Laboratory Experiments on Sexual Isolation between the two Species, *Ostrinia furnacalis* (Guenee) and *Ostrinia scapulalis pacifica* Mutsuura & Munroe (Lepidoptera : Pyralidae) [1]

Kunikichi YOSHIDA and Yutaka NISHIJIMA

(*Laboratory of Entomology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido* 080, *Japan*)

Received November 29, 1975

アワノメイガとフキノメイガ間における性的隔離に関する室内実験

吉田　国吉*・西島　浩*

Introduction

For a long while in Japan, *Pyrausta nubilalis* Hubner had been known as a single polyphagous pest attacking various crops such as *Zea mays* L., *Staria italica* Beauv., *Vigna sinensis* Hasskarl. *Humulus lupulas* L. and so on. However TAKEUCHI (1959) found that in the morphological and biological characters this species included two different types, the one having a preference for gramineous plants and the other for legumina etc. Thereafter, MUTSUURA and MUNROE (1970) identified the former with *Ostrinia furnacalis* (Guenee) and the latter with *Ostrinia scapulalis pacifica* Mutsuura & Munroe. In Hokkaido, the distribution of the two species is sympatric and it is therefore an interesting problem to determine what isolation mechanisms, if any, are present between these species. Thus the present laboratory experiment was carried out on sexual isolation between the two speccies.

Materials and Methods

Unless otherwise stated, the young larvae of *O. furnacalis* were collected from sweet corn fields in the Naganuma district in early August in 1972, and those of *O. scapulalis pacifica* were collected from adzuki bean fields in the Obihiro district at the same time. Each species was reared separately with a meridic diet (BECK *et al.*, 1968) during two generations in the laboratory, and the progeny of the inbred colony was maintained.

1) *Contribution No.* 70, *from the Laboratory of Entomology, Obihiro University.*

* 帯広畜産大学昆虫学研究室

In this case, two thousand newly hatched larvae were treated aseptically, and individually in cotton-plugged vials (11×16.4 cm) in an incubator under conditions of 16 hr-lighting at 26°C with ca. 70% R. H. The pupae obtained were sexed and trnsferred into transparent plastic cups of which the inside was covered with a sheet of paraffin paper. The newly emerged males and females were kept separately for two days under conditions of 16 hr-lighting at 26°C with ca. 80% R. H. Then the two-days-old moths were used for all experiments under conditions of both artifical and natural lighting. In the experiments, the moths were put into petri dishes(6×8.5cm) with a sheet of wetted filter paper on the bottom and with a sheet of paraffin paper on the inside or into wire cages(23×23×27cm). With the aid of a red electric light, observations were made at intervals of 15 minutes in the laboratory conditions (ca. 24° C and 70% R.H.).

Results

Intensity of isolation

Just before the beginning of the experiment, 20 unmated males of *O. scapulalis pacifica* were caged with 20 virgin females each of *O. scapulalis pacifica* and *O. furnacalis*, whlie 20 unmated males of *O. furnacalis* were caged with 20 virgin females each of *O. scapulalis pacifica* and of *O. furnanalis*. These two tests were repeated 5 times. In order to simplify the discrimination of the species, the females of *O. scapulalis pacifica* were marked by cutting off their wing end. After the moths were kept overnight in the cage, the females were killed and preserved in 70% alcohol for subsequent inspection. Whether the female mated or not was determined by inspecting the copulatory sac in her abdomen.

The results are summarized in Table 1. When the males of *O. scapulalis pacifica* were caged with the females of both species, 56% of the pairs which mated were intraspecific and only 9% were interspecific. When the males of *O. furnacalis* were caged with the females of both species, 57% of the pairs which mated were intraspecific and only 11% were interspecific. The coefficiences of isolation were calculated from the results of the tests according to the method of LEVENE (1949). The coefficience of isolation of *O. scapulalis pacifica* was 0.794, while that of *O. furnacalis* was 0.765. The coefficience of joint isolation between the two species was 0.779. From these results, it is clear that there is a significant sexual isolation between *O. scapulalis pacifica* and *O. furnacalis*.

Mating behaviour

Ino rder to observe mating behaviour, a virgin female and an unmated male of the same species were kept together in a petri dish under the condition of natural lighting in late July in 1972 and under the condition of artificial lighting in early October of the same year.

In mating behaviours, no marked difference between the two species could be found. Their typical pattern was as follows. At the beginning of dusk, the virgin females and unmated males were at rest with the antennae put along the anterior margin of the forewings. A few hours before copulation, both the female and the male repeated randomly

an intense flying, active wandering and resting. During the resting time, they turned their antennae upward and occasionally moved regularly and alternately forward and backward. As mating time drew near, the female moved her antennae continuously and bent ventrally the tip of her extended abdomen for a moment. After that, female repeated this movement a few times and then moved about actively again. The male turned his antennae or his head and antennae to the female which was resting near by. Before long the male stood his wings upward, vibrated them rapidly, extended the posterior part of his abdomen and bent it dorsally maintaining this movement, the stimulated male approached the resting female and positioned himself behind her. The male held the wing end of the female between his antennae and then moved to a position which was at right angles to the female, followed by bending the posterior part of his abdomen toward the abdomen of the female to copulate with her. Thereafter the male and female which were paired continued to rest with the antennae put along the anterior margin of the forewings (Fig. 1).

As a rule, the mating behaviour of lepidopterous insects is started in the calling position of the female, followed by the mating dance of the male which was stimulated by her movement, culminating in copulation. It has been said that a series of such behaviour

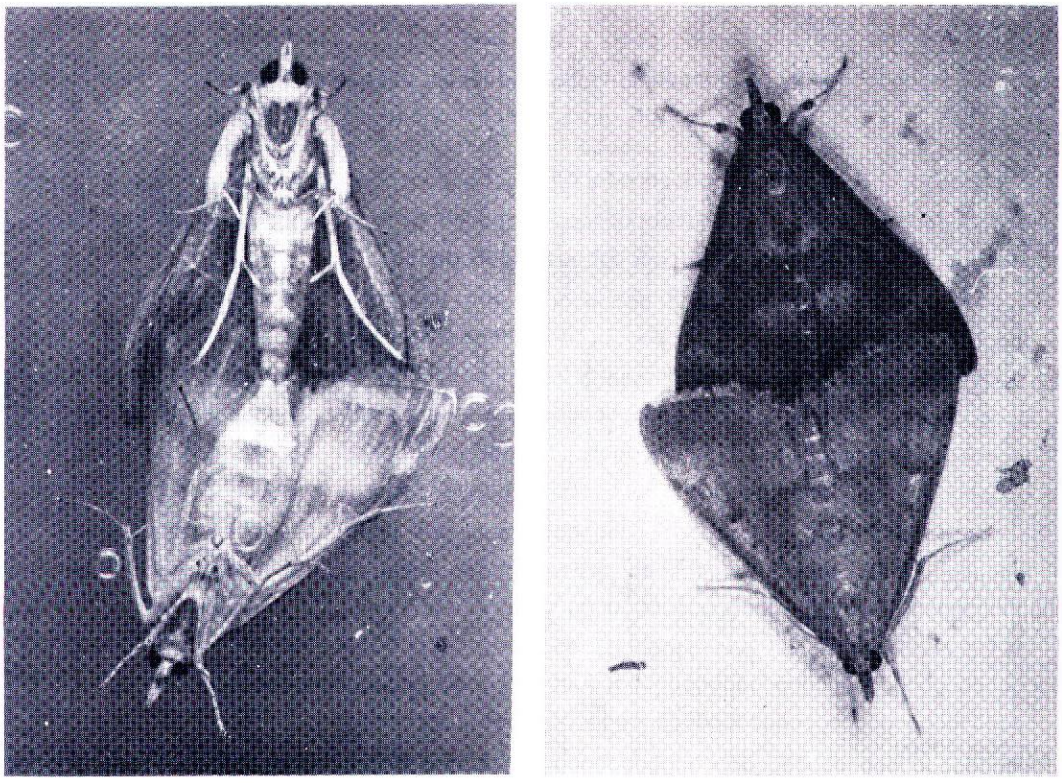

Fig. 1 Mating of the adult *Ostrinia scapulalis pacifica*: the upper part: male, the under part: female

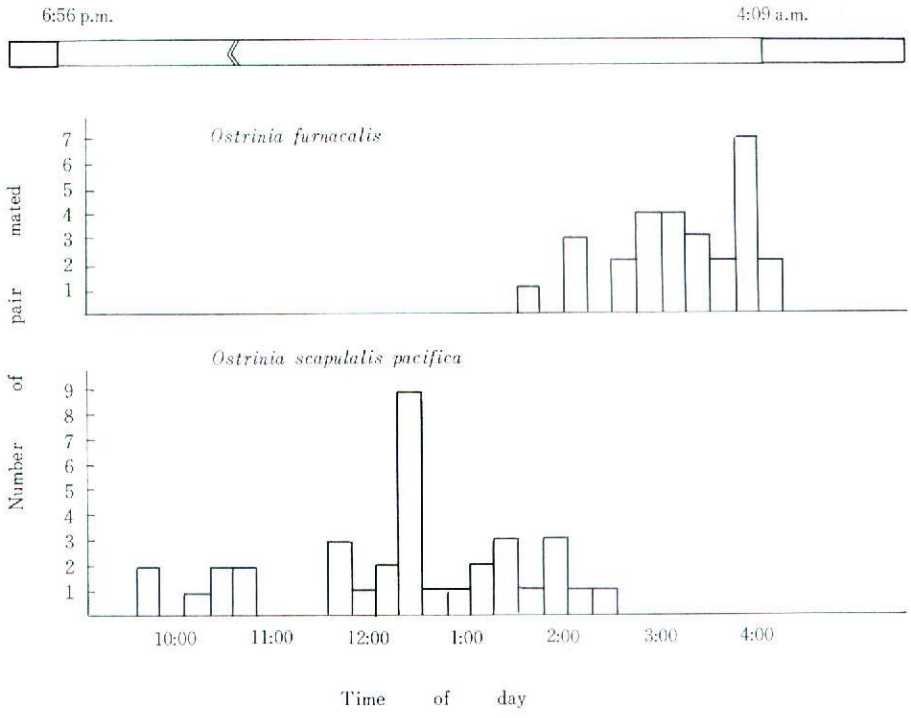

Fig. 2 Mating time of 2-days-old *O. scapulalis pacifica* and *O. furnacalis* under the condition of natural lighting.

is common to lepidopterous insects (JACOBSEN, 1965). The present observation indicated that the mating behaviuor of both species resembled that of many lepidopterous insects.

Mating time under the condition of natural lighting

The hibernating larvae of *O. furnacalis* were collected from withered corn stalks in the Naganuma district in early May in 1972. The mature larvae of *O. scapulalis pacifica* were collected from adzuki bean fields in the Obihiro district in late fall in 1971 and were made to hibernate under room temperature. The insects were reared through two generations under the condition of natural lighting at 26°C and ca. 80% R.H. Their progeny was used for the experiment. Immediately before the start of the experiment, a virgin female and an unmated male were put into a petri dish. The observation was performed from before sunset of the 25th of July to after sunrise of the 26th of July.

Fig. 2 shows the mating time of 35 pairs in *O. scapulalis pacifica* and of 28 pairs in *O. furnacalis*. The mean mating time of *O. scapulalis pacifica* was at 11 : 47 p.m. (4 hr and 51 min after sunset or 4 hr and 22 min before sunrise), while that of *O. furnacalis* was at 2 : 47 a.m. (8 hr and 51 min after sunset or 1 hr and 22 min before sunrise). That is, the mean mating time of *O. scapulalis pacifica* was 3 hr earlier than that of *O. furnacalis*. It was found also that the mating time of both species overlapped to some extent.

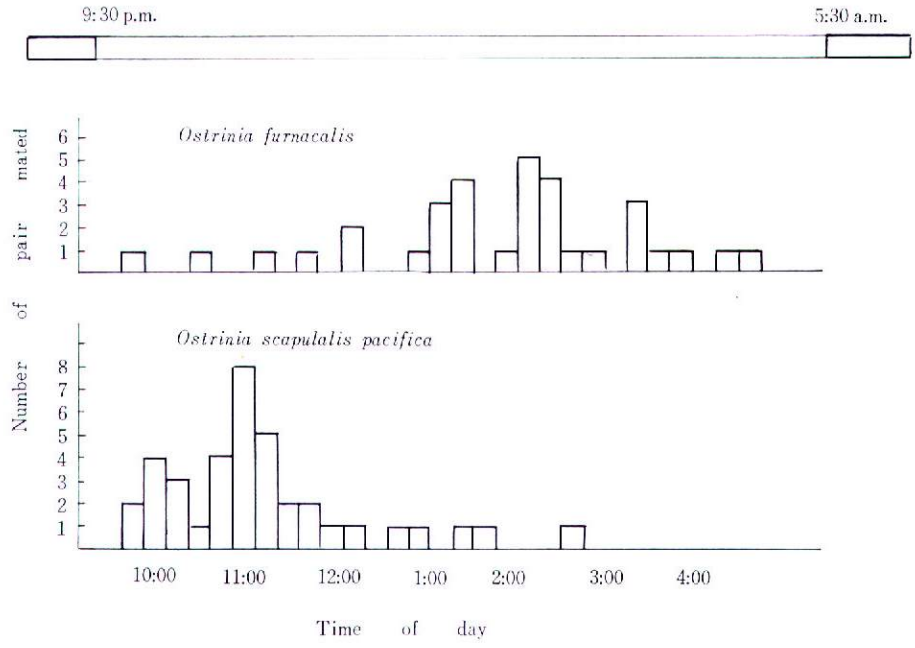

Fig. 3 Mating time of 2-days-old *O. scapulalis pacifica* and *O. furnacalis* under the condition of artifical lighting.

Mating time under the condition of artfical lighting

Under the condition of 8 hr dark-time (from 9 : 30 p.m. to 5 : 30 a.m. the next day) at 24°C with ca. 70% R.H., the experiments were performed on the 11th and 12th of October.

The results are shown in Fig. 3. Thirty nine pairs of *O. scapulalis pacifica* mated, while 28 pairs of *O. furnacalis* mated. The mating time of *O. scapulalis pacifica* was, on the average, 1 hr and 42 min after the beginning of the dark-time (6 hr and 18 min before the end) while that of *O. furnacalis* was, on the average, 4 hr and 25 min after the initiation of the dark-time (3 hr and 25 min before the end). The mean mating time of *O. scapulalis pacifica* was 2 hr 40 min earlier than that of *O. furnacalis*. These results were similar to those under natural lighting.

Number and time of mating of the two species

Three tests were carried out to clarify the number and time of the mating of the two species by means of the mate choice test. Twenty moths from each of the two species were caged in the following combinations. In the first tests : (a) the males and females of *O. scapulalis pacifica* were caged with the males of *O. furnacalis*, and (b) the males and females of *O. scapulalis pacifica* were caged with the females of *O. furnacalis*. In the second tests : (a) the males and females of *O. furnacalis* were caged with the males of *O. scapulalis pacifica*, and (b) the males and females of *O. furnacalis* were caged

with the females of *O. scapulalis pacifica*. In the third test : the males and females of *O. scapulalis pacifica* were caged with the males and females of *O. furnacalis*. When mating occurred, the mated pairs were removed from the cages and replaced by other virgin females and unmated males.

Table 1 Sexual isolation between the two species of *Ostrinia*.

Combination (♀) (♂)	Homogamic N	+	−	%	Heterogamic N	+	−	%	I	X	P
O.f. O.s.p. ×O.s.p.	100	56	44	56	100	9	91	9	0.794	50.3	0.001
O.f. O.s.p. ×O.f.	94	57	37	60.6	94	11	83	12.8	0.765	52.5	0.001

N: total number of female
+: number of mated female
−: number of unmated female
I: coefficience of isolation
O.f.: *O. furnacalis*
O.s.p.: *O. scapulalis pacifica*

Table 2 Number and mean time of the mating when the males and females of *O. scapulalis pacifica* were caged with the males or the females of *O. furnacalis*.

(a)

O.s.p. ♀ \	O.s.p. ♂	O.f. ♂
	12 *	4 **
	1:30	2:18

(b)

O.s.p. ♂ \	O.s.p. ♀	O.f. ♀
	15	4
	11:04	11:18

* number of pair which mated intraspecifically
** number of pair which mated interspecifically

Table 3 Number and mean time of the mating when the males and females of *O. furnacalis* were caged with the males or the females of *O. scapulalis pacifica*.

(a)

O.f. ♀ \	O.f. ♂	O.s.p. ♂
	9	3
	2:16	11:40

(b)

O.f. ♂ \	O.f. ♀	O.s.p. ♀
	13	2
	1:12	12:30

Table 4 Number and mean time of the mating when the males and females of *O. scapulalis pacifica* were caged with the males and females of *O. furnacalis*.

	O.s.p. ♀	O.f. ♀
O.s.p. ♂	10 12:34	1 1:45
O.f. ♂	1 12:00	8 1:54

The number and mean time of mating are summarized in Tables 2, 3 and 4. In the first test (a), the intraspecific mating time of *O. scapulalis pacifica* was at 1 : 30 a.m. on the average (Table 2(a)), while in the first test (b) the intraspecific mating time of *O. furnacalis* was at 11 : 04 a.m. on the average (Table 2 (b)). In the third test, the intraspecific mating time of *O. scapulalis pacifica* was at 12 : 34 a.m. on the average (Table 4). In the preceding exeriment, when a virgin female and unmated male of *O. scapulalis pacifica* were put into a petri dish, the mean mating time was at 11 : 12 a.m. under the condition of artifical lighting. From these results, it is clear that the intraspecific mating time of *O. scapulalis pacifica* is independent of the presence of the females of *O. furnacalis* but is delayed by the presence of its males. On the contrary, the intraspecific mating time of *O. furnacalis* was not affected by the presence of either the males or the females of *O. scapulalis pacifica* (Tables 3(a) and 4).

CAMBELL (1967) tried tests similar to those of the present study, using two related species, the spruce budworm *Choristoneura fumiferana* (Clem.) and the western two-year cycle budworm *C. biennis* Free., and found that the mating times both within and between species were quickened by half an hour when the males of *C. fumiferana* were caged with the males and females of *C. biennis*. He concluded that this phenomenon was due to the competition between the males of two different species. In the present study, it is obscure why the intraspecific mating time of *O. scapulalis pacifica* was delayed by the presence of the males of *O. furnacalis*. The number of matings was always much more in intraspecific mating than in interspecific mating, regardless of the species (Tables 2, 3 and 4). It is obvious that mating occurs within the same species with a significantly strong preference.

Duration of copulation

The duration of copulation of the two species was investigated under conditions of both artifical and natural lighting. The duration of the copulation of *O. scapulalis pacifica* was 101 min on the average (min. 60 min and max. 165 min) under the condition of matural lighting, and was 106 min on the average (min. 60 min. and max. 150 min) under the condition of artifical lighting, while that of *O. furnacalis* was 115 min on the average (min. 60 min and max. 195 min) under the condition of natural lighting, and was 127 min on the average (min. 75 min and max. 180 min) under the condition of

artifical lighting. In respect to the duration of copulation, there was no significant difference between the two species or the two conditions. It was ascertained that all the mated females used for the present tests laid fertilized eggs during the 2 days after mating. These results suggest that when *O. scapulalis pacifica* mated with *O. furnacalis*, the spermatophores of one species were delivered successfully to the female of another species.

Discussion

SMITH (1953) tried the mate choice tests by using the eastern spruce budworm *C. fumiferana* (Clem.) and the jack-pine budworm *C. pinus* Free. and found that there was a strong isolation between the two species. It should be noted that 82% of the pairs which mated were intraspecific and the isolation index was 0.65. CAMBELL (1967) stated that this strong sexual isolation brought about in a small cage would be attributable to the difference in the mating time of the two species.

In the present study, the mate choice tests showed the presence of a strong sexual isolation (the coefficience of joint isolation=0.779) between *O. scapulalis pacifica* and *O. furnacalis*. It was also found that the male always copulated with the female of the same species with an intense preference, and that the mating time differed markedly between the two species under conditions of both artifical and natural lighting. It is probable that the isolation between the two species is stronger in the natural population than in the artifical population (in the small cage tests), because of various spatial, ecological and other unknown factors in the field. In addition to these factors, the mating time must be an important factor in the sexual isolation between *O. scapulalis pacifica* and *O. furnacalis* Thus, the delivery of the genus between the two species may be so rare in the natural population that they are probably two reproductive populations independent of each other.

MATSUMOTO *et al.* (1965) reported an interesting result that a hybrid was produced between the two species, when one crossed these two species. It is sure that more detailed studies on the mechanism of the reproductive isolation in these closely related species may be of very great importance in the consideration of the process of sympatric speciation.

References

1) BECK, S. D., G. M. CHIPPENDALE and D. E. SWINTON (1968) Nutrition of the European corn borer, *Ostrinia nubilalis*. VI. A larval rearing medium without crude plant fractions. Ann. Ent. Soc. Amer. 61 : 459-462.

2) CAMPBELL, I. M. (1967) On coniphagous species of *Choristoneura* in North America. IV. Sexual isolation between three species. Cand. Ent. 99 : 482-486.

3) CHIANG, H. C., A. J. KEASTER and G. L. REED (1968) Differences in ecological reponses of three biotypes of *Ostrinia nubilalis* from the North Central United States. Ann. Ent. Soc. Amer. 61 : 140-146

4) ELLIS, W. O. (1925) Some lepidopterous larvae resmbling the European corn borer. J. Agr.

Res. 30 : 777-792.

5) GUTHRIE, W. D., RAUN, E. S., DICKE, F. F., G. R., PESHO and S. W., CARTER (1965) Laboratory production of European corn borer egg masses. Iowa State J. Sci. 40 : 65-83.

6) HEINRICH, C. (1919) Note on the European corn borer (*Pyrausta nubilalis* Hubner) and its nearest American allies, with description of larvae, pupae and one new species. J. Agr. Res. 18 : 171-178.

7) HIDAKA, T. (1972) Biology of *Hyphantria cunea* Drury (Lepidoptera : Arctiidae) in Japan. XIV. Mating behavior. Appl. Ent. Zool. 7 : 116-132.

8) HONMA, K. (1972) Studies on two forms of the smaller tea tortrix, *Adoxophyes orana* Fischer von Röslerstamm. Bull. Hort. Sta,. Japan, ser. C. 1-33 (in Japanese)

9) KIM, K. C., H. C., CHIANG and B. W, .BROWN (1967) Morphometric differences among four biotypes of *Ostrinia nubilalis* (Lepidoptera : Pyralidae). Ann. Ent. Soc. Amer. 60 : 756-801.

10) KLUN, J. A. (1968) Isolation of a sex pheromone of the European corn borer. J. Econ. Ent. 61 : 484-487.

11) KUWAYYAMA S. (1930) Studies on the corn borer, *Pyrausta nubilalis* Hubner, in Japan. Rperint from the report no. 25 of the Hokkaido Agricaltual Experiment Station, Japan. 140 pp. (In Japanese)

12) LEVENE, H. (1942) A new mesure of sexual isolation. Evolution 3 : 315-321.

13) MATSUMOTO, S. and T., SHIMAZAKI (1957) on the development of the European corn borer, *Pyrausta nubilalis* Hubner (Preliminary report) Res. Bull. Hokkaido Nat. Agr. Exp. Stat. 72: 22-27.

14) MATSUMOTO, S. and T., KUROSAWA (1961) Host-plants of *Ostrinia nubilalis* and *O. varialis* in Japan. Res. Bull. Hokkaido Nat. Agr. Exp. Stat. 82 : 80-85. (In Japanese)

15) MATSUMOTO, S., T., KUROSAWA and S., TAKEUCHI (1965) Ecological studies on *Ostrinia varialis* Bremer. II. Life history of *O. varialis* as compared with European corn borer. Res. Bull. Hokkaido Nat. Agr. Exp. Stat. 86 : 44 56. (In Japanese)

16) MAYR, E. (1942) Systematics and the origin of species from the viewpoint of a zoologist. Dover ed., New York. 334 pp.

17) METTLER, L. E. and T. G., GREGG (1969) Population genetics and evnlution. Prentice-Hall, Inc. Englewood cliffs New Jersey, U. S. A.

18) MUTUURA, A. (1952) On the morphological characters of the larvae of Japanese Pyraustinae. Bull. Naniwa Univ. ser. B. (2) 25-34. (In Japanese)

19) MUTUURA A., and E., MUNROE (1970) Taxonomy and distribution of the European corn borer and allied species: Genus *Ostrinia* (Lepidoptera: Pyralidae) Mem. Ent. Soc. Canad. 71: 112pp.

20) ONO, T., K., IYATOMI and T. SAITO (1972) Mating behavior of the potato tuber moth, *Phthorimaea operculella* Zeller. Jap. J. Appl. Ent. Zool. 16 : 51 53. (In Japanese)

21) SMITH. S. G. (1953) Reproductive isolation and the integrity of two sympatric species of *Choristoneura* (Lepidoptera: Tortricidae). Canad. Ent. 85 : 141-151.

22) SMITH. S. G. (1954) A partial breakdown of temporal and ecological isolation between *Choristoneura* species (Lepidoptera: Tortricidae). Evolution 8 : 206-224.

23) SPARKS, A. N., CHIANG, H. C., KEASTER, A. J., M. L., FAIRCHILD and T. A., BRINDLEY (1966) Field studies of European corn borer biotypes in the midwest. J. Econ. Ent. 59 : 922 927.

24) TAKEUCHI, S. (1959) Comparative studies on the corn borer attacking corn and bean. Res.

Bull. Hokkaido Nat. Agr. Exp. Stat. 74 : 80-86. (In Japanese)

25) TAMAKI, Y. (1959) Studies on nutrition and metabolism of the smaller tea tortrix, *Adoxophes orana* I. Aseptic rearing of the larva on synthetic diets. Jap. J. Appl. Ent. Zool. 3 : 286-290.

26) TAMAKI, Y. (1966) Mass rearing of the smaller tea tortrix, *Adoxophyes orana*, on a simplified artifical diet for successive generations (Lepidoptera: Tortricidae). Appl. Ent. Zool. 1 : 120-124. (In Japanese)

27) TAMAKI, Y., H., NOGUCHI and T., YUSHIMA (1969) Mating behaviors of the smaller tea tortrix, *Adoxophyes orana* Fisher von Röslerstamm, and evidence of sex pheromone production. Botyu-Kagaku 34 : 97-102. (In Japanese)

28) TAMAKI, Y., H., NOGUCHI and T., YUSHIMA (1969) Artifical of mating activity of the smaller tea tortrix, *Adoxophyes orana*, and a quantitative bioassay for the sex pheromone. Botyu-Kagaku 34 : 107-110. (In Japanese)

29) WONG, T. T. M. L., CLEVELAND and D. G., DAVIS (1969) Sex attraction and mating of lesser peach tree moths. J. Econ. Ent. 62 : 789-793.

Summary

The coefficiences of isolation calculated from the male multiple choice method indicated that there was a strong sexual isolation between *Ostrinia furnacalis* and *O. scapulalis pacifica*. In the mate choice tests, the males mated with the females of their own species with an intense preference. Under the conditions of both artifical and natural lighting, there was significant difference in the mean mating time of the two-days-old adult between the two species. The mating time of *O. scapulalis pacifica* was delayed owing to the coexistence of *O. furnacalis* males. In mating behavior and duration of copulation, no marked difference could be found between either of the species.

摘　　要

飼料作物などの大害虫であるアワノメイガとフキノメイガは形態的にきわめて近縁でかつ同所性分布を示す。両者の性的隔離機構の一端を知るため，人工飼料により無菌的に得た多数の成虫を使って室内実験をくり返した。その結果，両者の交尾の行動および継続時間にはほとんど差異を認めなかったが，雄の雌選好性および交尾時刻に著しい差異を認めた。また雄の multiple choice 法による実験結果は，両種間に強い性的隔離があることを示した。