



Formosan Entomologist

Journal Homepage: entsocjournal.yabee.com.tw

Copulation Competition of Male *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida (Acarina: Tetranychidae) with conspecific and Heterospecific Females and Their Isolation Mechanism 【Research report】

雄性二點葉蟎 (*Tetranychus urticae* Koch) 及神澤葉蟎 (*Tetranychus kanzawai* Kishida) (Acarina : Tetranychidae) 對同種及異種雌蟎之交尾競爭及隔離機制 【研究報告】

Chain-Ing T. Shih* and Sheuan-Ping Shiue
施劍鏗*、薛烜坪

*通訊作者E-mail :

Received: Accepted: 1995/08/23 Available online: 1995/09/01

Abstract

More than 96.7% of male *Tetranychus urticae* Koch (TSM) and *Tetranychus kanzawai* Kishida (KSM) copulated with conspecific or heterospecific females (CF or HF) in 30 min of pairing. The rates of second matings of CF and HF with TSM males were 66.7% and 69.0%, respectively, and 36.7% and 13.3% with KSM males, respectively. The rate of second matings of KSM females with conspecific males was lower than that of TSM females. With precopulation guarding, KSM males guarded a teleiochrysalis at the detention position-the first guarding status. KSM males exhibited a higher tendency than TSM males to mate first with a guarded female upon her emergence, where at the coguarding position the male generally got a second mating chance in sequence with the female. The first guarding males gave up their guarding status to mate first with intruding females and then immediately resumed the first guarding status-mate-first strategy. Consequently, KSM performed better in population competition than TSM when both populations existed in the same ecological habitat because of a higher tendency to perform precopulation guarding, the mate-first strategy and second mating. Under conditions with the presence of females of both species, the males' first and second copulation rates with CF or HF were not significantly different ($\chi^2 = 1.914$, $df = 1$). The copulated females produced a 10.3%-13.6% non-insemination rate which revealed that these two spider mites retain a control mechanism on the sex ratio in their populations. During heterospecific pairing and female guarding, 80% of KSM males were interrupted and 75% were replaced by heterospecific rival males, while 81% and 100% of TSM males were interrupted and replaced, respectively. With conspecific pairing and female guarding, males were interrupted and replaced by a conspecific rival male 57% and 24% of the time for TSM and 73% and 18% for KSM, respectively; 13.3% of once-mated females mated again with their coguarding males. TSM and KSM males did not show any preference of mating choice between CF or HF, and conspecific rival males interrupted conspecific mating pairs at a low rate (20-23%) and their replacement rate was lower than that of heterospecific mating pairs. Consequently, there must be intraspecific communication

摘要

雄二點葉蟎 (*Tetranychus urticae* Koch, TSM) 及雄神澤葉蟎 (*Tetranychus kanzawai* Kishida, KSM) 於30分鐘內與同種雌 (Conspecific female, CF) 或異種雌 (Heterospecific female, HF) 之首次總交尾率均達96.7%以上；CF或HF與雄TSM第二次交尾率分別為66.7%及69.0%，與雄KSM則為36.7%及13.3%。KSM雌蟎之第二次與雄交尾率均低於TSM者，於護雌位雄KSM具較高“交尾第一”(雄蟎放棄護雌位與接近另一雌蟎先交尾再重獲護雌位)行為。首次、第二次交尾率及“交尾第一”行為，似可解釋KSM在田間與TSM共同存在一寄主時，常為優勢族群。此二種雌蟎共同存在時，雄蟎首與CF交尾率為55.2-57.6%，首先與CF或HF交尾率間無差異($\chi^2 = 1.914$, $df = 1$)。雌蟎與同種雄交尾後，尚有10.3-13.6%未受孕率(雌蟎未接受精包，只產生雄性子代)；因此，此二葉蟎似擁有控制族群性比之機制力。異種配對護雌時，80.0%雄KSM受情敵雄TSM干擾及75%的取代，81%雄TSM受雄KSM干擾及100%的取代；同種配對護雌時，73%被異種TSM雄干擾及91%被取代，另有84%被異種KSM雄干擾及56%被取代。同種配對被同種TSM雄情敵干擾及取代率分別為57%及24%，被KSM雄者分別為73%及18%；並且有13.3%交尾一次後雌蟎與“共同護雌”之雄蟎交尾。

Key words: Behavior, interruption, replacement, fertility, sperm transference.

關鍵詞: 生殖、干擾、取代、生育、授精

Full Text:  PDF(9.28 MB)

下載其它卷期全文 Browse all articles in archive: <http://entsocjournal.yabee.com.tw>

Copulation Competition of Male *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida (Acarina: Tetranychidae) with Conspecific and Heterospecific Females and Their Isolation Mechanism

Chain-Ing T. Shih* and Sheuan-Ping Shiu Department of Entomology, National Chung-Hsing Univ. Taichung, Taiwan 402, R.O.C.

ABSTRACT

More than 96.7% of male *Tetranychus urticae* Koch (TSM) and *Tetranychus kanzawai* Kishida (KSM) copulated with conspecific or heterospecific females (CF or HF) in 30 min of pairing. The rates of second matings of CF and HF with TSM males were 66.7% and 69.0%, respectively, and 36.7% and 13.3% with KSM males, respectively. The rate of second matings of KSM females with conspecific males was lower than that of TSM females. With precopulation guarding, KSM males guarded a teleiochrysalis at the detention position—the first guarding status. KSM males exhibited a higher tendency than TSM males to mate first with a guarded female upon her emergence, where at the coguarding position the male generally got a second mating chance in sequence with the female. The first guarding males gave up their guarding status to mate first with intruding females and then immediately resumed the first guarding status—mate-first strategy. Consequently, KSM performed better in population competition than TSM when both populations existed in the same ecological habitat because of a higher tendency to perform precopulation guarding, the mate-first strategy and second mating.

Under conditions with the presence of females of both species, the males' first and second copulation rates with CF or HF were not significantly different ($\chi^2=1.914$, $df=1$). The copulated females produced a 10.3%~13.6% non-insemination rate which revealed that these two spider mites retain a control mechanism on the sex ratio in their populations. During heterospecific pairing and female guarding, 80% of KSM males were interrupted and 75% were replaced by heterospecific rival males, while 81% and 100% of TSM males were interrupted and replaced, respectively. With conspecific pairing and female guarding, males were interrupted and replaced by a conspecific rival male 57% and 24% of the time for TSM and 73% and 18% for KSM, respectively; 13.3% of once-mated females mated again with their coguarding males. TSM and KSM males did not show any preference of mating choice between CF or HF, and conspecific rival males interrupted conspecific mating pairs at a low rate (20~23%) and their replacement rate was lower than that of heterospecific mating pairs. Consequently, there must be intraspecific communication

and affinity mechanisms existing in both TSM and KSM, and the evidence of high interruption and replacement rates in heterospecific pairing by conspecific males also reveals a behavioral isolation mechanism existing between TSM and KSM. The KSM males competed with and excluded the TSM population by wasting mating chances, times and energy of TSM females.

Durations of the first mating between a TSM male and a CF or a HF lasted 199 and 116 sec, while those of a KSM male lasted 156 and 80 sec, respectively. No males of a conspecific mating pair were replaced by a rival male. Although a short interruption might have occurred, the males immediately resumed a second part of copulation which was much shorter than the initial part. Female spider mites during heterospecific pairing exhibited escaping, idiosoma lowering, body turning and hitting, and facing the males to prevent the male from entering the copulation position, while during conspecific pairing females did perform any one of these behaviors but conformed to the males' movements and approaches. Copulation durations between heterospecific TSM and KSM varied from 31~235 sec. The amount of sperm transferred during conspecific copulation was positively correlated with copulation duration in TSM but not in KSM, where a 30 sec copulation duration was enough for female insemination. Relationships between male ages and female insemination rates need further study. The morphological studies of seminal receptacles on con- and heterospecific-copulated females, showed no heterospecific sperm transfer from male to female. Consequently, isolation mechanisms between TSM and KSM would probably not include mating plug, genetic incompatibility between sperm and ovum, rejection of sperm from seminal receptacle, or antagonistic reactions against the heterospecific sperm in the receptacle and/or during the sperm migration in the haemocoel, but should be due to physical differences or un-suitability between the aedeagus and the receptacle, or due to the omission of one or more chemical or pheromonal cues which induce the male to transfer sperm.

Key words: Behavior, interruption, replacement, fertility, sperm transference.

Introduction

Tetranychus urticae Koch (TSM) and *Tetranychus kanzawai* Kishida (KSM) are closely related and highly polyphagous species which commonly live on many kinds of host plants (Osakabe, 1967). The females of both of these two species produce webbing and pheromones to attract the male to engage in pre-copulation guarding (Penman and Cone, 1972, 1974). Most tetranychid mite species produce effective insemination during their first copulation; however, the effectiveness and authenticity of the second copulation depend upon the duration and sufficiency of sperm transfer during the

first copulation of the male (Helle, 1967; Overmere, 1973; Potter and Wrensch, 1978, Shih *et al.*, 1994).

Feldmann (1977) and Potter and Wrensch (1978) reported that the second copulation of two spotted spider mites (TSM) produced diploid female offspring, however, Shih and Pai (1994) and Shiue *et al.* (1995) demonstrated that TSM females received sufficient sperm during their first mating, and the second and third copulations were ineffective.

Tetranychid spider mite populations colonize into new habitats generally with a single dispersal by an inseminated female (Mitchell, 1973) and then development into several complex strains (De-

Boer, 1985). The eggs produced by cross insemination between strains influences the sex ratio, fertilization and hatching rates of offspring (Overmeer, 1973; DeBoer, 1982a,b,c, 1983; DeBoer and Veerman, 1983; Young *et al.*, 1985; Takafuji, 1988). A high frequency of interspecific copulation among tetranychid mites was also observed (Collins and Margolies, 1991; Collins *et al.*, 1993). The minute size of spider mites makes the techniques to decide the transfer of sperm difficult, so this was assessed from diploid female offspring (Keh, 1952; Dillon, 1958; Cagle, 1962; Saba, 1975; Murtaugh and Wrensch, 1978; Zaher *et al.*, 1981; El-Enany *et al.*, 1983; Hill and O'Donnell, 1991; Shih and Pai, 1994; Shiue *et al.*, 1995). The isolation mechanisms among spider mites are mainly due to features of ethological function, mechanical incompatibility, physiological or genetic isolation, and ecological or phenological asynchroniza-tion. TSM and KSM seem to remain isolated from each other. Precopulation guarding and agonistic or aggressive behavior, genetic isolation, and gametic or mechanical incompatibility, besides the morphohopical identity of the aedeagus (Grant, 1966; Shih and Pai, 1994, 1995), seem to play a significant role in species isolation between TSM and KSM.

The second copulation between conspecific males and females of TSM does not result in a high fertilization rate of offspring (Boudreaux, 1963; Helle, 1967; Overmeer, 1972; Feldmann, 1977; Potter, 1978; Potter and Wrensch, 1978; Shih and Pai, 1994, 1995). The duration and frequency of the second copulation were shortened and lowered if the first copulation of TSM female lasted longer (Potter *et al.*, 1976a, b). Shih and Pei (1995) and Shiue *et al.* (1995) reported that more than 90% of TSM females mated two or more times but increasing the number of copulations did not result in high female proportions in the offspring. Mated females produced a higher number of offspring

than virgin females, while virgin females produced a greater number of daily progeny in the early stages of the oviposition period than mated ones (Wrensch and Young, 1975). A high incidence of heterospecific copulation between TSM and KSM and a high number of secondary or multiple conspecific and heterospecific copulations between these two species were reported by Ozawa and Takafuji (1987) and Shiue *et al.* (1995).

The influence of the ineffective second copulation or heterospecific copulation on reproductivity is therefore studied here. In other words, does heterospecific copulation affect the insemination or fertilization of eggs? Do preferences of TSM or KSM females exist in selecting their first mating partner conspecifically and why does the male prefer a conspecific female? Do TSM and KSM males experience different mating potentials (number of matings) between conspecific females and heterospecific ones? The evidence of transferring spermatophore from the male to the female during conspecific or heterospecific copulation is also morphologically studied to determine whether eggs are actually fertilized and to verify the mechanical isolation mechanisms between heterospecific copulation and ineffective copulations of the second or multiple matings.

Materials and Methods

Material preparation

Two-year-old laboratory colonies of TSM and KSM were reared on 6" potted lima bean plants *Phaseolus vulgaris* L. at $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH, and 12h D. A 9 cm petri dish contained a 7 cm dia. X 1 cm cotton pat covered with a 7 cm dia. filter paper and a 4 cm dia. lima bean leaf disc. Water was added daily to the petri dish up to the level of the filter paper to prevent the mites from escaping and to maintain the leaf disc freshness. The petri dish with the cotton pat, filter paper, and

leaf disc is hereafter called 'leaf-rearing-disc, or LRD' and the leaf surface of the disc in the LRD is called the 'arena'. Twenty-five 3- to 6-day-old female and male of TSM and KSM from a stock colony were individually transferred onto each arena for 24 hours of egg laying by the females, and then the females and males were removed. When the eggs developed into teleiochrysalises they were individually transferred onto a new LRD to obtain virgin females which were used to lay haploid progeny. Whenever a pairing of a female teleiochrysalis with a detained male was found in an LRD, both were transferred onto a new LRD for copulation and then the male was removed. These inseminated females were kept on the LRDs to lay eggs which would develop into teleiochrysalises thus producing virgin females and unmated males of known ages. These teleiochrysalises, virgin female and unmated male adults are hereafter called 'test batches'.

Specific female-guarding and mate-first strategy of a male

A total of 25 either TSM or KSM female deutonymphs from test batches were individually transferred onto each LRD. When the deutonymph developed into a teleiochrysalis, either a conspecific or a heterospecific male was added to the arena. Whenever the male was found to perform female-teleiochrysalid-guarding, an additional 12-hour-old conspecific virgin female was added to the male. The male and the female teleiochrysalis were removed 1 hour after transfer of the second virgin female. The mating and copulation between the detained male with the female teleiochrysalis or with the intruder rival female were determined by assessing the female progeny produced by the intruder about 8 to 10 days later. Following the same procedures, a virgin female, instead of a female teleiochrysalis was added with a conspecific or heterospecific male. Then a conspecific virgin

female was also transferred onto the LRD with the previous female. Both the transferred male and female were removed 1 hour after introduction of the second virgin female. The assessment of mating and copulation of the second female with the detained male were determined from the female progeny produced by the intruder 8-10 days later.

Mate selection by TSM and KSM

1. The second copulation preferences of TSM and KSM females A 12-hour-old virgin TSM or KSM female was transferred onto one of 30 sets of LRDs with a 2- or 3-day-old conspecific or heterospecific male for mating and copulation. After the first mating, the male was removed and another conspecific or heterospecific male to the removed male was added onto the arena for 30 min. Any mating and copulation of the second male with the once-mated female were recorded to estimate the second conspecific or heterospecific mating frequency of the female.

2. Copulating preferences of a TSM or KSM male in the presence of females of both species Using similar procedures, both TSM and KSM female-deutonymphs from test batches were transferred onto an LRD to obtain uniquely developing teleiochrysalises. Whenever both species of female adults were found in the arena, a young and unmated TSM or KSM male was then added onto the arena to test and to record the duration of first mating pair and the species of the copulating female. Female progeny from mated females were recorded in the first 3 days of the oviposition period in order to estimate the sex ratio.

3. Copulating preferences of and competition between TSM and KSM males in the presence of either or both species of females A 12-hour-old TSM or KSM virgin female was transferred onto an LRD arena, and then both

young (≤ 2 -day-old) and unmated TSM and KSM males from test batches were placed onto the arena. Each male mating with a conspecific or heterospecific female within 30 min was individually recorded. By the same procedures, both a TSM and a KSM virgin female were kept on an LRD with young and unmated males of both species. The mating and copulation competition between the rival males and pairing females were determined for 30 min after the transfer of males onto the arena.

Effect of copulation duration on the sex ratio of progeny

Twenty 12-hour-old TSM and KSM virgin females were individually transferred onto an LRD with a conspecific or a heterospecific 2-day-old unmated male. The mating and copulation duration of each pair was observed and recorded under a 20-power dissection microscope. By the same procedures, but after removal of the mated male, another once-mated and conspecific male to the former rival one was transferred onto the LRD for observation of secondary mating and copulation duration of the female. Another 16 sets of LRDs were individually stocked with a 12-hour-old virgin TSM or KSM females and a conspecific 2-day-old unmated male. The pairing and copulation of each pair on each LRD were interrupted at 30, 60, 90 and 120 sec with a 00 camel brush. The females with different copulation durations were then separately transferred onto an LRD for 3 days of egg laying and finally the females were removed. LRDs with eggs were kept in a growth chamber and the female sex ratios of progeny were estimated 10 days later.

Mating and copulation potential of TSM and KSM males

Twelve sets of LRDs consisted of 16 one-day-old virgin TSM or KSM females and a 3-day-old unmated male. After 24

hours, the males were removed while the females were individually transferred onto a new LRD. The females were removed again, after 3 days of egg laying, from the LRDs. The numbers of successful copulations and inseminations of females by a male in a 12-hour period were estimated from those females producing female progeny.

Spermatophore transfer

Conspecific and heterospecific copulation was studied between an unmated male and a young virgin TSM or KSM females individually paired on an LRD. The copulation between the pair was observed and results assessed under the microscope (20X) after pairing. The males and females which had copulated were individually submerged in formalin-alcohol fixation (70% alcohol: formalin conc.: glycerol acetic acid=100: 5: 5) for 12-24 hours. The specimens were then dehydrated with an alcohol series and were kept in a solution of 1 part anhydrite alcohol and 1 part paraffin (56-58°C m.p.) for 12-14 hours. The specimens were finally embedded in pure paraffin in an oven at 60°C for 12-24 hours. The paraffin-embedded specimens were microtomed into 4 μ m slides with a Leica 2035 microtome and the slided sections of the specimen were kept on 30°C hot-spreader-plate for 2 hours and the de-waxing process was performed. After dyeing with haematoxym and Eosin the specimens were examined under the microscope for sperm in the seminal receptacle of the female.

Results and Discussion

Heterospecific copulations between TSM and KSM

1. Copulation between an unmated male and virgin or mated female

An unmated 2- to 3-day-old male successfully copulated 96.7% of the time

with either conspecific or heterospecific 12-hour-old virgin females in 30 min. The copulation rates of the second mating of a female with conspecific unmated 2- to 3-day-old males immediately after her first mating were 66.7% for TSM and 69.0% for KSM, while the rates for second matings of females with heterospecific unmated males after their first conspecific mating were 36.7% for TSM and 13.3% for KSM (Table 1). Therefore the frequencies of TSM females mating again with second conspecific males were higher than with heterospecific males. Shih and Pai (1994) reported that a TSM female mated and copulated two or more times during her life. They stated that, besides the first copulation, the second or multiple copulations were not effective for female insemination and egg fertilization. The second and multiple copulations of a female did not increase the egg fertility rate or the survival rates of diploid eggs during embryonic development of progeny (Boudreaus 1963; Helle 1967; Overmeer 1972; Feldmann 1977; Potter 1978; Potter and Wrensch 1978; Shih and Pai 1994), copulation was not observed and a rapidly increasing population would not be obtained either. The second conspecific copulation having higher frequency than heterospecific copulation with TSM and KSM females would favor production of diploid progeny and assure that males' sperm and energy would be transferred to and spent on conspecific females rather than on heterospecific ones.

Within 2 hours of pairing, although

KSM females produced almost the same copulation rates with unmated and free-walking heterospecific males at the first ($\chi^2=2.023$, $df=1$) and the second mating ($\chi^2=0.077$, $df=1$) as TSM females did (Table 1), detained KSM males (72.2%) exhibited a greater tendency than TSM males (28.6%) to release themselves from the guarded heterospecific female to first mate with an intruding conspecific female (Shiue *et al.*, 1995). The frequency of KSM males (66.7%) releasing themselves from conspecific guarding to first mate with a conspecific female was not significantly higher than that for TSM males (47.8%) ($\chi^2=2.478$, $df=1$) (Shiue *et al.*, 1995). Therefore the chances of conspecific copulation from detained males on heterospecific and conspecific females of TSM ♂ × KSM ♀ and TSM ♂ × TSM ♀ pairing are not significantly different from those males with KSM ♂ × TSM ♀ and KSM ♂ × KSM ♀ pairing, if both the species and population are ecologically homologous. The tandem-detention guarded males showed a first priority to mate with guarded females. A very low frequency of secondary copulation of a TSM or KSM female was found after having a conspecific copulation (Table 1). With heterospecific guarding by a male, the intruding conspecific KSM females experienced a higher copulation rate to the guarding KSM males than the rate of TSM males releasing from guarding to mate with TSM females (Shiue *et al.*, 1995). Consequently, KSM and TSM females exhibit better fitness in mating behavioral responses than their males do, but their males

Table 1. Influences of interspecific and intraspecific mating sequences of *T. urticae* (TSM) and *T. kanzawai* (KSM) on fertility rate of offspring at a half hour mating duration

Female species	Male species		N	Male mating rates (%)		Fertility rates (%)	
	1st mating	2nd mating		1st mating	2nd mating	1st mating	2nd mating
TSM	TSM	KSM	30	100 %	13.3 %	100	100
	KSM	TSM	30	100 %	66.7 %	—	100
KSM	TSM	KSM	30	96.7 %	69.0 %	100	100
	KSM	TSM	30	100 %	36.7 %	—	100

decide the mating preference to conspecific rather than heterospecific females. It is logical to infer that the selection favors the female of a species to avoid extra consumption of energy in ineffective copulation and favors the male to perform a higher rate of conspecific second copulations which is induced by the male instead of the female. The results reasonably explain why the KSM population is usually dominant in the field when KSM and TSM coexist in the same habitat in central Taiwan.

2. Preference of an unmated male to mate with a conspecific or a heterospecific virgin female

Under conditions of the presence of both virgin TSM and KSM females, the rate of first mating of a female with a conspecific male was 55.2% (32/58) for unmated TSM males which was not significantly different from that of KSM males (57.6%, 34/59) ($t=0.51$, $p=0.25$) (Table 2). There was no significant difference between unmated males copulating with either conspecific or heterospecific TSM and KSM females ($\chi^2=1.914$, $df=1$). In 24 hours of detention of an unmated male with a female in an arena, 9.4% (6/(58+6)) of TSM and 11.6% (8/(59+8)) of KSM females were not copulated with nor inseminated by conspecific males. A high percentage of non-insemination and/or non-copulation in TSM and KSM females may result from limitations of conspecific communication and/or identification or from the species regulation within populations

to maintain a certain level of haploid male progeny, all of which indicate some important questions to be investigated by further experiments.

3. Copulation competition of an unmated male to conspecific and heterospecific females

The total copulation rates of TSM and KSM males with conspecific and heterospecific females were 100% which indicated that the males of these two species have a high searching ability. Since males were found to copulate with females within 3~5 seconds after their transfer onto the arena, the actual practice of transferring males onto the arena were, in accordance with the female species, done by alternating conspecific and heterospecific males to avoid bias due to the sequence of introduction. Further, the second male was introduced at the same distance as that of the first male from the female. Before introducing the second rival male onto the arena, any set up was replaced by a new one if the female had already mated with the first transferred male. The conspecific and heterospecific copulation rates between TSM females and TSM or KSM males were both 50% (15/30), however, KSM females produced a higher rate in conspecific than heterospecific copulation (63.3% (19/30) and >36.7% (11/30)) ($\chi^2=4.374$, $p=0.05$) (Table 3). During pairing, the frequency of interference and disturbance from a rival male was high since (1) the TSM males interrupted 80% (12/15) of heterospecific detained males

Table 2. Interspecific and intraspecific mating preferences of males of *T. urticae* (TSM) and *T. kanzawai* (KSM)

Male	N	First-mated female species		Sex ratio ¹ (♀/(♀+♂))		No. of unfertilized females
		TSM	KSM	TSM	KSM	
TSM	58	32	26	0.64	0	6
KSM	59	25	34	0	0.55	8

1. Sex ratio was calculated only from the offspring produced in the first 3-day oviposition period of the parental female.

Table 3. Interspecific and intraspecific disturbance or replacement rate of *T. urticae* (TSM) and *T. kanzawai* (KSM) males in the presence of only one virgin female

Female species	N	Mating partner	Rival male	Disturbance rate	Replacement rate
TSM	30	TSM	KSM	11 / 15	1 / 11
TSM	30	KSM	TSM	12 / 15	9 / 12
KSM	30	TSM	KSM	9 / 11	9 / 9
KSM	30	KSM	TSM	16 / 19	9 / 16
Mating pair species (♀ × ♂)	N	Conspecific rival male disturbance rate	Conspecific rival male replacement rate	Immediate 2nd mating rate	Non- disturbance rate
TSM × TSM	30	17 / 30	4 / 17	4 / 30	13 / 30
KSM × KSM	30	22 / 30	4 / 22	4 / 30	8 / 30

(KSM) and replaced 75% (9 / 12) of them before mating with the TSM females; (2) interruption rates on conspecific pairings and copulation between TSM males and females (73.3% (11 / 15)) were not significantly lower than those of females with the heterospecific detained males (KSM) (80% (12 / 15)), however only 9.1% (1 / 11) of conspecific detention males (TSM) were replaced, which was much lower than heterospecific pairing with a conspecific male to female (75% (9 / 12)) ($\chi^2=20.65$, $df=1$); (3) with conspecific detention between KSM males and females, 84.2% (16 / 19) of pairs were interfered with and interrupted, and 56.3% (9 / 16) of the males among them were replaced by heterospecific males (TSM); (4) of the pairing between TSM males and KSM females, 81.8% (9 / 11) of pairs were interfered with and interrupted by KSM rival males and all of these males (9 / 9) were replaced with conspecific rival males (KSM) (Table 3). Competition for the first mating position in order to have a chance to mate first with the guarded conspecific females involved conspecific rival males interrupting TSM pairs at a rate of 56.7% (17 / 30) with a 23.5% (4 / 17) replacement rate, while 73.3% (22 / 30) of KSM pairs were interrupted and 18.2% (4 / 22) of the males were replaced (Table 3). Subsequently,

after the first mating, 13.3% (4 / 30) of rival males of both species copulated with mated females within a few seconds (Table 3).

With the presence of a TSM female and a KSM female in a test arena, TSM males showed no preference for a conspecific or a heterospecific female for their first mating partner (13 / 31), while KSM males chose 13 and 12 of their first mating partner from conspecific and heterospecific females, respectively, out of 32 trials (Table 4). The results demonstrated that TSM and KSM males did not show preference in choosing to mate with a conspecific or a heterospecific female. In the tests of copulation of a TSM male in the presence of females of both species, 4 and 2 (4 / 13 and 2 / 13) conspecific and heterospecific matings, respectively, were interrupted by the rival heterospecific male with a total interference rate of 20.0 (5 / 25) (Table 4). Consequently, in the presence of both species of females the male shows a lower rate of interference than it does in the presence of only one species of females but with 2 males of the same or different species (Tables 3, 4). The replacement by a rival male was only demonstrated during heterospecific pairing or mating. The replacement rate by a TSM rival male was 1 / 13 and 2 / 12 for KSM males (Table 4), and the replace-

Table 4. Interspecific and intraspecific disturbance or replacement rate of *T. urticae* (TSM) and *T. kanzawai* (KSM) males in the presence of virgin females of both species

Rival male species	N	Rate of disturbance on ² copulation pairs (No. disturbed / No. tested pairs)		Rate of replacement on ³ copulation pairs (No. replaced / No. tested pairs)		No. of disturbed and non-copulating males
TSM ¹	31	4 / 13 (TSM ♂ × TSM ♀)	2 / 13 (KSM ♂ × TSM ♀)	—	1 / 13 (KSM ♂ × TSM ♀)	5
KSM ¹	32	3 / 12 (TSM ♂ × KSM ♀)	2 / 13 (KSM ♂ × KSM ♀)	2 / 12 (TSM ♂ × KSM ♀)	—	7

1. Both 13 con- and heterospecific copulation pairs of TSM females are tested for disturbance and replacement for the rival male of TSM, while 13 and 12 con-and heterospecific copulation pairs of KSM females, respectively, are tested for the rival males
2. Rate of disturbance of a rival male on copulation pair is indicated by 'No. of disturbance by rival of TSM ♂ or KSM ♂ / No. of tested copulation pairs'.
3. Rate of replacement of a rival male on copulation pair is indicated by 'No. of replacement by rival of TSM ♂ or KSM ♂ / No. of tested copulation pairs'.

ment rates of detained males were also lower than those of males in the presence of only one species of female with another conspecific or heterospecific male (Table 3).

TSM and KSM males exhibited a higher conspecific female guarding behavior and protected their female mating partners more aggressively than those in heterospecific detention. This high level of specific communication and affinity between conspecific males and females is therefore interpreted as species identity with which the males exhibit a highly aggressive and defensive guarding behavior of mating first to the guarded conspecific female. In spite of heterospecific mating and copulating, those mating and copulations were completely interrupted and replaced by conspecific rival males which revealed that, as to mating behavior, species isolation mechanisms exist between these two spider mites, in addition to genetic isolation during copulation (no sperm transference during heterospecific copulation). KSM males interrupted most heterospecific copulating mates (9/11) and competed successfully to mate with a conspecific female (9/9) (Table 3) which demonstrated that

KSM has an advantage in competition for population success. Consequently, KSM impedes TSM population growth by a strategy of wasting the heterospecific females' copulation chances, time and energy (Shiue *et al.*, 1995).

Conspecific and heterospecific copulation duration and behavior and their influence on progeny sex ratio

1. Duration and behavioral variation of copulation

Among 119 pairs of conspecific and heterospecific copulations between TSM and KSM, only one pair of a TSM male and a KSM female did not mate within 30 minutes in an arena (Table 5). Copulation duration of a TSM female mating with a conspecific or a heterospecific unmated male lasted 198.8 or 116.0 sec, respectively, and after 1-2 hours from the first copulation, the female mated again with a conspecific once-mated male and the second part of copulation duration lasted 164.8 sec (Table 5). The variations among the copulation duration between conspecific and heterospecific females with once-mated males were significantly different (Table 5). Copulation duration of KSM females mating with conspecific

Table 5. Interspecific and intraspecific copulation duration between *T. urticae* (TSM) and *T. kanzawai* (KSM)

Female species	Male	N	No. mating	Copulation duration (sec) ¹ (Mean (SD))
TSM	Chaste TSM	20	0	198.8 (30.7)a
	Chaste KSM	19	1	116.0 (42.8)c
	Once-mated TSM	20	0	164.8 (17.9)b
KSM	Chaste TSM	20	0	81.7 (35.1)d
	Chaste KSM	20	0	155.9 (50.7)b
	Once-mated KSM	20	0	106.2 (33.1)c

1. Means followed by different letters within the same column are significantly different at the 5% level by Duncan's new multiple range test.

unmated males (155.9 sec) was significantly longer than those mated heterospecifically (81.7 sec), while copulation duration of females mated with once-mated males within 1-2 hours after his first copulation lasted 106.2 sec which was shorter than that of the first copulation between conspecific pairs ($t=3.60$, $p=0.001$) but longer than that between heterospecific pairs ($t=2.27$, $p=0.025$) (Table 5).

Males engaged in conspecific copulation were not replaced by intruding rival males, no matter whether the intruder was conspecific or heterospecific, before the mating male had transferred spermatophore. The male mating with a conspecific female showed a highly aggressive defensive behavior toward its mate against intruding rival males. Although there was about 10% temporary interruption of the mates by the intruder, the mating male overruled and excluded the intruding rival males and reassumed mating immediately. After defending the female and excluding the rival male, the male performed a second part of copulation immediately after the interruption. This second part of copulation duration was much shorter than the first part, but was not significant enough to show the relationship between the duration of the first and the second parts of copulation. During heterospecific pairing and mating between TSM and KSM females and

males, the females exhibited some rejection behaviors against her mates by: (1) escaping, (2) bending and lowering her idiosoma to resist the entrance of the male from the posterior, (3) hitting or turning her body horizontally to prevent the male from entering the copulation position, and (4) facing the male to prevent him from entering the copulating position posteriorly. The heterospecific copulation duration of TSM and KSM females varied from 31 to 235 sec. No rejection or very few interruptions were found during conspecific mating and copulating and the female did not exhibit any avoidance behavior, abdominal bending and resisting, body turning or hitting the conspecific male mate.

2. Relation between conspecific copulation duration and sex ratio of progeny

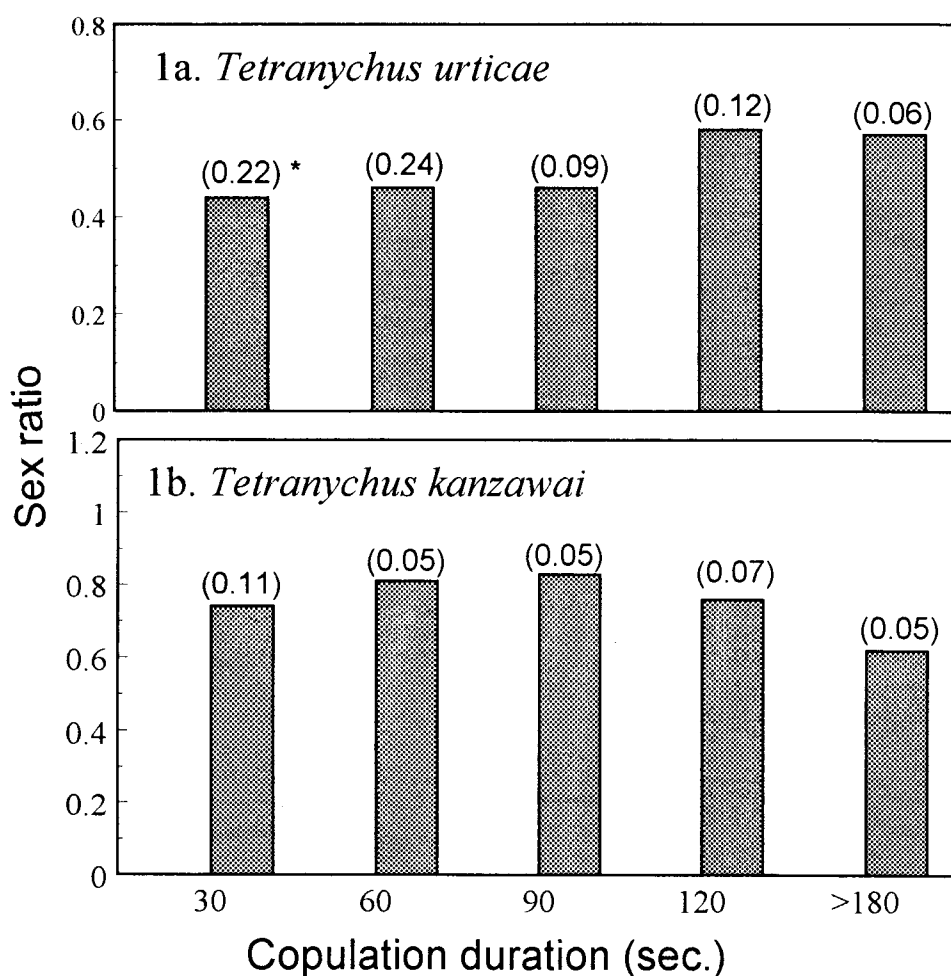
In the tests of interruption after 30, 60, 90, 120, and 180 sec of copulation between conspecific mates, the female sex ratio of progeny of TSM increased with copulation duration beyond 120 sec ($LSD=0.15$, $p=0.05$) but no such relationship was found for KSM (Fig. 1). Thirty seconds of copulation was enough time to produce 100% effective copulation, i.e., accepting transferred sperms and producing female progeny. A TSM female increased her reception of sperm volume from her mate in terms of enhancing her female progeny sex ratio with prolonged

copulation beyond 30 sec but KSM females did not (Fig. 1).

Effectiveness of copulation potential of an unmated TSM or KSM male

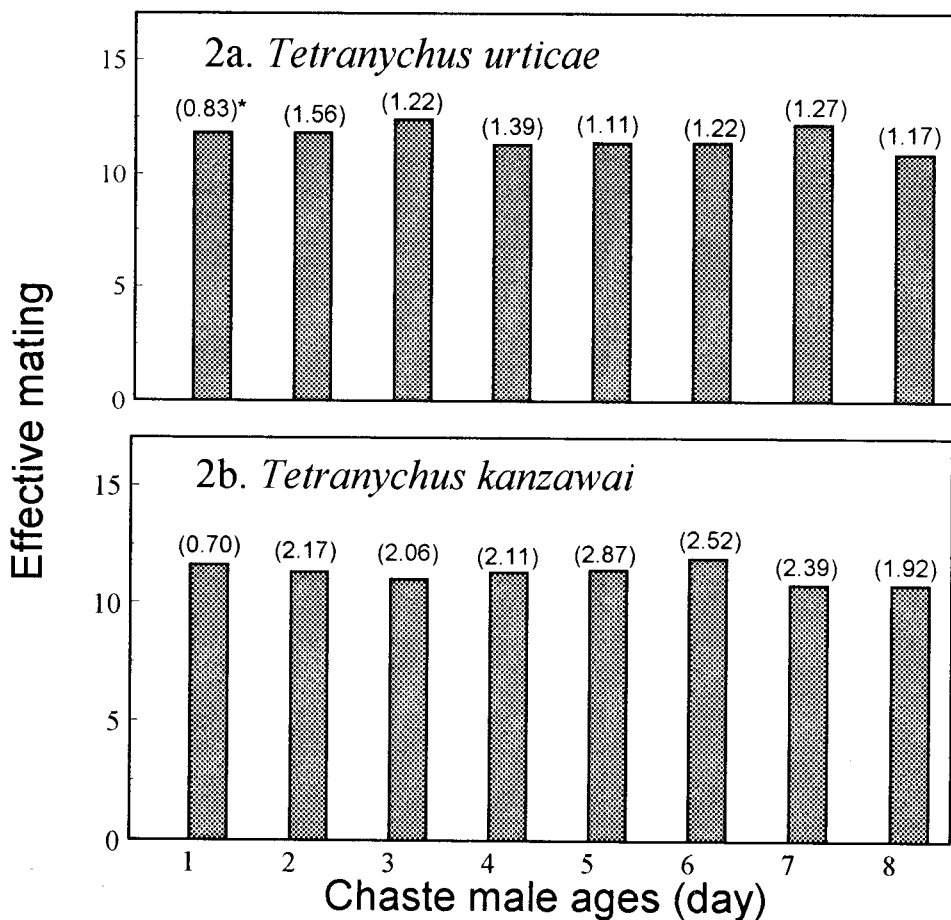
In the presence of 16 females of both TSM and KSM in an arena, the numbers of effective copulations of a male mating with virgin females in 24 hours did not significantly vary with male age (1 to 8 days old) ($LSD=2.01$, $p=0.05$) (Fig. 2). Shih and Pai (1994) reported that the age

of the unmated male (1, 4, 7 and 10 days old) affected the insemination rate (daily number of virgin females inseminated by an unmated male) and in the presence of only conspecific females, males produced 12.96, 9.73, 8.5, and 4.56 female-inseminations per male per day, respectively. The presence of heterospecific females seems effectively to enhance male sexuality to produce a higher rate of conspecific copulation and insemination than its absence. This strongly implies further



* Values in the parenthesis are SE's values

Fig.1. Effects of different copulating durations on the sex ratio of progeny in the first 3-day of oviposition of *Tetranychus urticae* and *Tetranychus kanzawai*.



* Values in the parenthesis are SE's values

Fig.2. Effects of chaste male age of *Tetranychus urticae* and *Tetranychus kanzawai* on mating effectiveness.

study on the significance of sexuality of spider mites which vary with the presence or absence of a conspecific or heterospecific rival male.

Spermatophore transference

Fig. 3a and Fig. 3b show the reproductive organs of virgin females with well-developed ovaries and seminal receptacles. The seminal receptacles of virgin TSM and KSM females are condensed and compacted with mono-layer epithelium cells and enclosed lumen.

Successful conspecific copulation and the transference of male spermatophore resulted in thinning of the epithelial layer and enlargement of the lumen due to a heavy load of sperm which were shown by heavily stained nuclei (Figs. 3c and 3d). The cross-section of the lumen of the seminal receptacle of a TSM or KSM female with heterospecific copulation were annularly or elliptically enlarged which seems to have been caused by the physical activity and intrusion of the

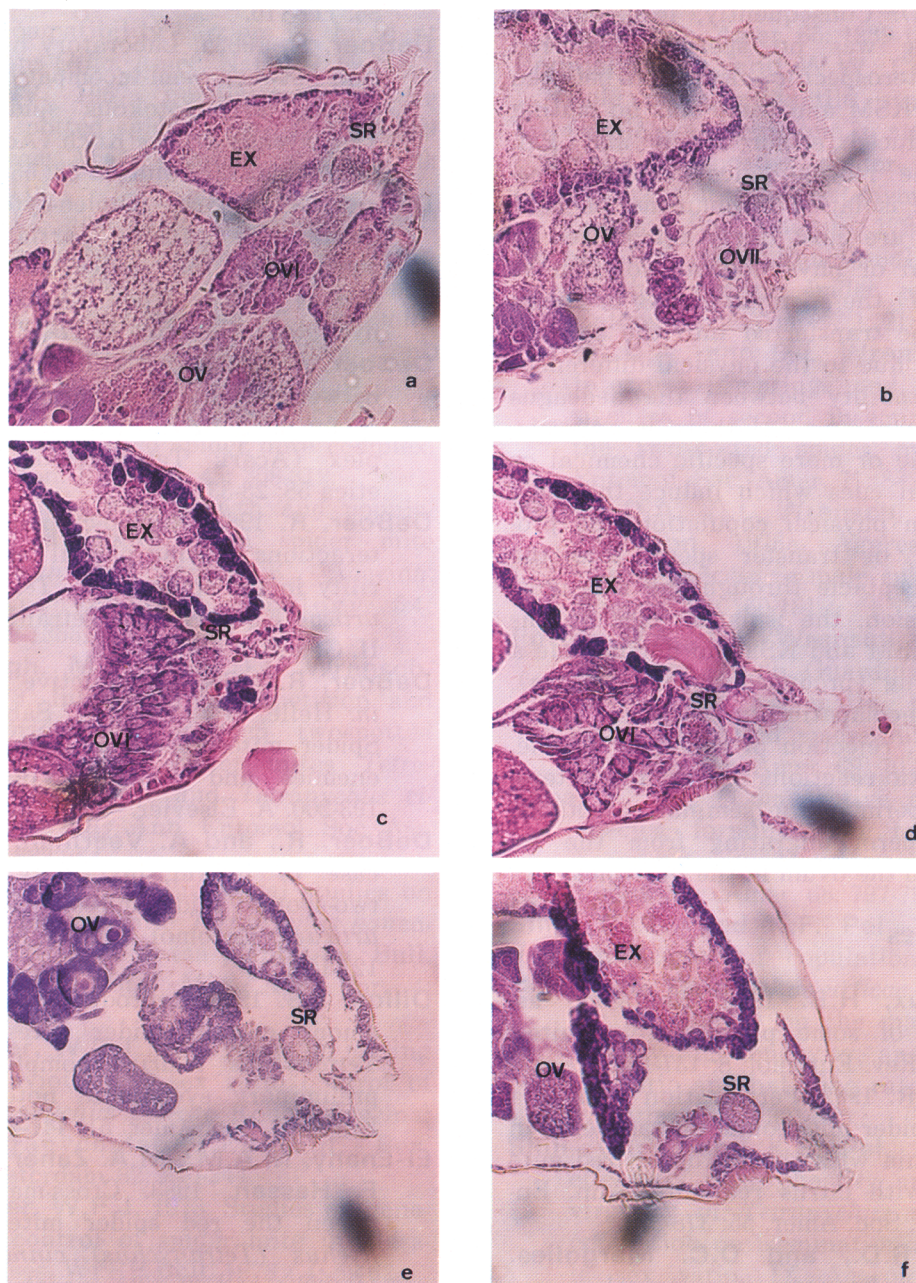


Fig.3. Spermatophore transfer between *T. urticae* and *T. kanzawai*. a. seminal receptacle constrictum of *T. urticae* virgin female; b. seminal receptacle constrictum of *T. kanzawai* virgin female; c. seminal receptacle constrictum of *T. urticae* after intraspecific copulation and sperm reception; d. seminal receptacle constrictum of *T. kanzawai* after intraspecific copulation and sperm reception; e. seminal receptacle constrictum of *T. urticae* after interspecific copulation; f. seminal receptacle constrictum of *T. kanzawai* after interspecific copulation. Abbreviations: E=egg; EX=excretory organ; OV=ovary; OV^I, OV^{II}= anterior and posterior oviduct; SR= seminal receptacle. (700 x)

aedeagus of the heterospecific male (Figs. 3e and 3f). Consequently, it is evident that there was no sperm transferred during heterospecific copulation between TSM and KSM and there was no mating plug produced either (Shih and Pai, 1994). The morphological evidence reveals that isolation mechanisms of TSM and KSM are most likely categorized as mechanical or physiological isolation as follows: (1) the spermatophores or sperm can not be transferred into the seminal receptacle due to the physical differences or unsuitability between the aedeagus and receptacle; or (2) there is an omission of one or more specific chemical or pheromonal cues which induce the male to perform physical copulation and produce and/or transfer sperm into the female receptacle through the aedeagus. In conclusion, the isolation mechanisms between TSM and KSM do not include a mating plug (produced by male mates), genetic incompatibility between sperm and ovum, rejection of sperm by female, antagonistic reactions against the heterospecific sperm in the seminal receptacle, or the sperm migrating from the receptacle to the ovum in the ovarioles.

References

- Boudreaux, H.B.** 1963. Biological aspects of some phytophagous mites. *Ann. Rev. Entomol.* 8: 137-153.
- Cagle, L.R.** 1962. Fertilization tests of a red spider mite found on raspberry in Virginia with the European red mite and with citrus red mite. *Ann. Entomol. Soc. Amer.* 55: 378-379.
- Collins, R.D., and D.C. Margolies.** 1991. Possible ecological consequences of heterospecific mating behavior in two tetranychid mites. *Exp. Appl. Acarol.* 13: 97-105.
- Collins, R.D., D.C. Margolies, and S. Rose.** 1993. Guarding behavior and reproductive isolation in two tetranychid mite species (Acari: Tetranychidae). *Ann. Entomol. Soc. Amer.* 86: 111-116.
- DeBoer, R.** 1982a. Laboratory hybridization between semi-incompatible races of the arrhenotokous spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Evolution* 36: 553-560.
- DeBoer, R.** 1982b. Nucleo-cytoplasmic interactions causing partial female sterility in the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Genetica* 58:17-22.
- DeBoer, R.** 1982c. Partial hybrid sterility between strains of the arrhenotokous spider mite, *Tetranychus urticae* complex (Acari: Tetranychidae). *Genetica* 58: 23-33.
- DeBoer, R.** 1983. Nucleo-cytoplasmic interactions causing partial female sterility in the spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). II. *Genetica* 61: 107-111.
- DeBoer, R.** 1985. Reproductive barriers. in: Helle, W., and M.W. Sabelis eds. Spider mites: their biology, natural enemies and control. Vol. 1 pp: 193-200. A. Elsevier.
- DeBoer, R., and A. Veerman.** 1983. A case of hybrid inviability in the two-spotted spider mites, *Tetranychus urticae*. *Entomol. Exp. Appl.* 34: 127-128.
- Dillon, L.S.** 1958. Reproductive isolation among certain spider mites of the *Tetranychus telarius* complex, with preliminary systematic notes. *Ann. Entomol. Soc. Amer.* 51: 441-448.
- El-Enany, M.A.M., M.A. Zaher, and A. F. Hassan.** 1983. Cross-mating between the red spider mites *Tetranychus (Tetranychus) cinnabarinus* (Boisd.) and *T. (T.) cucurbitacearum* (Sayed). *Z. Angew. Entomol.* 96: 1-3.
- Feldmann, A.M.** 1977. Mating competitiveness and the effect of X-rays and ageing on males of *Tetranychus urticae* (Acarina: Tetranychidae) in relation to genetic control. *Ent. Exp. Appl.* 21: 182-191.

- Appl. 21: 182-191.
- Grant, V.** 1966. The selective origin of incompatibility barriers in the plant genus *Gilia*. Amer. Nat. 100: 99-118.
- Helle, W.** 1967. Fertilization in the two-spotted spider mite (*Tetranychus urticae*: Acari). Ent. Exp. Appl. 10: 103-110.
- Hill, R.L., and D.J. O'Donnell.** 1991. Reproductive isolation between *Tetranychus lintearius* and two related mites, *T. urticae* and *T. turkestanii* (Acarina: Tetranychidae). Exp. Appl. Acarol. 11: 241-251.
- Keh, B.** 1952. Mating experiments with the two-spotted spider mite complex. J. Econ. Entomol. 45: 308-312.
- Mitchell, R.** 1973. Growth and population dynamics of a spider mite (*Tetranychus urticae* K., Acarina: Tetranychidae). Ecology 54: 1349-1355.
- Murtaugh, M.P., and D.L. Wrensch.** 1978. Interspecific competition and hybridization between twospotted and carmine spider mites *Tetranychus urticae* and *Tetranychus cinnabarinus*. Ann. Entomol. Soc. Am. 71: 862-864.
- Osakabe, M.** 1967. Biological studies on the tea red mite *Tetranychus kanzawai* Kishida in tea plantation. Bull. Tea Res. Sta. Min. Agr. For. 4: 35-156.
- Overmeer, W.P.J.** 1972. Notes on mating behaviour and sex ratio control of *Tetranychus urticae* Koch (Acarina: Tetranychidae). Entomol. Ber. Amsterdam 32: 240-244.
- Overmeer, W.P.J.** 1973. Studies on genetic control of spider mite in glass-houses. WPRP Bull. 1973 / 4: 59-62.
- Ozawa, K., and A. Takafuji.** 1987. Interspecific crosses between *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida. Jap. J. Appl. Entomol. Zool. 31: 51-54.
- Penman, D.R., and W.W. Cone.** 1972. Behavior of male twospotted spider mites in response to quiescent female deutonymphs and to web. Ann. Entomol. Soc. Amer. 65: 1289-1293.
- Penman, D.R., and W.W. Cone.** 1974. Role of web, tactile stimuli, and female sex pheromone in attraction of male twospotted spider mites to quiescent female deutonymphs. Ann. Entomol. Soc. Amer. 67: 179-182.
- Potter, D.A.** 1978. Functional sex ratio in the carmine spider mite. Ann. Entomol. Soc. Amer. 71: 218-222.
- Potter, D.A., and D.L. Wrensch.** 1978. Interrupted matings and the effectiveness of second inseminations in the twospotted spider mite. Ann. Entomol. Soc. Amer. 71: 882-885.
- Potter, D.A., D.L. Wrensch, and D.E. Johnston.** 1976a. Aggression and mating success in male spider mites. Science 193: 160-161.
- Potter, D.A., D.L. Wrensch, and D.E. Johnston.** 1976b. Guarding, aggressive behavior, and mating success in male twospotted spider mites. Ann. Entomol. Soc. Amer. 69: 707-711.
- Saba, F.** 1975. Comparative studies of species forming two tetranychid complexes in Morocco. Ann. Entomol. Soc. Amer. 68: 797-799.
- Shih, C.I.T., and K.F. Pai.** 1994. The significance of copulation ages of female *Tetranychus urticae* (Acarina: Tetranychidae) on the maternal fecundity and longevity, and progeny sex ratio and hatching rate. Chinese J. Entomol. 14: 291-305.
- Shih, C.I.T., and K.F. Pai.** 1995. The effects of male chastity and female virginity of *Tetranychus urticae* at copulation on isemination and female sex ratio. IX Acarology. (Columbus, Ohio) (in press).
- Shiue S.P., C.I.T. Shih, and K.F. Pai.** 1995. Guarding and arresting behavioural responses of males *Tetranychus urticae* Koch and *Tetranychus kanzawai* Kishida (Acarina: Tetranychidae) to the interspecific and

- conspecific females. Chinese J. Entomol. 15: 137-148.
- Takafuji, A.** 1988. Mating between diapausing and nondiapausing strains of citrus red mite, *Panonychus citri* (McGregor). Mem. Entomol. Soc. Can. 146: 181-189.
- Wrensch, D.L., and S.S.Y. Young.** 1975. Effects of quality of resources and fertilization status on some fitness traits in the two-spotted spider mite, *Tetranychus urticae* Koch. Oecologia 18: 259-267.
- Young, S.S.Y., D.L. Wrensch, and M. Kongchuensin.** 1985. Geographic variations and combining abilities in the two-spotted spider mite, *Tetranychus urticae*. Entomol. Exp. Appl. 39: 109-113.
- Zaher, M.A., E.A. Gomaa, and M.A.M. El-Elenany.** 1981. Cross-breeding between the green two-spotted spider mite, *Tetranychus urticae* Koch and *T. arabicus* Attiah. Z. Angew. Entomol. 92: 527-529.
- Received for publication April 25, 1995;
Revised manuscript accepted August 23, 1995.

雄性二點葉蟎 (*Tetranychus urticae* Koch) 及神澤葉蟎 (*Tetranychus kanzawai* Kishida) (Acarina: Tetranychidae) 對同種及異種雌蟎之交尾競爭及隔離機制

施劍瑩*、薛烜坪 國立中興大學昆蟲研究所 台中市國光路250號

摘 要

雄二點葉蟎(*Tetranychus urticae* Koch, TSM)及雄神澤葉蟎(*Tetranychus kanzawai* Kishida, KSM)於30分鐘內與同種雌(Conspecific female, CF)或異種雌(Heterspecific female, HF)之首次總交尾率均達96.7%以上;CF或HF與雄TSM第二次交尾率分別為66.7%及69.0%,與雄KSM則為36.7%及13.3%。KSM雌蟎之第二次與雄交尾率均低於TSM者,於護雌位雄KSM具較高“交尾第一”(雄蟎放棄護雌位與接近另一雌蟎先交尾再重獲護雌位)行為。首次,第二次交尾率及“交尾第一”行為,似可解釋KSM在田間與TSM共同存在一寄主時,常為優勢族群。此二種雌蟎共同存在時,雄蟎首與CF交尾率為55.2~57.6%,首先與CF或HF交尾率間無差異($\chi^2=1.914$, $df=1$)。雌蟎與同種雄交尾後,尚有10.3~13.6%未受孕率(雌蟎未接受精包,只產生雄性子代);因此,此二葉蟎似擁有控制族群性比之機制力。異種配對護雌時,80.0%雄KSM受情敵雄TSM干擾及75%的取代,81%雄TSM受雄KSM干擾及100%的取代;同種配對護雌時,73%被異種TSM雄干擾及91%被取代,另有84%被異種KSM雄干擾及56%被取代。同種配對被同種TSM雄情敵干擾及取代率分別為57%及24%,被KSM雄者分別為73%及18%;並且有13.3%交尾一次後雌蟎與“共同護雌”之雄蟎交尾。TSM及

KSM雄蟎對CF或HF交尾選擇無偏好，同種情敵雄對同種交尾對之干擾率甚低(20~23%)，其取代率遠低於對異種交尾對者。此種種顯示了此二葉蟎之種內專一而有效的訊息交換(Intraspecific communication)行為及親和力(Affinity)機制，而種間交尾時受異種雄情敵高干擾及取代率等，證實了此二葉蟎之種間行為隔離機制之存在。雄KSM借浪費TSM雌蟎交尾機會，時間及精力(能量)促進其族群發展優勢。

TSM第一次同異種間之交尾分別長達199秒及116秒，而且雄蟎能於1~2小時內可再次交尾，再次交尾時間平均達165秒，KSM交尾時間則分別為156, 82及106秒。同種交尾時，受同異種情敵雄取代率為零，即使受干擾而暫時中斷其交尾，但均迅速重獲其第二階段之交尾；此第二階段之交尾時間一般均甚短。異種配對時，雌蟎表現“逃避(escaping)”，“胴部下降(Indiosoma lowering)”，“旋體閃擊(Body turning and hitting)”及“面對(facing)”等行為以阻礙雄蟎進入交尾位，而同種配對時，則無此等阻礙及規避行為發生。異種間交尾時間長短變異極大(31~235秒)。TSM同種交尾時間及其精子(精包)傳遞量及雌性子代率成正比，但KSM則無此種顯著關係；而令此二種雌蟎受孕僅需30秒之交尾。雄蟎日齡對雌蟎受孕率間關係，尚待更進一步之探討。同異種間交尾後之雌蟎及處女雌蟎之生殖系統及受精囊解剖形態，證實異種間無精包或精子之傳遞；此二種葉蟎隔離機制應不包含交尾栓，精卵間遺傳或生理之不適性，雌蟎受精囊或血腔內等之排斥作用等，最可能係陽具與受精囊口或道之不適配性或雄蟎因化學訊息或生理因素不傳遞精子或精包給雌蟎。

關鍵詞：行為，干擾，取代，受精，精子傳遞。