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Geographical structure of morphological characters in *Semanotus japonicus* (Coleoptera: Cerambycidae) in Japan

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Abstract

The sugi bark borer, *Semanotus japonicus*, occurs naturally in Honshu, Shikoku, Sado Is., Oki Is., and Yaku Is. in Japan, and its main host tree is the Japanese cedar, *Cryptomeria japonica.* Because of a correspondence between the present distribution of geographical variation in the borer and the refugia of the Japanese cedar during the last full-glacial period, isolation of the cedar is thought to have caused differentiation in the borer between local populations. In this study, we used morphological information to elucidate the geographical differentiation of *S. japonicus* populations. Multivariate analyses were performed based on ten morphological characters for ten populations. Populations between the Pacific coast side and the Wakasa Bay area showed remarkable variation in morphological characters. Because the distribution of the refugia of the cedar coincided with the patterns of morphological variations in *S. japonicus*, it is suggested that the geographical structure of the present *S. japonicus* population was caused by isolation during the last glacial period. While vicariance is no longer a factor with the expansion of *C. japonica*'s distribution, its past effects may have been important enough to influence the present genetic structure of *S. japonicus.*

Key words: Sugi bark borer; *Cryptomeria japonica*; glacial refugia; morphological characters; multivariate analysis

INTRODUCTION

The sugi bark borer, *Semanotus japonicus* Lacordaire, occurs in Honshu, Shikoku, Kyushu, Sado Is., Kanmuri Isles., Oki Is., Awaji Is., Iki Isles., and Yaku Is. in Japan, and its main host tree is the Japanese cedar, *Cryptomeria japonica* D. Don (Niisato, 1992; Makihara, 2000). Intensive establishment of cedar plantations occurred during the 1960–70's and due to poor management of stands, this borer has become a serious forest pest (Kobayashi and Shibata, 1985). Makihara (2000) inferred its natural distribution as Honshu, Shikoku, Sado Is., Oki Is., and Yaku Is., because there is little natural cedar distributed outside these areas. Geographical variation in this species has been recognized in the size of spots on the elytra and from body color. *S. japonicus* living in Honshu and Shikoku have been separated into four morphological types; Pacific coastal, Wakasa Bay, San-in, and Kochi types (Makihara, 1988). Pacific coastal and Kochi types are distributed along the Pacific coast, and the others on the Japan Sea side. The San-in region is part of the Chugoku area, on the southwestern Japan Sea coast. Wakasa Bay and San-in types have larger spots on the elytra than Pacific coastal and Kochi types. Body color of the Wakasa Bay type was brighter than other types.

Whereas the present limits of the natural distribution of cedar are Ajigasawa Town in Aomori Prefecture (Lat 40°42′N) in the north and Yaku Island in Kagoshima Prefecture (30°15′N) in the south (Hayashi, 1960; Maeda, 1983), its range was restricted to coastal areas during the last glacial period because of low temperatures and humidity (Tsukada, 1982). From analysis of pollen diagrams from 37 sites throughout Japan, the refugia of *C. japonica* and the route of its postglacial migration have been located in detail (Tsukada, 1982). During the full-glaciation, the refugia in Honshu and Shikoku so far determined are: 1) the broad coastal plain of the present Wakasa Bay area; 2) scattered distributions offshore of San-in; 3) discontinuous distributions in the lowland along the Pacific coast (Fig. 1). Locations 1) and 2) face the Japan Sea, with 2) occupying the western region.

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Because of the correspondence between the present geographical variations in *S. japonicus* and the refugia of the cedar, isolation of the Japanese cedar is thought to have caused differentiation of the borer among local populations (Makihara, 1988). However, the extent of morphological differentiation and the structure of variations among them are unknown. Unless the geographical structure of the populations is elucidated, it is impossible to test the hypothesis that the beetle has diverged in response to the glacial refugia of its host. In addition to analysis for qualitative characters conducted in Makihara (1988), it is therefore essential to quantitatively examine the morphological variations among populations of *S. japonicus* to elucidate their geographical structure.

This study aimed to explain the geographic structure of present *S. japonicus* populations using morphological multivariate data to elucidate the factors that have influenced them in the past.

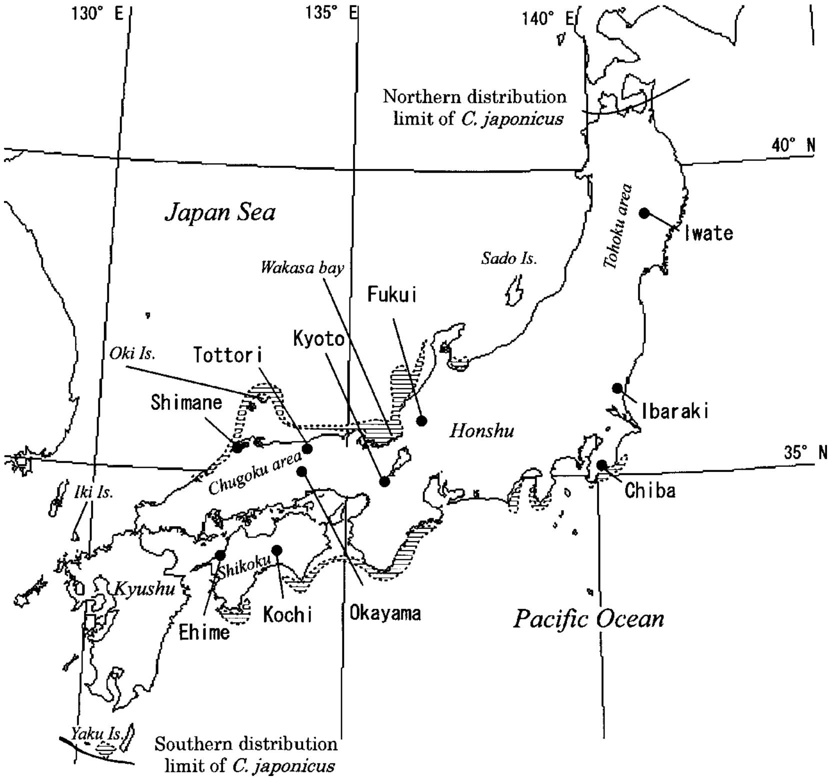

Fig. 1. Inferred refugia of *Cryptomeria japonica* (arranged from Tsukada, 1982) and collection sites of the *Semanotus japonicus*. Refugia are indicated by hatched lines.

Table 1. Collection localities and sample sizes

Collection site	Latitude	Longitude	Male	Female
Inase, Iwate Pref.	39°14′	141°08′	15	14
Naka-machi, Ibaraki Pref.	36°25′	140°29′	11	15
Kiyosumi-yama, Chiba Pref.	35°10′	140°09′	6	5
Mitsuzuka, Fukui Pref.	35°55′	136°30′	12	28
Momoyama-chou, Kyoto Pref.	34°56′	135°46′	10	10
Kawara-machi, Tottori Pref.	35°25′	134°13′	8	0
Sho-ou-chou, Okayama Pref.	35°04′	134°07′	8	5
Shinji, Shimane Pref.	35°25′	132°55′	5	5
Futami-chou, Ehime Pref.	33°38′	132°34′	7	2
Ohtoyo-chou, Kochi Pref.	33°44′	133°41′	12	12

MATERIALS AND METHODS

Ten morphological characters were measured in adult beetles collected from Iwate, Ibaraki, Chiba, Fukui, Kyoto, Tottori, Okayama, Shimane, Ehime, and Kochi prefectures in Japan (Fig. 1, Table 1). All individuals were collected in areas of the natural distributions of *S. japonicus*. Populations which were evidently artificially introduced were omitted from the analysis. With digital calipers (unit 0.01 mm), body length (BL), pronotum length and width (PTL, PTW), elytral length and width (ELL, ELW), length and width of both upper and lower spots on the right elytrum (USL, USW, LSL, LSW), and head width (HW) were measured (Fig. 2). Numbers of individuals measured for each population are shown in Table 1. Since individual variation in body size in *S. japonicus* is large, measurements were adjusted with body size. To consider the appropriateness of adjusting the measurements by body length, allometric relationships between body length and the other characters were examined. The allometric relationship between two characters can be described by the ordinary allometric model,

$$\mathrm{Log}_{10} Y = a \log_{10} X + b$$

where X is body length and Y is the measurement of one of the other characters, a being the allometric coefficient and b a constant. If a is one, the relationship between the two variables is linear and the character can be adjusted by the body length. The significant level of correlation between body length and the other characters was adjusted by the Bonferroni method. Since the allometric coefficient for each character that had a high coefficient of determination did not vary from 1 (see Table 2), $-\log_{10}$(measurement/BL) was used for analysis to exclude the effect of variation in body size. Because some individuals completely lacked an upper and/or lower spot, $-\log_{10}\{1-(\text{measurement})/\text{BL}\}$ was used for measurements of the spots. Analyses were conducted separately for each sex.

Principal component analysis (PCA) was used to examine the intensity of the associations between morphological characters, and to determine characters which could explain differences between indi-

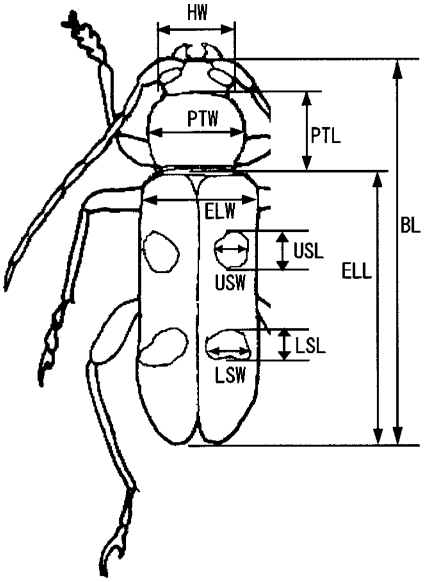

Fig. 2. Dimensions of *Semanotus japonicus* measured.

Table 2. Allometric coefficients (slopes of regression line) a and coefficients of determination for morphological characters and body length

Characters	Male		Female	
	Slope of regression line (a)	Coefficient of determination	Slope of regression line (a)	Coefficient of determination
PTL	0.88	0.90***	1.08	0.82***
ELL	0.98	0.97***	1.01	0.96***
USL	1.17	0.13*	1.20	0.14***
LSL	1.18	0.19***	1.20	0.17*
HW	0.86	0.94***	0.86	0.89***
PLW	0.99	0.93***	0.93	0.90***
ELL	0.94	0.95***	1.03	0.91***
USW	1.19	0.20***	1.23	0.25***
LSW	1.18	0.20*	0.92	$0.10^{\mathrm{N.S.}}$

N.S.=not significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$, adjusted with Bonferroni method.

viduals. The number of factors was determined by correspondent eigenvalues: Factors were adopted when eigenvalues were greater than one. We based our interpretation of each component on those variables with loadings greater than 0.50 or less than −0.50, and placed most emphasis on those with loadings greater than 0.80 or less than −0.80. To determine characters contributing to morphological differentiation among populations, canonical variate analysis, in which variables are calculated so that the ratio of among group variance to within-group variance is largest, was performed. We adopted first and second canonical variates for determination of contributing characters. Significances of morphological differentiation among populations were examined by multivariate analysis of variance (MANOVA) (Scheiner, 2001). In the canonical variate analysis and MANOVA, female individuals in Ehime were omitted because the sampling number was too small. Pairwise differences between population combinations were tested with the Bonferroni adjusting method. Characteristic cohesiveness of the population was then estimated using classification done with the canonical variates, and morphology of the female individuals in Ehime were also classified in this analysis. Statistical analyses were calculated with SYSTAT ver. 9.01 (SPSS Inc., 1998).

RESULTS

Significant correlations between body length and other characters were detected except for female lower spot length (LSW) (Table 2).

Nine morphological measurements of adults were combined into two principal components for males and three for females. In the PCA, the two principal components explained 66.6% of variation of the morphological characters for males, and the three principal components 77.0% of the variation for females (Table 3). The first principal component was highly correlated to the relative size of spots (USL, LSL, USW, LSW) for both sexes. All variables of spot size characters showed loadings greater than 0.80 or less than −0.80, and each character varied synchronously. In females, head width (HW) and pronotum width (PTW) showed loadings of over 0.50. The second principle component (PC2) primarily represented the relative size of body width (HW, PTW, ELW) for both sexes. The pronotum length (PTL) was also a considerable character for PC2 in males. In females, pronotum length (PTL) and elytral length (ELL) had a high contribution to the third principle component.

Using canonical variate analysis, 76.1% of the variation among the populations was explained for males and 77.6% for females by the first two

Table 3. First two and three principle component factors calculated for morphological characters and first two canonical coefficients standardized by within variance for each character

Character	Principal component analysis					Canonical variate analysis			
	Male		Female			Male		Female	
	Component 1	Component 2	Component 1	Component 2	Component 3	Canonical 1	Canonical 2	Canonical 1	Canonical 2
PTL	0.356	0.561	0.128	0.251	0.738	0.243	0.120	0.254	0.261
ELL	0.447	0.283	0.253	−0.095	0.732	−0.060	0.071	0.090	0.724
USL	−0.881	0.344	−0.861	0.329	0.036	−0.440	−0.982	−0.361	0.691
LSL	−0.886	0.282	−0.882	0.337	−0.048	−0.503	0.080	−0.121	−0.702
HW	0.495	0.695	0.501	0.672	−0.120	0.446	0.029	0.374	0.047
PTW	0.376	0.585	0.577	0.705	−0.069	0.213	−0.100	0.123	−0.075
ELW	0.427	0.636	0.456	0.718	−0.052	−0.413	0.690	−0.681	−0.397
USW	−0.828	0.415	−0.877	0.267	0.116	0.000	1.384	−0.330	−0.206
LSW	−0.876	0.303	−0.847	0.312	0.078	−0.156	−0.206	−0.497	0.418
Eigenvalue	3.91	2.09	3.88	1.93	1.13	2.12	0.74	2.89	0.78
Contribution (%)	43.4	23.2	43.1	21.4	12.5	56.3	19.7	61.1	16.5
Cumulative contributions (%)	43.4	66.6	43.1	64.5	77.0	56.3	76.1	61.1	77.6

Table 4. Wilk's Λ (above diagonal) and F-value and df (below diagonal) for each pairwise comparison between populations

(a) In males

	Iwate	Ibaraki	Chiba	Fukui	Kyoto	Tottori	Okayama	Shimane	Ehime	Kochi
Iwate	—	0.338	0.337	0.431	0.238	0.398	0.327	0.260	0.163	0.258
Ibaraki	6.53 (9, 30)**	—	0.471	0.306	0.172	0.428	0.215	0.102	0.241	0.581
Chiba	5.48 (9, 25)*	2.74 (9, 22)$^{\mathrm{N.S.}}$	—	0.280	0.176	0.122	0.168	0.155	0.459	0.492
Fukui	4.54 (9, 31)*	10.3 (9, 41)***	10.3 (9, 36)***	—	0.407	0.432	0.297	0.299	0.156	0.259
Kyoto	10.3 (9, 29)***	11.2 (9, 21)***	8.33 (9, 16)**	3.56 (9, 22)$^{\mathrm{N.S.}}$	—	0.310	0.305	0.120	0.123	0.228
Tottori	4.53 (9, 27)*	3.56 (9, 24)$^{\mathrm{N.S.}}$	3.19 (9, 4)$^{\mathrm{N.S.}}$	5.55 (9, 38)**	4.44 (9, 18)$^{\mathrm{N.S.}}$	—	0.347	0.197	0.097	0.417
Okayama	6.18 (9, 27)**	5.68 (9, 14)$^{\mathrm{N.S.}}$	4.94 (9, 9)$^{\mathrm{N.S.}}$	3.94 (9, 15)$^{\mathrm{N.S.}}$	3.28 (9, 13)$^{\mathrm{N.S.}}$	2.30 (9, 11)$^{\mathrm{N.S.}}$	—	0.388	0.183	0.268
Shimane	4.74 (9, 15)$^{\mathrm{N.S.}}$	10.8 (9, 11)*	3.65 (9, 6)$^{\mathrm{N.S.}}$	3.13 (9, 12)$^{\mathrm{N.S.}}$	8.13 (9, 10)$^{\mathrm{N.S.}}$	3.62 (9, 8)$^{\mathrm{N.S.}}$	1.40 (9, 8)$^{\mathrm{N.S.}}$	—	0.092	0.309
Ehime	6.84 (9, 12)$^{\mathrm{N.S.}}$	2.80 (9, 8)$^{\mathrm{N.S.}}$	0.393 (9, 3)$^{\mathrm{N.S.}}$	5.41 (9, 9)$^{\mathrm{N.S.}}$	5.54 (9, 7)$^{\mathrm{N.S.}}$	5.16 (9, 5)$^{\mathrm{N.S.}}$	2.48 (9, 5)$^{\mathrm{N.S.}}$	2.20 (9, 2)$^{\mathrm{N.S.}}$	—	0.313
Kochi	9.89 (9, 31)***	2.25 (9, 28)$^{\mathrm{N.S.}}$	2.30 (9, 20)$^{\mathrm{N.S.}}$	13.4 (9, 42)***	8.27 (9, 22)**	1.55 (9, 10)$^{\mathrm{N.S.}}$	4.56 (9, 15)$^{\mathrm{N.S.}}$	2.98 (9, 12)$^{\mathrm{N.S.}}$	2.20 (9, 9)$^{\mathrm{N.S.}}$	—

(b) In females

	Iwate	Ibaraki	Chiba	Fukui	Kyoto	Okayama	Shimane	Kochi
Iwate	—	0.293	0.534	0.351	0.229	0.404	0.239	0.259
Ibaraki	9.12 (9, 34)***	—	0.373	0.284	0.143	0.303	0.268	0.459
Chiba	2.33 (9, 24)$^{\mathrm{N.S.}}$	3.92 (9, 21)$^{\mathrm{N.S.}}$	—	0.314	0.269	0.062	0.097	0.491
Fukui	9.67 (9, 47)***	12.6 (9, 45)***	8.51 (9, 35)***	—	0.390	0.387	0.393	0.206
Kyoto	10.9 (9, 29)***	16.6 (9, 25)***	4.52 (9, 15)$^{\mathrm{N.S.}}$	6.60 (9, 38)***	—	0.232	0.101	0.199
Okayama	3.94 (9, 24)$^{\mathrm{N.S.}}$	4.61 (9, 18)$^{\mathrm{N.S.}}$	13.5 (9, 8)*	5.45 (9, 31)**	4.79 (9, 13)$^{\mathrm{N.S.}}$	—	0.192	0.231
Shimane	4.96 (9, 14)$^{\mathrm{N.S.}}$	4.55 (9, 15)$^{\mathrm{N.S.}}$	5.16 (9, 5)$^{\mathrm{N.S.}}$	4.81 (9, 28)*	9.88 (9, 10)*	2.34 (9, 5)$^{\mathrm{N.S.}}$	—	0.223
Kochi	9.84 (9, 31)***	3.66 (9, 28)$^{\mathrm{N.S.}}$	2.19 (9, 19)$^{\mathrm{N.S.}}$	17.9 (9, 42)***	9.85 (9, 22)***	5.54 (9, 15)$^{\mathrm{N.S.}}$	4.65 (9, 12)$^{\mathrm{N.S}}$	—

N.S. = not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$, adjusted with Bonferroni method.

Table 5. Identification matrix for morphology of population *Semanotus japonicus*

(a) In males

Collected sites	Group into which each individual was classified										
	Iwate	Ibaraki	Chiba	Fukui	Kyoto	Tottori	Okayama	Shimane	Ehime	Kochi	% correct
Iwate	11	1	0	1	0	0	0	1	1	0	73
Ibaraki	1	5	1	0	0	0	0	0	1	3	45
Chiba	1	0	4	0	0	0	0	0	1	0	67
Fukui	2	0	0	8	0	0	0	2	0	0	67
Kyoto	0	0	0	1	6	0	3	0	0	0	60
Tottori	1	1	0	2	0	3	0	0	1	0	38
Okayama	1	0	0	0	1	0	5	1	0	0	63
Shimane	0	0	0	0	0	0	0	4	1	0	80
Ehime	0	2	0	0	0	0	1	0	3	1	43
Kochi	1	2	1	0	0	1	0	0	0	7	58
Total	18	11	6	12	7	4	9	8	8	11	60

(b) In females

Collected sites	Group into which each individual was classified								
	Iwate	Ibaraki	Chiba	Fukui	Kyoto	Okayama	Shimane	Kochi	% correct
Iwate	10	0	2	1	0	0	1	0	71
Ibaraki	0	8	1	0	0	2	2	2	53
Chiba	1	0	4	0	0	0	0	0	80
Fukui	3	0	0	20	1	1	3	0	71
Kyoto	1	0	0	0	9	0	0	0	90
Okayama	0	0	0	1	0	3	1	0	60
Shimane	0	1	0	1	1	0	2	0	40
Ehime	0	1	0	0	0	0	0	1	—
Kochi	1	0	2	0	0	1	0	8	67
Total	16	9	9	23	11	7	9	10	68

canonical variates. Upper spot length (USL), lower spot length (LSL), head width (HW), elytral width (ELW), and upper spot width (USW) were demonstrated to contribute most to morphological differentiation among populations in males, whilst upper spot length (USL), elytral width (ELW), and lower spot width (LSW), did so for females (Table 3). In MANOVAs, morphological differentiations among populations were highly significant for both sexes (Wilk's $\Lambda=0.0820$, $F_{81,500}=2.917$, $p<0.0001$ for males, Wilk's $\Lambda=0.0617$, $F_{63,445}=4.523$, $p<0.0001$ for females). Using pairwise comparisons among populations, morphological differentiations between populations were examined (Table 4). According to the canonical variates, 60% of the individuals could be classified correctly to their original populations for males, and 68% for females (Table 5). Female individuals in Ehime were classified into Ibaraki and Kochi populations.

For the first canonical variate, gradual morphological changes among populations were found (Fig. 3). Individuals from Kochi, Ibaraki, Chiba, and Ehime showed high scores for the first canonical variate, whereas individuals from Kyoto showed the lowest scores. In females, the Fukui population was also shown to have the lowest scores. Okayama, Shimane, Iwate and Tottori (only males) populations showed intermediate morphology on the first canonical variate. The second canonical variate showed morphological differences within populations having similar first canonical variate scores. In females, the Iwate population was divided from the Okayama and Shimane populations by the second canonical variate.

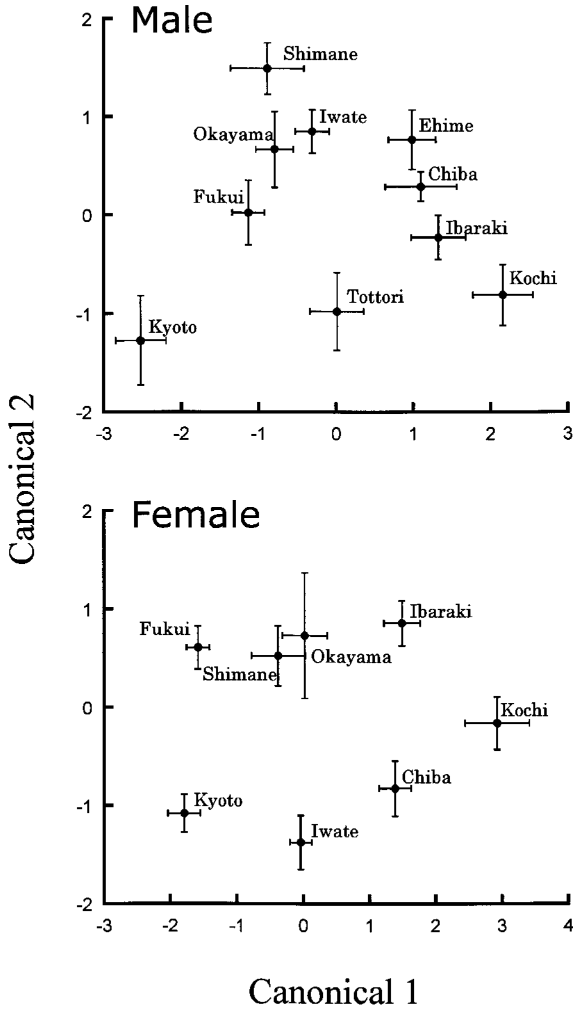

Fig. 3. Mean scores of first canonical variate (canonical 1) and second canonical variate (canonical 2) for morphological characters at each collection site. Bars represent standard errors.

DISCUSSION

Since the allometric coefficient for each character that had a high coefficient of determination did not vary from one, the adjustment methods were considered to be acceptable. As the principal component analysis showed, spot size characters were correlated with each other and they explained morphological differentiation between individuals of *S. japonicus* the best. Relative sizes of head, pronotum, and elytra width also expressed variations between individuals. Previously, individual variation in *S. japonicus* was known only for spot sizes (Fujishita et al., 1968; Nakamura and Fujishita, 1968), but multivariate analyses also indicate variations in the relative sizes of body length and width.

With regard to the geographical variation among populations, the high ratio of correct classification by the canonical variate analysis suggested that each population has a unique combination of morphology. Whereas Pacific coast side populations (Ibaraki, Chiba, Ehime, and Kochi: subsequently denoted 'PA' populations) had high first canonical variates, i.e. had small spots on the elytra and wide head, populations near Wakasa Bay (Fukui and Kyoto: subsequently denoted 'WB' populations) had opposite trends in morphology. In MANOVAs post hoc test between each population, PA populations and WB populations showed relatively high *F*-values for each degree of freedom, and most of these were significantly different for both sexes. As there was no misclassification between PA populations and WB populations in the canonical variate analysis, morphological differences were manifest between PA and WB populations. This morphological divergence between PA and WB populations concurs with the hypothesis that there was differentiation among the refugia of *C. japonica* as the host of *S. japonicus*, being divided into a Pacific coastal area and a Japan Sea side. Thus, these clear differences in morphology were probably caused by their different evolutionary histories. Since Honshu and Shikoku were joined during the last glaciation (Suzuki, 1962), populations in Shikoku and on the Pacific coastal side of Honshu can be considered equivalent to the PA population.

From typical morphological information from the first canonical variates, the morphologies of the populations of Tottori, Okayama and Shimane in the Chugoku area, and Iwate in the Tohoku area were intermediately located between PA and WB lineages. Chugoku and Iwate area populations have frequently been misclassified into PA and WB populations in the canonical variate analysis. Therefore, they are thought to have intermediate morphological characters between PA and WB populations. To hypothesize their origin, we should treat populations in the Chugoku area and Iwate population separately, because the histories of these populations must be quite different due to their geographical separation. While cedar existed in the Chugoku area (offshore of San-in) during the last glacial period, cedar was not distributed in Iwate before colonization from the refugial populations (Tsukada, 1982).

Among the Chugoku area populations, the first canonical variate showed a high similarity, and no significant differences were detected in multiple comparisons of MANOVAs for both sexes. Morphologies of populations in the Chugoku area may not be so heterogeneous and so we discuss them together. Why do populations in the Chugoku area have intermediate morphologies? Since the backbone range in the Chugoku area may not have been high enough to prevent borer movement between the Japan Sea and Pacific Ocean sides, it is possible that lineages of PA and WB made contact and formed hybrids in the Chugoku area. Another possibility is that the intermediate morphology is of an endemic population that persisted in cedar stands along the San-in region during the last glaciations.

In contrast, the Iwate population cannot have been endemic since the last glaciation. A population of the beetle probably did not exist in Iwate before the cedar reached the Tohoku area, so the immigration routes of the cedar may be the most important factor for the creation of *S. japonicus* populations there. Tsukada (1982) suggested two routes of postglacial colonization into the Tohoku area for *C. japonica*, and the Pacific and the Japan Sea populations of the cedar were suggested to have intermingled in this area. Considering the dispersal route of the cedar, the Iwate population could thus be an offspring of the PA and/or WB populations. The intermediate morphology suggests the population may be a hybrid between them. The *S. japonicus* population of Iwate seems to have been created as a consequence of the two distinct dispersal processes of *C. japonica* in the area.

Since Shoda (2000) showed spot patterns to be a heritable trait in rearing experiments, some of the variation in characters of spots is due to genetic difference and the morphological variations elucidated above are probably due to genetic differentiation. However, as food qualities and climatic conditions differ among localities, environmental effects may also be responsible for the morphological differences. The environmental effects could be assessed by further investigations, for example, using rearing experiments under constant conditions, measuring heritability for each character or contrasting morphological variation to genetic divergence in molecular data.

While vicariance is no longer a factor due to the expansion of the distribution through intensive plantation of the cedar, past vicariance events may have been important enough to influence the present genetic structure. Concerning the last ice age, processes of genetic diversification and formation of phylogeographic structures have been deduced with recent advances in molecular makers (e.g. Hewitt, 1996, 1999). Molecular analysis is needed to trace the diversification in *S. japonicus* in more detail.

Finally, we should exercise some caution in applying the results of previously conducted experiments. In addition to the morphological variation mentioned above, physiological traits in *S. japonicus* might have differentiated among populations. The sum of effective temperatures and lower developmental thresholds of the egg stage of the borer differed between Kyoto and Ibaraki (Kitajima, 1993). Kitajima suspected this difference to be due to experimental errors, but they might instead have been ascribable to genetic differentiation between PA and WB populations. In applying the results of experimental studies to decision-making concerning protection of the cedar from the borer, we should thus be aware of the location borers were collected.

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