult progenies resulted from the cross CHI x FSS at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

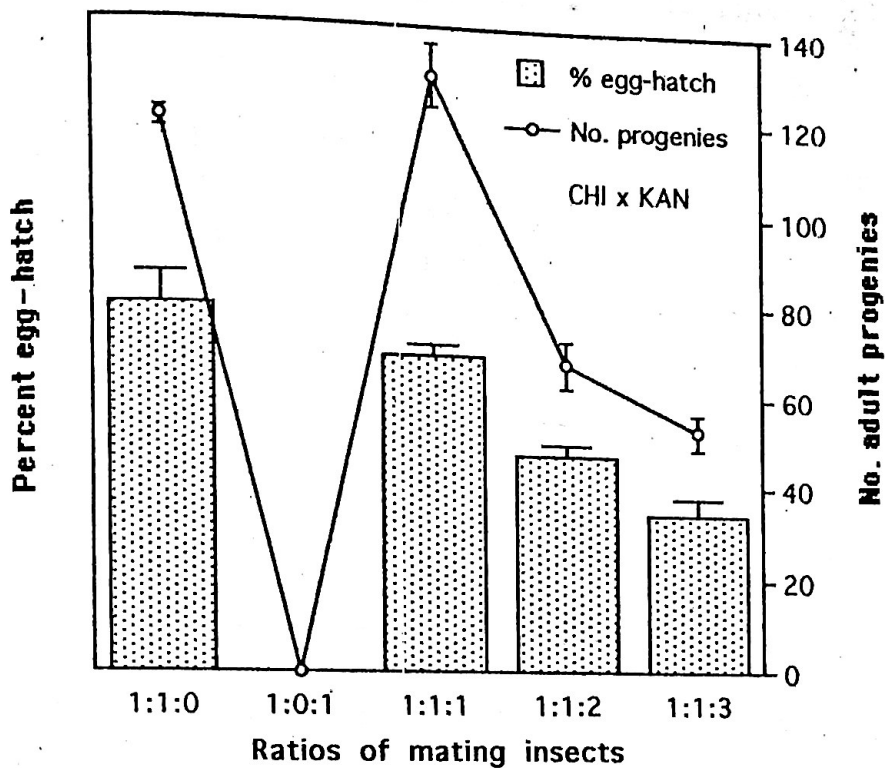


Fig. 11. Percentage of egg-hatch and number of adult progenies resulted from the cross CHI x KAN at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

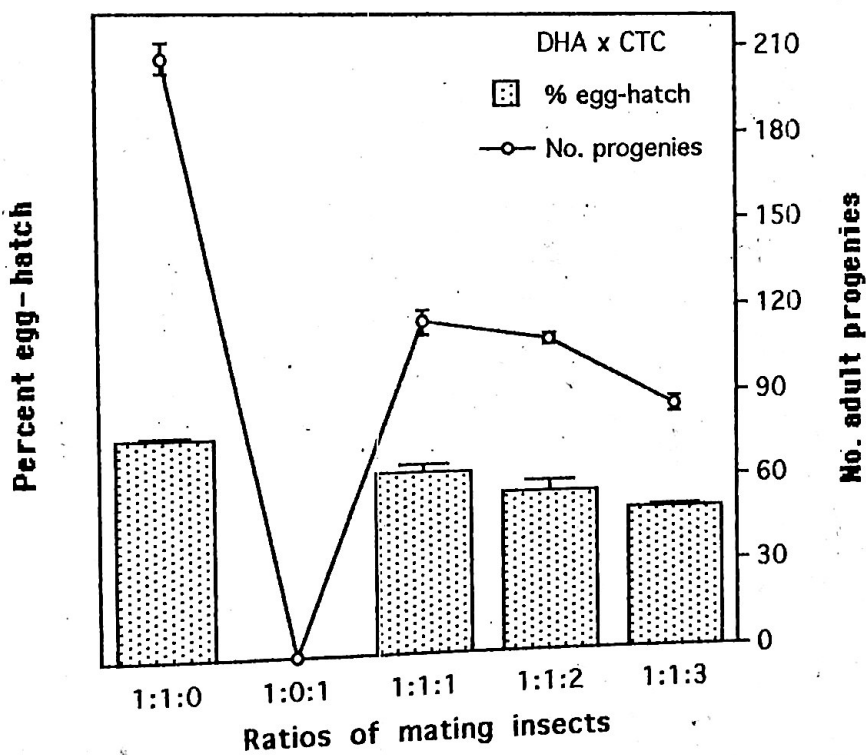


Fig. 12. Percentage of egg-hatch and number of adult progenies resulted from the cross DHA x CTC at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

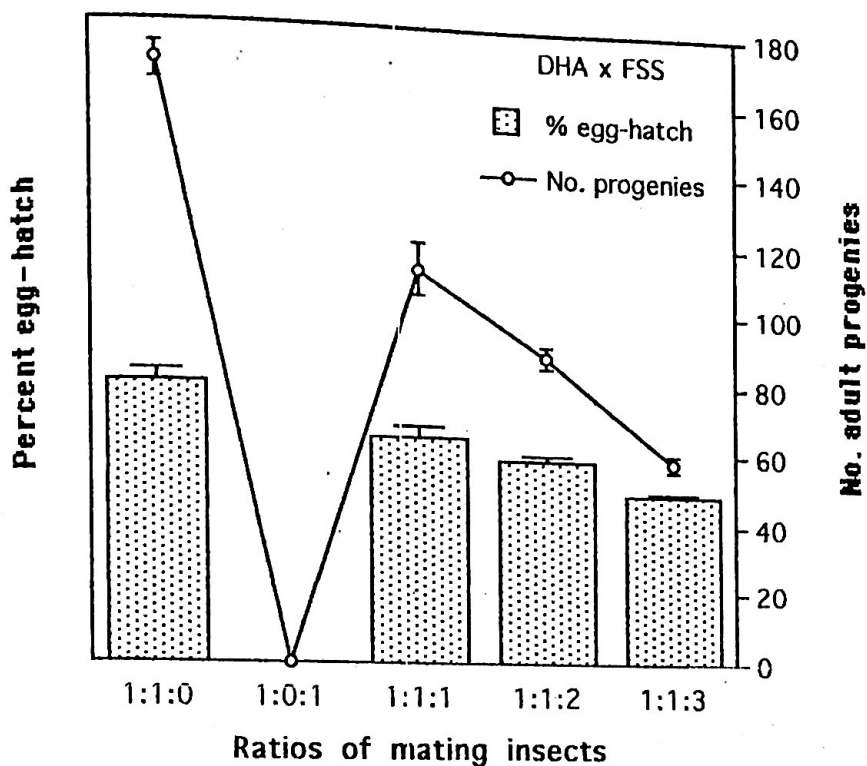


Fig. 13. Percentage of egg-hatch and number of adult progenies resulted from the cross DHA x FSS at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

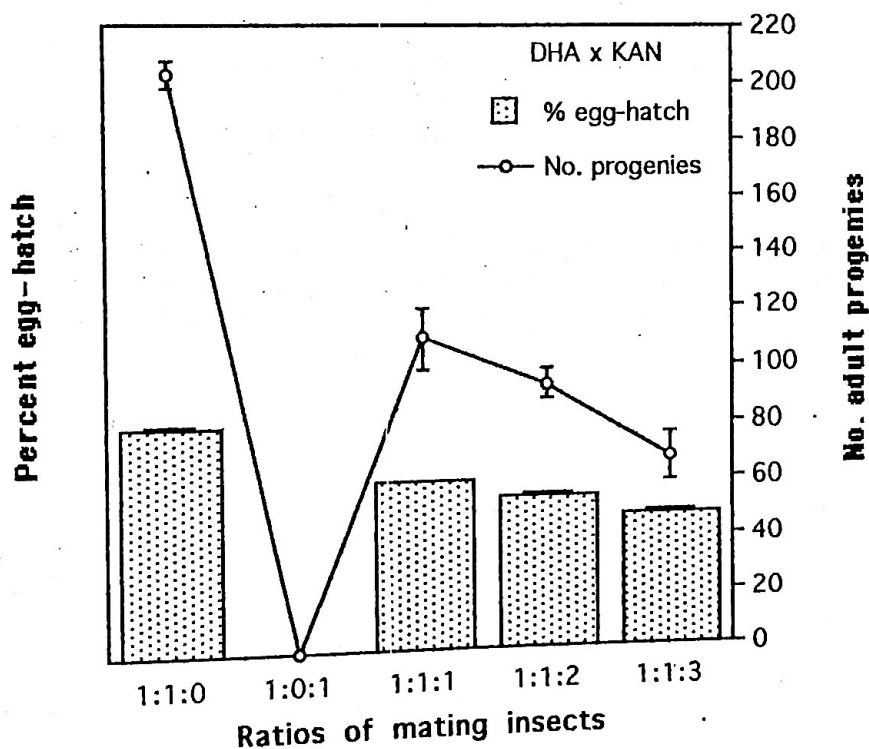


Fig. 14. Percentage of egg-hatch and number of adult progenies resulted from the cross DHA x KAN at various ratios in *T. castaneum*. Bars indicate $\pm$ SE.

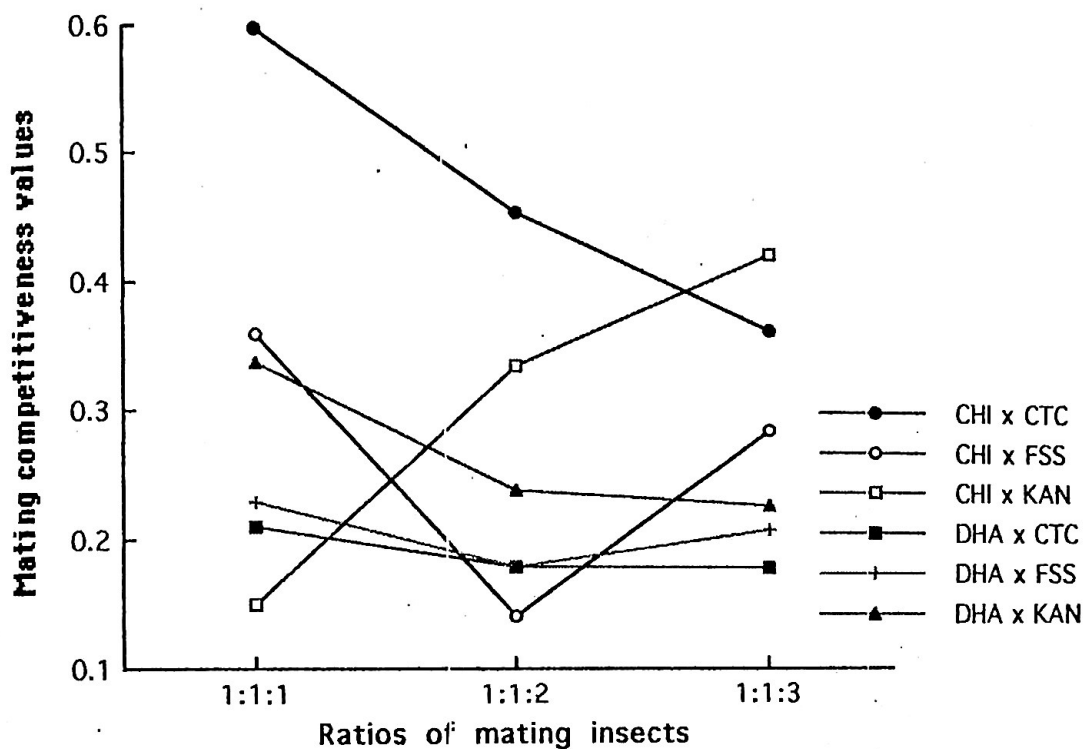


Fig. 15. Mating competitiveness values (CVs) estimated from percentage of egg-hatch data of the crosses involving exotic males strains (CTC, FSS and KAN) x indigenous female strains (CHI and DHA) of *T. castaneum*.

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