Incipient Post-Copulatory Isolation and Morphological Divergence in the Melon Fly, Bactrocera cucurbitae

Author: Kasuya, Eiiti

Incipient Post-Copulatory Isolation and Morphological Divergence in the Melon Fly, *Bactrocera cucurbitae*

Eiiti Kasuya[1]

Entomological Laboratory, Faculty of Agriculture, University of the Ryukyus, Nisihara, Okinawa 903-01, Japan

ABSTRACT—Post-copulatory isolation and the difference in morphological traits between populations of the melon fly, *Bactrocera* (*Dacus*) *cucurbitae* on Okinawa and Isigaki Islands in the Ryukyu Archipelago, which shared the common ancestor in 1929 or later, were studied. The hatchabilities of eggs from inter-population crosses were lower than those from intra-population crosses. Of four morphological traits studied, two measures of wing length and width between eyes of females in the Okinawa population were larger than those of the Isigaki population. Measures of wing length and midleg length of males in the Okinawa population were larger than those of the Isigaki population. The degrees of sexual dimorphism in two measures of wing length and width between eyes in Okinawa population were larger than in the Isigaki population. There were differences between the populations in allometry of several pairs of the morphological traits.

INTRODUCTION

The melon fly, *Bactrocera (Dacus) cucurbitae* has expanded its distribution to the Ryukyu Archipelago in this century (for the history of invasion and control, see e.g. Iwahashi [6]). In 1919, this was first observed on Isigaki Island. After this, the fly expanded its distribution from southwest to northeast within the Ryukyu Archipelago. The fly invaded into Miyako Island in 1929, Kume Island in 1970, and Okinawa Island in 1972, respectively. As the population of the melon fly on Okinawa Island is considered to be descendants of immigrants to Miyako from Isigaki Islands, two fly populations on Okinawa and Isigaki Islands at the time of the present study (1989) had the common ancestor when the fly had invaded into Miyako Island (for further discussion of the timing of sharing the common ancestor, see Discussion). Therefore, their difference showed the divergent evolution during several decades. This is a kind of "natural experiments" (e.g. [11]). Comparison between the traits of the flies on the two islands enables to estimate the rate of evolution in this short scale of time in nature. This is the main aim of the present study.

There was another difference between the Okinawa and Isigaki populations (abbreviated O and I, respectively, hereafter) other than the difference of their natural habitats. Sterile Insect Release Technique (SIT, hereafter) has been applied to the population on Okinawa Island, but not to that on Isigaki. As SIT releases large number of artificially raised sterilized male flies to the field, response of the wild population to this artificial selection pressure is expected. This can provide opportunity to test hypotheses of sexual selection by field experiments of a large scale.

Though the melon flies on Okinawa and Isigaki Islands present the opportunity to measure the rate of evolution and response to sexual selection pressure in the field on a large scale, this was exploited only by Hibino and Iwahashi [4]. They showed the difference in female mate preference between I- and O-populations and hypothesized that this difference was caused by the application of SIT on Okinawa Island. Difference in other traits between I- and O-populations has not been studied, however. In the present study, I report the difference in several morphological traits of adult flies and post-copulatory isolation between the two populations.

MATERIALS AND METHODS

Melon fly

I-population was sampled at Omoto and Mikawa on Isigaki Island in May, 1989 (P flies). I used F1 (the offspring of P flies) and F2 (the offspring of the F1) flies maintained under laboratory conditions (25°C and natural day length) for experiments. Eggs were collected by sliced or crushed pumpkin because P and F1 flies oviposited only a small number of eggs into pumpkin juice that was used in the usual technique of rearing.

O-population was sampled by Okinawa Prefectural Fruit Fly Eradication Office in the Tyubu district of Okinawa Island in May, 1989 (P flies). F1 and F2 flies maintained under the same conditions as those of I- population were used for experiments.

I used F1 flies to test hatchabilities of eggs from intra- and inter-population crosses, and F2 flies for the morphological comparison of the two populations.

Post-copulatory isolation: hatchability

F1 adult flies from 18 to 28 days after emergence were used to test post-copulatory isolation between the two populations. An unmated female and an unmated male were put in a small plastic cup with a moist filter paper for water supply and food [5]. There were 4 combinations of crosses: O female ×O male, O female ×I male, I female ×O male, and I female ×I male. Thirty pairs of each cross were prepared. Copulation was checked every night with a red light, for 11 nights. Because *B. cucurbitae* does a prolonged copula-

tion [12], a single observation during a night was sufficient to determine copulation status.

Females that were observed to copulate was used for the following two investigations of hatchability of eggs. In the first one, the male in a cup was removed and the eggs oviposited by the female into crushed pumpkin under the cup were collected (the cup had small holes at the bottom). Eggs were put on a moist gause and filter paper in a petri dish. The number of eggs which hatched was counted on the next day (27–31 hr after setting of the eggs in a petri dish) and 2 days later (51–55 hr after setting). Eggs of *B. cucurbitae* usually hatch on the next day after oviposition. No egg hatched between the first and the second observations in the present study.

After this, all the females observed to copulate of a given cross were put in a cage (20 cm×20 cm×20 cm) with pumpkin slices (the second investigation of hatchability). The number of eggs that hatched was counted in the same manner as the first one.

Morphological comparison

I measured morphological traits of F2 adult flies of both populations. 0.6g (wet weight) pumpkin per egg was given as larval food to control the larval food condition that could affect the resultant adult body size. I measured four morphological traits, that is, the length of midleg (the tibia length of midleg), wing length 1 (the distance between humeral vein and the point where R_{2+3} reached the costal margin), wing length 2 (the distance between humeral vein and the point where A1 reached wing margin), and length(width) between eyes. The morphological traits of adult flies were measured with a ruler on the screen of Nikon profile projector model 6CT2® to the nearest 0.05 mm.

RESULTS

Hatchability

All the females that had been observed to copulate oviposited at least one egg that hatched. This showed that all copulated females were inseminated. Mating frequency and the number of eggs deposited per female were not different among crosses (chi-square=0.181, $P>0.9$ for mating frequency, and H'=1.239, $P>0.74$ in Kruskal-Wallis' test for the number of eggs per female). In the first investigation (Table 1a), hatchabilities of eggs obtained from intra-population crosses were significantly higher than those of inter-population ones (Mann-Whitney's U test, $U=25$ and 13.5 in O-population *vs* inter-population crosses, $U=14.5$ and 9 in I-population *vs* inter-population crosses, $P<0.05$ in all the comparison). In the second investigation (Table 1b), hatchabilities of eggs obtained from intra-population crosses were also significantly higher than those of inter-population ones (Fisher's exact probability test, O-population *vs* inter-population crosses, $P<0.05$ and $P<0.001$, I-population *vs* inter-population croses, $P<0.001$ and $P<0.01$).

The hatchabilities of eggs obtained from the two intra-population crosses were not significantly different ($U=73.5$ in Mann-Whitney's U test, $P>0.05$ and Fisher's exact probability test, $P>0.5$). Those of the two inter-population ones were also not significantly different ($U=59.5$ in Mann-Whitney's U test, $P>0.05$ and Fisher's exact probability test, $P>0.1$). The lower hatchabilities of inter-population crosses showed that there was a weak but significant post-copulatory isolation between O- and I- populations.

Difference of morphology between two populations

I compared morphological traits of I- and O- populations in two manners, i.e., comparison of each trait and that of relationship between a pair of traits by allometry.

Simple comparison

The differences in the four morphological traits of each sex between populations (Table 2) were tested by Mann-Whitney's U test and the sequential Bonferroni method [10]. Because I studied 4 morphological traits at once, the conventional procedure of statistical tests gives higher risk of type 1 error. I used the sequential Bonferroni method to avoid this risk.

In both sexes, three traits of O-population were significantly larger than those of I-population. There was not a significant difference in the length of midleg in females, and in width between eyes in males. As shown in Table 2, the

TABLE 1. Hatchability of eggs obtained from 4 crosses

(a) Investigation 1: single females

	Mean hatchability±SD	
O female×O male	94.8±5.9%	(14)
O female×I male	80.8±7.4%	(13)
I female×O male	82.6±10.3%	(12)
I female×I male	94.6±5.4%	(11)

(b) Investigation 2: mass females

	No. of hatching eggs	No. of total eggs	Hatchability	
O female×O male	140	149	94.0%	(13)
O female×I male	121	150	80.7%	(13)
I female×O female	166	191	86.9%	(12)
I female×I male	148	154	96.1%	(11)

Number in parenthesis shows the number of females

TABLE 2. Comparison of 4 morphological traits (mm, Mean±SD)

	O female (n=33)	I female (n=35)	Mann-Whitney U-test
Length of midleg	1.93±0.09	1.91±0.10	z=0.80
Wing length 1	5.50±0.13	5.21±0.21	z=5.98***
Wing length 2	2.96±0.06	2.79±0.15	z=4.98***
Width between eyes	0.84±0.04	0.73±0.04	z=6.51***
	O male (n=32)	I male (n=41)	Mann-Whitney U-test
Length of midleg	1.90±0.05 (1.02)	1.84±0.07 (1.04)	z=3.34**
Wing length 1	5.02±0.11 (1.10)	4.96±0.13 (1.05)	z=4.67***
Wing length 2	2.50±0.07 (1.18)	2.44±0.05 (1.14)	z=2.99**
Width between eyes	0.71±0.03 (1.18)	0.71±0.04 (1.14)	z=0.17

Number in parenthesis: ratio of mean of females to that of males.
** and ***: significant at the 1% and 0.1% level by the sequential Bonferroni method [10], respectively.

mean of each trait of females was larger than that of males. I used the difference between means of females and males as the measure of the sexual dimorphism of these traits in this study. The sexual dimorphism was more distinct in O-population than in I-population except length of midleg (Table 2). I tested the degrees of sexual dimorphism between O- and I-populations by randomization tests [2] (random data permutation, 10000 permutations). The degree of sexual dimorphism in O-population was significantly larger than that in I-population in three traits (P=0.021 for wing length 1, P=0.003 for wing length 2, P=0.0001 for width between eyes), but not significantly different in the midleg length (P=0.093).

Allometry

Next, I analysed the relationships among the four morphological traits by using regression of allometric equation, $y=ax^b$. I made linear regression of log-transformed data to estimate the parameters a and b. Table 3 shows the parameters and correlation coefficients. The regression was significant at the 5% level in all the cases. The differences in allometric equations were tested with the comparison of slope (b) and elevation of regression (log a) by the conventional F tests. In this case, I tested six pairs of traits in each sex. Because this causes the higher risk of type 1 error, I used again the sequential Bonferroni method [10].

In females, both slope and elevation were significantly

TABLE 3. Allometry between 4 morphological traits

(a) Female

x	y	Okinawa			Ishigaki		
		a	b	r	a	b	r
Length of midleg	Wing length 1	4.49	0.32	(0.64)	3.29	0.72	(0.89)
Length of midleg	Wing length 2	2.62	0.17	(0.37)	1.63	0.81	(0.79)
Length of midleg	Width between eyes	0.60	0.53	(0.50)	0.45	0.72	(0.72)
Wing length 1	Wing length 2	1.00	0.63	(0.67)	0.15	1.14	(0.89)
Wingth length 2	Width between eyes	0.31	0.92	(0.41)	0.35	0.73	(0.75)

(b) Male

x	y	O			I		
		a	b	r	a	b	r
Length of midleg	Wing length 1	3.59	0.53	(0.66)	3.70	0.44	(0.61)
Length of midleg	Wing length 2	1.68	0.60	(0.61)	1.79	0.52	(0.61)
Length of midleg	Width between eyes	0.40	0.91	(0.58)	0.44	0.75	(0.44)
Wing length 1	Wing length 2	0.52	0.96	(0.77)	0.58	0.90	(0.78)
Wing length 1	Width between eyes	0.16	0.92	(0.47)	0.13	1.06	(0.45)
Wing length 2	Width between eyes	0.36	0.73	(0.47)	0.27	1.08	(0.53)

different in 2 pairs of six (midleg vs wing length 1: $P<0.001$ for slope and elevation, midleg vs wing length 2: $P<0.001$ for slope and elevation), only slope was significantly different in a pair (wing length 1 *vs* wing length 2: $P<0.05$ for slope) and only elevation was significantly different in 3 pairs (midleg vs width between eyes: $P<0.001$ for elevation, wing length 1 *vs* width between eyes: $P<0.001$ for elevation, wing length 2 *vs* width between eyes: $P<0.001$ for elevation). In males, only elevation was significantly different in a pair (midleg *vs* wing length 1: $P<0.01$ for elevation). Neither slope nor elevation was significantly different in other 5 cases.

DISCUSSION

The results of the present study showed that there were significant morphological differences (including those in the morphological sexual dimorphism) and weak post-copulatory isolation between I- and O-populations.

Rate of evolution

The rate of evolution in processes of a speciation event is difficult to estimate because of its long duration. The evolutionary rate of isolation in a field population does not have been reported except studies where the duration during divergence of two populations was determined by the electrophoretic data [1]. The duration that can be dealt with in electrophoretic studies is confined to the order of 10^5 years or longer. An evolutionary rate in a shorter time scale cannot be detected by this method. In the present study, however, it is possible to estimate the evolutionary rate of post-copulatory isolation during some decades. It should be necessary to determine the number of generations passed after the recent common ancestor of the O- and I-populations, before estimation of the rate of evolution.

The years and the number of generations after the recent common ancestor of O- and I- populations is determined as follows. The history of invasion of the melon fly into islands of the Ryukyu Archipelago has been recorded because this fly is an important agricultural pest. From the records of introduction, the fly population on Okinawa Island in 1989 are reasonably assumed to be descendants of the flies invaded from Isigaki into Miyako Islands in 1929, (and into Kume Island in 1970 and Okinawa Island in 1972). Because the distance between Miyako and Kume Islands is more than 200 km, the rate of migration between these two islands is probably very low [8]. No migration of sterilized flies (they were marked) between the two islands has been observed (Kawasaki, personal communication). Forty one years (from 1929 to 1970) between the first discoveries of the fly on these two islands also support the very low rate of migration between the islands. There might be, on the other hand, repeated migration from Isigaki to Miyako Islands. At the time of the present study (1989), the melon fly populations on Kume and Miyako Islands were extinguished by SIT in 1978 and 1987, respectively, while SIT was being applied to the population on Okinawa Island [6].

Thereby, the melon fly populations on Isigaki and Okinawa islands are considered to share the common ancestor in 1929 or later and to experience the different environmental conditions after this time. *B. cucurbitae* in Ryukyu Archipelago have 7 generations in a year [7]. Thereby 420 or less generations (60 or less years from 1929 to the present study, 1989) have passed after the most recent common ancestor of the two populations of *B. cucurbitae*.

The evolution of the post-copulatory isolation in the present case seems to be very rapid though the effect of an artificial factor, SIT, on Okinawa Is. was involved . In the data of isolation in *Drosophila* reviewed by Coyne and Orr [1], the post-copulatory isolation of the degree of 0.25 or 0.5 (unity means the full isolation) needs the order of 0.1 in the genetic distance. Under the assumptions that the observed decrease in the egg hatchability (ca. 10%) in the present study is one tenth of the post-copulatory isolation of index 0.25 or 0.5 and that the unity in the genetic distance corresponds to 5×10^6 years [9], the observed decrease in hatchability corresponds to roughly 5×10^4 years of divergence. This means that the observed reproductive isolation has evolved very faster (roughly 10^3 times) than other cases [1]. The present result suggests that the rapid evolution of post-copulatory isolation is possible in field populations.

In the present study, the difference in the morphology between two populations is less than 3 standard deviations (SD, hereafter). The rate of evolution necessary for the observed degree of divergence is thus smaller than 0.01 SD per generation. Standardized selection differential necessary for this order of evolution is less than 0.04 SD under the assumption of $h^2 \cong 0.3$ (Nakamori's unpublished data on the width of thorax, the point estimate was 0.327). This figure means that the selection intensity necessary in this case is rather weak in comparison with those observed in field studies [3].

Effects of SIT

The situation of the melon fly in the Ryukyu Archipelago can be viewed as another natural experiment to study the response to selection pressure by SIT. On Okinawa Is., two populations of the flies coexisted, i.e., O-population and sterile fly population (S, hereafter). S-population consisted of sterile flies that were reared and released artificially. The traits of life-history and behavior of S-flies were different from wild flies (O and I) to a large extent (e.g. [12]). In the natural experiment to study the effect of SIT, O-population can be viewed as the "experimental group" while I-population as the "control group" because SIT was not made on Isigaki Is.. As O-females that mate with S males do not produce viable offspring, the evolution of the female mate preference to avoid S males by reinforcement can be expected. This is the hypothesis of Hibino and Iwahashi [4], and their result is consistent with it.

The results of the present study seem to support the hypothesis of Hibino and Iwahashi [4] and to mean that their hypothesis can be applicable to morphological traits. The

morphological divergence of the two populations, particularly the differences in morphological sexual dimorphism, which suggest the different pressure of sexual selection between populations, is consistent with their hypothesis. It is also supported by the results of allometric analyses that the allometric regression was different in slope and/or elevation in all six pairs of traits in females, but only one in males.

Nakamori (personal communication) found that female mate preference was suppressed when body size of females were small in his laboratory study of the melon fly. Though females exhibited mate preference, large females were able mate with the preferred males while small females were not (Nakamori, personal communication). If this process occurred in the field, selection favors the large female body size that enable the female mate preference to avoid S-males. The present result of more distinctive sexual dimorphism in O-population is consistent with this version.

However, the present results do not exclude other possibilities, for example, random drift, bottleneck of population and selection by factors other than SIT. It is highly likely that the fly population experienced the bottleneck at the introduction to Kume Island.

The results of the present study together with Hibino and Iwahashi suggest that SIT is also useful as a large scale field experiment to study the response to sexual selection. They also show that SIT can change the genetical constitute of pest populations as in the case of the evolution of resistance to pesticides.

ACKNOWLEDGMENTS

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