Geographic Variation in Esterase Isozymes of the Yellow-Spotted Longicorn Beetle, *Psacotha hilaris* (PASCOE) (Coleoptera: Cerambycidae)

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The esterase isozymes of eggs of the yellow-spotted longicorn beetle, *Psacotha hilaris*, were separated by polyacrylamide gel electrophoresis into two major band groups, Est-A ($R_m=0.32-0.38$) and -B ($R_m=0.48-0.52$). All the newly-laid eggs produced by a female showed an identical esterase zymogram. Est-A was found in eggs laid by all females collected at various localities in the Japanese mainland. Almost all the females collected from the eastern districts of Japan produced eggs with Est-B (Electromorph-2, Em-2), while over 50% of the females from the west districts produced eggs lacking Est-B (Electromorph-1, Em-1). The distribution of Em-1 seems to resemble that of 'west Japan type' of this insect, which has been distinguished from 'east Japan type' by the difference of the spot pattern on the pronotum and the seasonal prevalence of adults. These characters, however, did not seem to be correlated with the presence of Est-B when examined individually.

Key words: *Psacotha hilaris*, geographic variation, esterase isozyme

INTRODUCTION

The yellow-spotted longicorn beetle, *Psacotha hilaris* (PASCOE), is one of the most injurious insect pests of mulberry trees in Japan. The female beetles lay eggs into the mulberry trunks and the larvae bore tunnels in the trunks. Moreover, the beetles feed on buds and leaves for maturation.

This species is distributed in eastern Asiatic Continent, Japan and Taiwan, and is classified by habitats into 13 subspecies. A subspecies, *P. hilaris hilaris*, widely distributed in the mainland of Japan (excluding Hokkaido), is considered to consist of two different populations with clearly different seasonal prevalence and spot patterns on the pronotum of adult insects (IBA et al., 1976; IBA, 1980; KUSAMA and TAKAKUWA, 1984; MAKIHARA, 1986). The boundary of the two populations seems to lie near the Yamanashi Prefecture (IBA, 1980), and the populations to the west and east of the boundary are called 'west Japan type' and 'east Japan type', respectively (MAKIHARA, 1986). Near this boundary, beetles with an intermediate spot pattern and beetles which have two prevalence peaks have also been recorded (YONEYAMA and OZAWA, 1979; IBA, 1980; TAKAHASHI and MIYASAKA, 1981; YONEYAMA and ICHIKAWA, 1981).

Generally, the genetic variation of coleopteran species is greater than that of the other insects, but few enzymatic studies have been made on Cerambycidae (HSIAO,

1989). KOBARA and KAI (unpublished) have found esterase isozyme variation in larvae of this subspecies. However, there is not enough information to formulate conclusions on the genetic status of the two populations. The purpose of this study is to genetically analyse the geographic variation of this subspecies by using esterase isozymes of the eggs as an indicator.

MATERIALS AND METHODS

Beetle collecting. Beetles were collected mostly in sericulture experiment stations or private mulberry fields at various localities, and partly from fig trees at two collection localities (Akita and Fukuyama). Most of the beetles used in this study were caught in 1990. At Annaka, Sagamihara and Nirasaki, beetles were repeatedly collected about every 30d in 1990. The collection localities ranged from Kyushu to the Tohoku district, covering most of the distribution area of the *P. hilaris hilaris* subspecies in Japan (Table 1 and Fig. 3).

Egg collecting. The beetles collected from a locality were reared in a plastic box (25 cm × 33 cm × 10 cm) for mating, with some mulberry leaves and trunks. A box accommodated about 15 beetles, and had a square hole with a net for ventilation on one side. Three or four days later, the females were transferred individually into transparent plastic cups (4.5 cm dia. × 4 cm), with identification labels on the lids. The females were provided with firmly-rolled mulberry leaves fastened with rubber bands. These rolls serve not only as food but also as oviposition substrate, and were replaced

Table 1. Collection locality and number of females assayed

Collection locality	Month collected	No. of females	Collection locality	Month collected	No. of females
1. Akita	Oct.	4	22. Yokoze	Aug.	19
2. Yanagawa	Sep.	41	23. Yayoi	Aug.–Oct.	41
3. Shiunji	Sep.	21	24. Hino	Sep.	6
4. Toyosaka	Sep.	5	25. Sagamihara	Jun.–Nov.	78
5. Kawaguchi	Sep.	8	26. Ebina	Oct.	16
6. Tsunan	Sep.	14	27. Sagamiko	Jun.	22
7. Katashina	Jul., Aug.	4	28. Uenohara	Jun.	34
8. Gokan	Sep.	13	29. Yanagawa	Jun.	16
9. Shikishima	Oct.	9	30. Hatsukari	Jul., Aug.	38
10. Maebashi	Oct.	12	31. Enzan	Jul., Aug.	10
11. Kamisato	Oct.	10	32. Kasugai	Jul., Aug.	6
12. Kokubunji	Sep.	20	33. Ryuoh	Aug.	14
13. Sekijo	Aug., Sep.	85	34. Nirasaki	Jul.–Sep.	81
14. Tsukuba	Oct.	14	35. Takamori	Jul.	24
15. Tako	Sep.	3	36. Shika	Jul.–Sep.	17
16. Ohamishirasato	Sep.	12	37. Ureshino	Jun., Jul.	15
17. Annaka	Jul.–Sep.	54	38. Hidaka	Jul.	15
18. Matsuida	Aug.	32	39. Fukuyama	Aug.	24
19. Yokokawa	Aug.	33	40. Ochi	Jul.	65
20. Komoro	Aug.	8	41. Isahaya	Jul.	17
21. Kumagaya	Jul.	10			

with new ones every day. The old oviposition substrates were unrolled and the eggs laid into them were collected. The eggs collected were put into microcentrifuge tubes identified by individuals, and kept at -80°C until use for electrophoresis.

Crossing between the beetles from different localities. Eight females which had already produced eggs were randomly selected and put into bigger transparent plastic cups (6 cm dia. $\times$ 6 cm) for crossing with males collected at a different locality for three days. Crossing combinations were Ochi (♀ , no. 40) $\times$ Kumagaya (♂ , no. 21), Ochi (♀) $\times$ Sagamihara (♂ , no. 25), Kumagaya (♀) $\times$ Ochi (♂) and Sagamihara (♀) $\times$ Ochi (♂). The females were then individually transferred into smaller cups for egg laying. The eggs laid were collected for 30d. The eggs collected on the 1st, 10th, 20th and 30th days were electrophoresed simultaneously with the eggs which had been produced by the same females before the crossing.

Spot pattern on the pronotum of the females. All the females collected in 1990 were checked for the spot pattern on the pronotum to examine the correlation to the electromorphs of their eggs. IBA (1980) classified the spot patterns into severed (SP-S), intermediate (SP-I) and continuous (SP-C) types (Fig. 1). SP-S and SP-C are typical of 'west Japan type' and 'east Japan type', respectively.

Electrophoresis and gel staining. An egg was ground in a concavity (20 mm dia.)

Fig. 1. Spot patterns on the pronotum of the yellow-spotted longicorn beetle. SP-S outnumbered others in the western districts in contrast with the diversity of spot patterns in the eastern districts of Japan. SP-I is considered to be intermediate between SP-S and SP-C.

Fig. 2. Esterase zymograms of the eggs of the yellow-spotted longicorn beetles collected from four localities. Each lane represents isozymes of an egg from different females.

of a porcelain spot plate with a glass pestle in 50 μ l of 50% (w/w) sucrose in 0.5 M Tris-HCl buffer (pH 6.7). A 5 μ l sample was loaded onto each sample well, and discontinuous vertical slab gel electrophoresis was conducted at a 30 mA constant current for 75 min with 7.5% polyacrylamide gel (1 mm thickness). Bromophenol blue was used as a dye front indicator. Gel was stained for esterase activity according to the method of SHAW and PRASAD (1970).

RESULTS

Electrophoresis and analysis of band patterns

The esterases of eggs of the yellow-spotted longicorn beetle were separated into two major band groups named Est-A and -B in the order of migration from the origin to

Fig. 3. The frequency of Em-1 and -2 at various localities in the Japanese mainland. The numbers in or near circles refer to the collection locality in Table 1. About 90% of beetles are those with SP-S in the western districts of the striped line, while beetles with SP-S, -I and -C are observed in the eastern districts.

anode (Fig. 2). It was very difficult to include other bands for analysis because they were very faint and their appearance did not seem reproducible. By the electrophoretic method used in this study, it was also impractical to individually analyse the respective bands in Est-A and -B. Analysis was thus made by grouping bands into Est-A and -B. All the eggs tested had Est-A ($R_m=0.32-0.38$), which consisted of at most eight bands. Some eggs had Est-B ($R_m=0.48-0.52$), which consisted of a maximum of three bands. Zymograms of all the eggs tested ($n=1010$) belonged to either of the two electromorphs; having only Est-A or having both Est-A and -B.

Effect of crossing with males from the other populations on the esterase zymograms

The eggs produced by a female showed an identical zymogram before and after the crossing with the other populations. Namely, genotypes of males had no effect on esterase zymograms of the newly-laid eggs. The result of this crossing experiment revealed that all the newly-laid eggs produced by a female showed the same esterase zymogram. Thus only an egg was needed to examine the esterase zymogram of the eggs of a female. Consequently, females were classified by the esterase zymograms of their eggs into two electromorphs; the females whose eggs showed only Est-A (Em-1) and the females whose eggs showed both Est-A and -B (Em-2).

Geographic variation

The frequencies of the two electromorphs at various collection localities are shown in Fig. 3. In the Kanto district and surrounding areas (nos. 2-18 and 21-29), almost all the females were of Em-2, while over 50% of the females were of Em-1 in the western districts (nos. 37-41). The intermediate frequencies tended to occur in the localities lying between the Kanto and western districts (nos. 19, 20 and 30-35). These results indicate that the 'east Japan type' and 'west Japan type' populations are genetically different in the composition of esterase phenotypes of the eggs in addition to the morphological difference of adults.

Table 2. Seasonal change in the frequency of Em-1

Collection locality	Date of collection	No. of females	No. of Em-1	% of Em-1
(17) Annaka	Jul. 14	19	0	0.0
	Aug. 17	18	0	0.0
	Sep. 20	17	0	0.0
(25) Sagamihara	Jun. 19	12	1	8.3
	Jul. 22	14	0	0.0
	Aug. 21	17	0	0.0
	Sep. 19	14	1	7.1
	Oct. 19	11	0	0.0
	Nov. 21	10	0	0.0
(34) Nirasaki	Jul. 21	32	9	28.1
	Aug. 20	34	14	41.2
	Sep. 17	15	5	33.3

Numbers in parentheses are the locality numbers from Table 1 and Fig. 3.

Table 3. Correlation between electromorphs and spot pattern on the pronotum of the adult

SP	-S	-I	-C	SP	-S	-I	-C	SP	-S	-I	-C
(39) Fukuyama				(36) Shika				(35) Takamori			
Em-1	14	0	0	Em-1	0	0	0	Em-1	10	0	0
Em-2	10	0	0	Em-2	15	2	0	Em-2	14	0	0
(30) Hatsukari				(19) Yokokawa				(12) Kokubunji			
Em-1	3	1	2	Em-1	6	3	4	Em-1	0	0	0
Em-2	24	5	3	Em-2	11	8	1	Em-2	2	6	12

Numbers in parentheses are the locality numbers from Table 1 and Fig. 3.

Seasonal change of the frequency of the electromorphs

The frequencies of Em-1 in the females periodically collected at Annaka, Sagami-hara and Nirasaki (nos. 17, 25, 34, respectively) are shown in Table 2. The percentages of Em-1 at each locality were not variable with regard to collection months and thus significant seasonal difference was not found.

Correlation between the electromorphs and the spot patterns on the pronotum of adults

There were no clear correlations between the spot patterns on the pronotum and the esterase zymograms when these two parameters were examined individually (Table 3). For example, 42% of the females from Fukuyama and 58% of the females from Takamori were of Em-2, while all of the females from these localities were those with SP-S. Furthermore, all of the females from Kokubunji were of Em-2 notwithstanding the diversity of their spot patterns on the pronotum.

DISCUSSION

Clear geographic variation was found in esterase electromorphs (Fig. 3). Nearly 100% of the females were of Em-2 in the Kanto districts, while less than 50% of the females were of Em-2 in the western districts. High frequency (50%) of Em-1 was observed at Katashina (no. 7), however, this value may be overestimated because only four females were assayed. Similarly the frequency of Em-1 may be an overestimate in the beetles from Komoro (no. 20). The beetles from Shika (no. 36) were also atypical in that no Em-1 was observed, but the reason is not clear. The boundary of the area predominated by the Em-2 females seems to lie between Yanagawa (no. 29) and Hatsukari (no. 30), and between Matsuida (no. 18) and Yokokawa (no. 19). This boundary lies close to that of the area where the beetles with SP-S are predominant; about 90% and 20% of the beetles were those with the SP-S to the west and east of the boundary, respectively (Fig. 3, SHINTANI, unpublished).

The difference in the seasonal prevalence of the 'west' and 'east' Japan types results from the difference in larval development (EMORI and KIKUCHI, 1969) and the presence of dormancy in last-instar larva (SAKAKIBARA and KAWAKAMI, unpublished). The 'west Japan type' has its prevalence peak from late June to mid-July (early summer), while the 'east Japan type' peaks in September (autumn) (IBA et al., 1976). Near the boundary of the two populations, outbreaks have been observed twice a year (in early summer and autumn) (YONEYAMA and OZAWA, 1979; TAKAHASHI and MIYASAKA, 1981; YONEYAMA and ICHIKAWA, 1981). In this study, the beetles were collected over most of

the emergence season (Table 2). However, seasonal change was not observed in the frequency of the electromorphs, though the collection localities (Annaka, Sagami-hara and Nirasaki) were close to the boundary of the two populations (Fig. 3).

It has been suggested that the genes controlling the seasonal prevalence and spot pattern on the pronotum were located on separate chromosomes (SAKAKIBARA and KAWAKAMI, unpublished). The distribution of Em-1 resembles that of the 'west Japan type'. But the presence of Est-B was not correlated with the spot patterns and seasonal prevalence of the beetles (Tables 2 and 3).

The presence of some diagnostic isozymes has been reported for geographically isolated populations of a species (AYALA et al., 1971; AYALA and TRACEY, 1973), and the genetic relations between the populations have been estimated based on isozyme variation data (HIGBY and STOCK, 1982; McDONALD et al., 1985). The 'east Japan type' is considered to have been accidentally introduced from outside of Japan into the Kanto district around the turn of the century, judging from its morphological similarity to the exotic subspecies of the same species (KUSAMA and TAKAKUWA, 1984; MAKIHARA, 1986). Est-B may have been introduced to Japan at the same time, considering the predominance of Em-2 in the Kanto district. To test this hypothesis, it is necessary to examine the exotic beetles morphologically and enzymatically. These studies may enable us to estimate the origin of the 'east Japan type'.

The yellow-spotted longicorn beetle is well-known for multiple mating behavior and sperm displacement (YOKOI, 1990). All eggs produced by each female allowed to copulate with males of different populations showed the same esterase zymogram as that of eggs produced before the interpopulational crossing, in spite of the last-male precedence at fertilization (YOKOI, unpublished). This indicates that multiple mating and sperm displacement has no effect on the esterase zymograms and that these esterases which appeared as bands were of maternal origin. Paternal effect had probably not yet manifested because the eggs used were in the earliest stages in the embryogenesis.

Considering that the beetles with SP-S and SP-I have extended their distribution eastwards in these decades (IBA, 1980; SHINTANI, unpublished), it is expected that the distributions of Em-1 and -2 will also change gradually. It will be of interest to make similar surveys some years later. Investigations on polymorphic enzymes other than esterase are under way to reveal the genetic status of this subspecies more clearly.

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REFERENCES

- AYALA, F. J., J. R. POWELL and T. DOBZHANSKY (1971) Polymorphisms in continental and island populations of *Drosophila willistoni*. *Proc. Nat. Acad. Sci. USA*. **68**: 2480-2483.
AYALA, F. J. and M. L. TRACEY (1973) Enzyme variability in the *Drosophila willistoni* group. VIII. Genetic differentiation and reproductive isolation between two subspecies. *J. Heredity* **64**: 120-124.

- EMORI, T. and M. KIKUCHI (1969) Geographic difference in larval development of the yellow-spotted longicorn beetle. *Abst. 20th Ann. Meeting Kanto branch Seric. Sci. Jpn.* p. 32 (in Japanese).
- HIGBY, P. K. and M. W. STOCK (1982) Genetic relationships between two sibling species of bark beetle (Coleoptera: Scolytidae), Jeffrey pine beetle and mountain pine beetle, in northern California. *Ann. Entomol. Soc. Am.* **75**: 668–674.
- HSIAO, T. H. (1989) Estimation of genetic variability amongst Coleoptera. In *Electrophoretic Studies on Agricultural Pests*. Clarendon Press, Oxford, pp. 143–180.
- IBA, M., S. INOUE and M. KIKUCHI (1976) Ecological studies on the yellow-spotted longicorn beetle, *Psacotha hilaris* PASCOE. I. The local difference in the seasonal prevalence of the adult insect. *J. Seric. Sci. Jpn.* **45**: 156–160 (in Japanese with English summary).
- IBA, M. (1980) Ecological studies on the yellow-spotted longicorn beetle, *Psacotha hilaris* PASCOE. IV. The geographic difference of the pattern on the pronotum of adult. *J. Seric. Sci. Jpn.* **49**: 429–433 (in Japanese).
- KUSAMA, K. and M. TAKAKUWA (1984) [The Longicorn-Beetles of Japan in Color.] Kodansha, Tokyo, 565 pp. (in Japanese).
- MAKIHARA, H. (1986) [Longicorn beetle. Extension of the distribution and geographic difference.] In [*Japanese insect. Ecology of invasion and disturbance.*] (K. KIRITANI, eds). Tokai Univ. Press, Tokyo, pp. 96–106 (in Japanese).
- MCDONALD, I. C., J. L. KRYSAN and O. A. JOHNSON (1985) Genetic variation within and among geographic populations of *Diabrotica barberi* (Coleoptera: Chrysomelidae). *Ann. Entomol. Soc. Am.* **78**: 271–278.
- SHAW, C. R. and R. PRASAD (1970) Starch gel electrophoresis of enzymes. A compilation of recipes. *Biochem. Gen.* **4**: 297–320.
- TAKAHASHI, I. and Y. MIYASAKA (1981) [Ecology of the yellow-spotted longicorn beetle in higher and cooler mulberry fields. II. Difference in the spot pattern on the pronotum and the seasonal prevalence of adult insects.] *Nagano Seric. Rep.* **17**: 31–36 (in Japanese).
- YOKOI, N. (1990) The sperm removal behavior of the yellow-spotted longicorn beetle, *Psacotha hilaris* (Coleoptera: Cerambycidae). *Appl. Entomol. Zool.* **25**: 383–388.
- YONEYAMA, M. and K. ICHIKAWA (1981) [Research on the prevalence and the control of the yellow-spotted longicorn beetle, *Psacotha hilaris* PASCOE. Ecology of the two populations.] *Rep. Seric. Exp. Stn. Yamanashi Pref.* **20**: 23–27 (in Japanese).
- YONEYAMA, M. and K. OZAWA (1979) [Research on the bionomics and the control of the yellow-spotted longicorn beetle, *Psacotha hilaris* PASCOE.] *Rep. Seric. Exp. Stn. Yamanashi Pref.* **18**: 75–79 (in Japanese).