Habitat-related mtDNA polymorphism in the stored-bean pest *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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Abstract

The genetic diversity of populations of the azuki bean beetle, *Callosobruchus chinensis* (Linnaeus) from natural, pre-harvest and post-harvest sites, was investigated to understand population structure and gene flow. A 522-bp fragment of the mitochondrial gene COI was sequenced for eight populations of *C. chinensis* from Japan, Korea and Taiwan collected from different habitats. Six haplotypes were detected, one of which, U1, occurred most frequently and widely. The following hypotheses were tested as a cause of the wide distribution of haplotype U1; (i) topographical separation (by national boundaries), (ii) host plant species, and (iii) habitat type (natural, pre-harvest crop, or post-harvest storage). Categorization of collection sites by country or by host species did not yield differences in the occurrence of haplotype U1, but habitat type did. Populations utilizing cultivated post-harvest hosts that were mass stored were highly likely to be the common haplotype, whereas host plants in natural habitats away from agriculture were utilized by populations with locally characteristic haplotypes. Sampling of commercial beans for quarantine and export purposes indicated that gene flow in *C. chinensis* was largely unidirectional into Japan at the present time.

Introduction

One characteristic of the ecology of seed-eating insects is their ability to develop into pests of post-harvest crops as a result of bulk storage of these natural food resources, i.e. as stored-product pests. This procedure facilitates the spread of these pests because they damage crops not only in the field but also in storage by primary infestation (i.e. storage of infested crops direct from the field) and secondary or 'cross' infestation (i.e. transfer of contaminated produce between stores) of intact stored products (Southgate, 1979). Whereas it is evident that storage populations have been initially established from populations in the wild, how frequently pest populations that develop in storage eventually return to wild hosts is less well understood.

International trade by humans has unintentionally spread such pests worldwide through the transportation of

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Table 1. Geographical origin and legume hosts of *Callosobruchus chinensis* populations used in the current study.

Collection site and year	Country	Host legume	Habitat of host	*n*
1 Ninohe, Iwate, 1999	Japan	*V. angularis*	Preharvest bean field, 2.2 adults m^{-2}	15
2 Kasukabe, Saitama, 1998	Japan	*V. angularis*	Preharvest bean field, > 30 adults m^{-2}	15
3 Mino, Gifu, 1998	Japan	*V. unguiculata*	Preharvest bean field, 22 adults m^{-2}	15
4 Imazu, Shiga, 1999	Japan	*V. angularis* var. *nipponensis*	Native, no bean fields nearby, 8% seed infestation	15
5 Seoul, Kyonggi, 2002	Korea	*V. radiata*	Market sample, 4% seed infestation	20
6 Hwaseong, Kyonggi, 2002	Korea	*V. angularis*	Postharvest storage, no crops nearby	15
7 Tainan, Tainan, 2002	Taiwan	*V. radiata*	Postharvest storage, nearest crop 3 km, 6% seed infestation	20
8 Kenting, Pingtung, 1997, 1999, 2002	Taiwan	*Rhynchosia minima*	Native, nearest crop 8 km, 8% seed infestation	5

n = the number of adult *C. chinensis* examined.

infested commercial crops. In some cases, the geographical origins of the pests are obvious because transportation has been from a native region to non-native regions. In other cases, however, transportation may include secondary exportation from non-native, invaded regions to uninvaded regions that confounds the detection of the route(s) of introduction (e.g. Tuda *et al.*, 2001). Recent applications of DNA sequence analysis of mitochondrial and nuclear genes have proved useful for resolving geographical population structure, inferring dispersal events from a common gene pool, and the phylogenetic relationship of widely distributed pests (Sperling & Hickey, 1994; Navajas *et al.*, 1998; Davies *et al.*, 1999; Landry *et al.*, 1999; Mun *et al.*, 1999; Cognato & Sperling, 2000; Shufran *et al.*, 2000; Tsutsui *et al.*, 2001; Tuda *et al.*, unpublished). Because the mitochondrial genome is maternally inherited and its effective population size is one quarter that of the nuclear genome, it is more subjected to random processes. Therefore, mitochondrial loci can track recent histories more precisely than nuclear loci (Moore, 1995).

The azuki bean beetle, *Callosobruchus chinensis* (Linnaeus) (Coleoptera: Bruchidae), is a pest of stored legumes, that originated in East Asia. *Callosobruchus chinensis* larvae utilize a variety of commercially distributed dried legume seeds as their hosts, primarily *Vigna* species (tribe Phaseoleae: subtribe Phaseolinae) and genera such as *Cajanus* (subtribe Cajaninae) and *Lens* (tribe Fabeae) (Johnson, 1981; Udayagiri & Wahdi, 1989; Nahdy *et al.*, 1998). Both cultivated and wild varieties of *Vigna angularis* (Willd.) Ohwi & Ohashi are major hosts of *C. chinensis* in Japan (Shinoda & Yoshida, 1990; Shinoda *et al.*, 1991; Tomooka *et al.*, 2000). The highest rates of survival (> 70%) to the adult stage of a Japanese population of *C. chinensis* under laboratory conditions were recorded on *V. angularis* and *V. radiata* (L.) Wilczek (Shinoda & Yoshida, 1990; Shinoda *et al.*, 1991; Tomooka *et al.*, 2000). As is typical of the life history of the Bruchidae, larvae of *C. chinensis* burrow into legume seeds on hatching and consume the cotyledons. They pupate and metamorphose into adults within the seed and emerge to seek mates and new hosts. Flight range is up to 2 km (Shinoda & Yoshida, 1990), and long-distance, transoceanic dispersal via air currents is assumed to be virtually impossible for weak-flying bruchid beetles, as they have never been observed crossing Asian oceans (Yoshimoto & Gressitt, 1959; Harrell & Holzapfel, 1966; Holzapfel & Perkins, 1969). The major consumption of azuki beans in the world today occurs largely in Japan, which not only produces the beans but also imports large quantities from China and Korea as well as

from several non-East Asian countries (Plant Quarantine Office, 2000; Watanabe, 2000).

The aim of the present study was to investigate factors affecting population structure of *C. chinensis* by examining the intraspecific mitochondrial COI variation of populations from pre- and post-harvest hosts. It was hypothesized that the detected distribution of haplotypes might be due to (i) topographical separation (by national boundaries), (ii) host plant species, and (iii) habitat type.

Materials and methods

Insect samples

Wild legume seeds, beans from cultivated fields (i.e. pre-harvest) and commercially stored beans (post-harvest) were collected from eight sites in Japan, Korea and Taiwan (table 1). The collected legumes were commercially grown beans of *Vigna angularis* (azuki), *V. radiata* (mung), *V. unguiculata* (L.) Walp. (previously named *V. sinensis* (L.) Savi ex Hassk.), and the wild legumes *V. angularis* var. *nipponensis* (Ohwi) Ohwi & Ohashi and *Rhynchosia minima* (L.) DC (Cajaninae) (table 1), all of which belong to the tribe Phaseoleae. Infestation of beans purchased in markets were low in all three countries investigated: in Taiwan for example, infestation by *C. chinensis* was detected in only three of 21 samples of purchased azuki and mung beans (each > 3000 beans) and the species that emerged most frequently from the beans was *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Each sample of stored beans consisted of approximately 1000–10,000 beans (samples 5–7 in table 1). For wild hosts, 100 pods or 900 seeds of *Vigna angularis* var. *nipponensis* (sample 4) and 40–100 seeds of *R. minima* (sample 8) were collected each year (Tuda *et al.*, in press). Live *C. chinensis* adults that emerged from collected seeds were preserved in acetone or subjected directly to DNA extraction. Laboratory populations (strains 1–3 in table 1) were established from adults collected from bean fields, in which case it was assumed that the plants were going to be utilized as hosts for the next generation. These populations were maintained as multiple isofemale lines (Kondo *et al.*, 2002) and single individuals of different maternal lines were subjected to DNA analyses.

Molecular methods

DNA was extracted from single adult (either live, dried or acetone-preserved) *C. chinensis* using the DNeasy tissue

kit (Qiagen, Japan), following the manufacturer's instructions. The primers, 5′-CTTTATCAACATT-TATTTTGATTTTTT-3′ (Tuda *et al.*, 1995) and 5′-TACTCCAATAAATATTATAATAAATTG-3′ were used to amplify a 522-bp segment of the cytochrome oxidase subunit I (COI) mtDNA gene. Reaction mixtures, in 50-µl total volumes [1.25-unit DNA polymerase (AmpliTaqR, Perkin Elmer), 5 µl dNTPs (10 mM each), 1 µl each of forward and reverse primers (10 ng), 5 µl 10× reaction buffer, 3 µl MgCl$_2$ (25 mM), 32 µl sterile water, and 3 µl DNA template (50–100 ng in total)] were subjected to 35 cycles of 94°C for 30 s, 45°C for 1 min, and 60°C for 3 min, in a thermal cycler (GeneAmp® PCR System 9600, Applied Biosystems Division of Perkin Elmer). Amplification products were excised from 1.0% agarose gels (SeaKem® GTG agarose, BioWhittaker Molecular Applications) following electrophoresis, and purified using glass powder (Easytrap ver. 2, Takara). The excised PCR products were then labelled using a BigDye™ terminator cycle sequencing ready reaction kit (Applied Biosystems) and purified using spin columns (Centri-sep, Applied Biosystems). The samples were electrophoresed using an automated DNA sequencer (ABI PRISM^R 377, Applied Biosystems). Pairwise genetic distances were estimated as Kimura's two-parameter distance (Kimura, 1980), with a transition/transversion ratio set at 2.0, by Dnadist program within PHYLIP 3.6a (Felsenstein, 2000) to compare to those from other studies. Uncorrected distances were also calculated for this purpose. The sequences were deposited in GenBank under Accession Nos. AY265224 (U1), AY265225 (U2), AY265226 (J), AY265227 (T1), AY265228 (T2) and AY265229 (T3).

Statistical analysis

Kruskal-Wallis tests were performed on the effects of national boundary, host species and habitat type on the frequency of the most common haplotype. A non-parametric multiple comparison test was applied when a significant among-group difference in the frequencies of the U1 haplotype was detected (Zar, 1996).

Results and Discussion

Haplotype distribution

There was no variation in length of the 522-bp COI segment amplified and the total span of the segment was aligned and subjected to analysis. The nucleotide frequencies were 66.0% A+T (30.4% A, 35.6% T) and 34.0% C+G (17.3% C, 16.8% G), and thus AT rich, as typically observed for insect COI. All base substitutions were confirmed as synonymous.

Six COI haplotypes referred to as U1, U2, J, T1, T2 and T3, were detected among the 120 individuals examined from the eight populations of *C. chinensis* obtained during the survey. Pairwise nucleotide divergence ranged from 0.19 to 5.1% (fig. 1, see the next section). Individuals collected at sites distant from areas of human population and bean production in Taiwan and Japan had locally characteristic haplotypes T1, T2, T3 and J (fig. 1). The U1 haplotype was the most frequent overall (70.0%), and was recorded from Taiwan, Korea and throughout Japan, from the northeastern part of the main-island Honshu to the Ryukyu Islands in the south (fig. 1, Tuda *et al.*, unpublished data).

The population frequencies of the U1 haplotype were independent of country ($H_{2,2,4} = 2.59$, $P > 0.1$) and host species ($H_{1,1,2,4} = 3.65$, $P > 0.1$). The frequencies of the U1 haplotype were significantly different according to habitat ($H_{2,3,3} = 6.40$, $P < 0.02$, fig. 2) and particularly between natural and stored samples, with populations infesting cultivated post-harvest legumes more likely to be of the U1 haplotype (nonparametric multiple comparison, corrected for tied ranks, $Q = 2.49$, $k = 3$, $P < 0.05$). There were no significant differences between natural and pre-harvest crop ($Q = 1.13$, $k = 3$, $P > 0.5$) and between pre-harvest and stored crops ($Q = 1.52$, $k = 3$, $P > 0.2$).

Genetic distance

The genetic distances among COI haplotypes of *C. chinensis* estimated by Kimura's two-parameter method, as well as uncorrected distances, are shown in table 2. Haplotypes from wild populations from Taiwan (T1, T2 and T3) were genetically distant from the other three haplotypes (U1, U2 and J) and variable within populations (up to 1.16%), whereas the genetic divergences between the haplotypes U1, U2, and J were small (0.38% at most) (table 2). The maximum genetic variability was 5.1%, observed between haplotypes J and T3 from Japan and Taiwan, respectively.

Possible causes of the haplotype distribution

Populations of *C. chinensis* found in commercial beans sold on the open market frequently shared a single common haplotype, U1, irrespective of geographical region and host plant species, while locally characteristic haplotypes were found under more natural conditions, distant from commercial bean fields (fig. 2). It is possible that in particular regions, populations are selected for their ability to utilize dried beans. For stored product pests such as the present species, international trade of crops by humans allows even longer transcontinental and transoceanic dispersal throughout the year. In the case of bruchids, dried infested beans serve not only as a vehicle but also as a protection against unfavourable conditions during transportation. Although typhoons can be a powerful means for seasonal transportation of migrant insects across Asia, as in air-borne transoceanic dispersal of planthoppers (Kishimoto, 1975; Mun *et al.*, 1999), Coleoptera have rarely been trapped above the East China Sea (e.g. Kishimoto, 1975). Long-distance, transoceanic dispersal in air currents would be even more difficult for weak-flying beetles such as bruchids, and significantly, they have never been observed on Asian oceans (Yoshimoto & Gressitt, 1959; Harrell & Holzapfel, 1966; Holzapfel & Perkins, 1969). Thus, it is concluded that long-range aerial dispersal of bruchids does not occur and that dispersal over long distances is a result of human assistance.

The population sampled from *R. minima* growing under natural conditions in Taiwan was genetically variable and the maximum genetic variability of 5.1% was found between this population and haplotype J from Japan. This level of intraspecific variation in COI in *C. chinensis* is of a similar order to that recorded from other Coleoptera (3.8–4.9% Funk *et al.*, 1995; Cognato & Sperling, 2000, the former corrected by Kimura's method, the latter uncorrected) and in
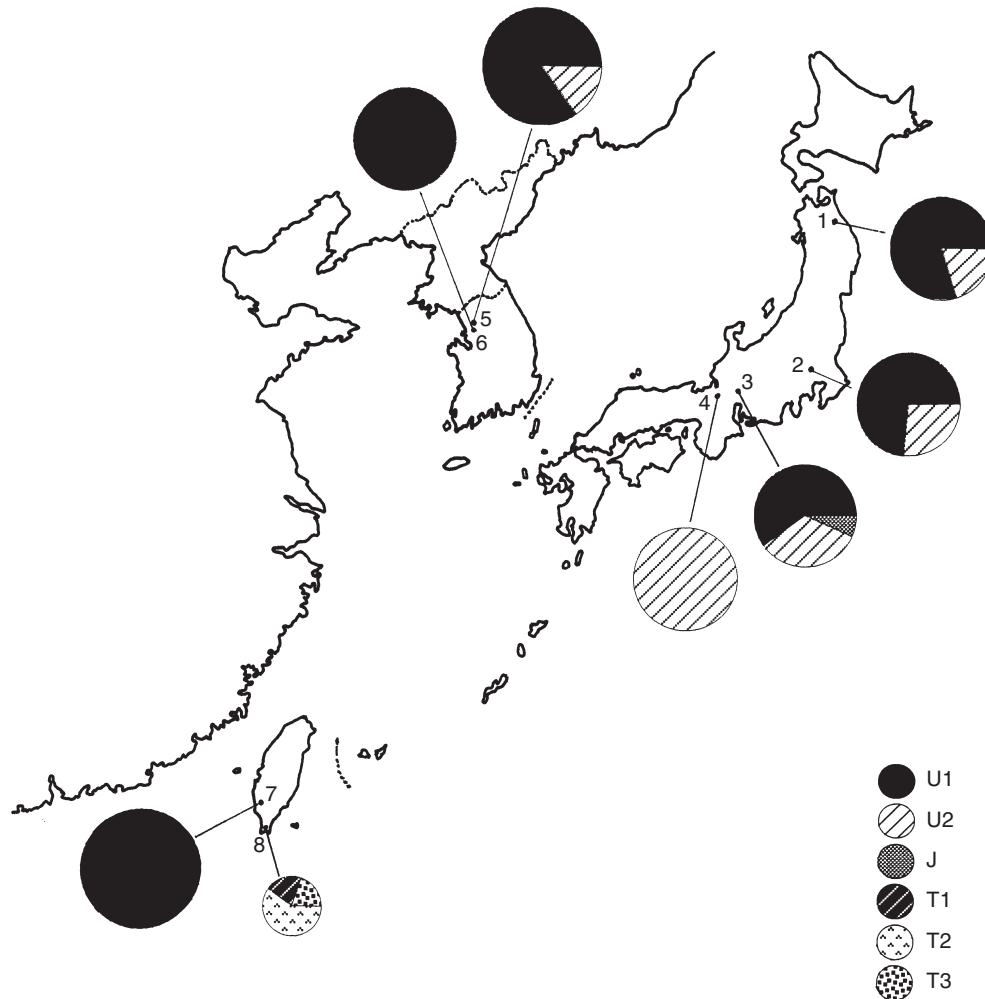
Fig. 1. Map showing the haplotype distribution of *Callosobruchus chinensis* in Japan, Korea and Taiwan.
Numbers for local populations correspond to those in table 1. Broken lines indicate national boundaries.

Lepidoptera (5.7%, corrected by Kimura's method, Brown *et al.*, 1994). The marginal difference in the COI haplotypes between the population from *R. minima* and the haplotype group with 1-bp difference from U1 suggests a measure of isolation and a possibility of the former becoming a cryptic species. Local association of one of the Taiwanese populations of *C. chinensis* with a wild host (*R. minima*) may have restricted gene flow from this population to pest populations that use cultivated beans as hosts (fig. 1).

Origin and maintenance of genetic homogeneity among populations of *C.* chinensis

Two conditions may have kept the pest populations of *C. chinensis* isolated from others found on wild hosts. Firstly, the low water content of stored beans may have benefited the flightless (sedentary) form of *C. chinensis* (Nahdy *et al.*, 1999) as in *C. maculatus* (Ouedraogo *et al.*, 1991). By having an abundant and often continuous supply of uninfested dry stored beans, the flightless variety may have prospered

Table 2. Genetic distances among haplotypes of *Callosobruchus chinensis* as estimated by Kimura's two-parameter method (above diagonal) and uncorrected distances (below diagonal).

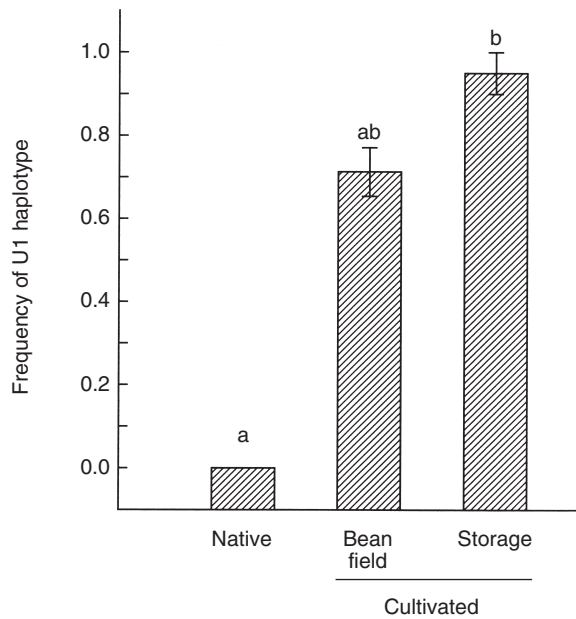|	U1	U2	J	T1	T2	T3
U1	–	0.0019	0.0019	0.0392	0.0453	0.0493
U2	0.0019	–	0.0038	0.0372	0.0432	0.0473
J	0.0019	0.0038	–	0.0412	0.0473	0.0513
T1	0.0383	0.0364	0.0402	–	0.0058	0.0096
T2	0.0441	0.0421	0.0460	0.0057	–	0.0116
T3	0.0479	0.0460	0.0498	0.0096	0.0115	–

Fig. 2. Mean frequencies of the U1 haplotype of *Callosobruchus chinensis* from hosts of different habitats (natural, pre-harvest crops and post-harvest storage). Error bars show ± SE. Letters above bars indicate the result of non-parametric multiple comparisons.

without a need to fly and locate a new supply of wild beans. Secondly, as this process is coupled with the international trade of commercial dried beans to non-native regions, then large-scale genetic mixing and homogenization would be accelerated in such pest species. Indeed, a reduction in genetic variability during colonization has been reported in the medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (e.g. Guglielmino, 1998; Malacrida *et al.*, 1998).

Although the universal occurrence of the U1 haplotype is probably the result of international trade in stored products, an ancestor hypothesis is also possible, in which the most abundant haplotype is considered ancestral as coalescent theory predicts (Watterson & Guess, 1977; Donnelly & Tavare, 1986). Underlying this latter hypothesis, however, is the implicit assumption of low levels of gene flow, which in the present species is often violated, especially in the pest populations.

Genetic variability in Japanese populations

Populations of *C. chinensis* from islands in Japan have low genetic variability (0.19–0.38%) relative to the population collected from a native legume, *R. minima*, on the island of Taiwan, in spite of their similar geographical isolation from mainland Eurasia. Whether *C. chinensis* is native or introduced to Japan is unknown but, by the 1830s, during the Edo Period, it had become one of the most commonly observed insects in Tokyo at a time which almost all international trade with Japan was forbidden (Tanaka, 1998). In more recent times, azuki beans and soybeans have been shipped repeatedly to Japan from the north-eastern province of China where azuki beans have long been cultivated, without any inspection for pests until 1950, when quarantine controls were introduced on imported produce

(Okajima & Shida, 1986). Today, the infestation levels of imported dried *V. angularis* and *V. radiata* by *C. chinensis* are among the highest found in commercial beans in Japan, about 90% of which come from mainland China (Plant Quarantine Office, 1964, 1965, 1966, 1967, 1973, 1974, 1993, 2000). By contrast, no *C. chinensis* has been detected in beans exported from Japan (Plant Quarantine Office, 1973, 2000). Therefore, present gene flow would appear to be highly unidirectional and limited source populations in combination with small effective population size of mitochondrial genes could account for the relatively low genetic variability found in Japanese populations.

Examination of genetic variability among native populations from mainland China is needed in order to determine possible source populations of the haplotype U1. Furthermore, comparison of the intraspecific population structure of this species with that of another widely distributed stored-product pest, *C. maculatus*, and of a non-pest closer relative *Callosobruchus utidai* Tuda (Tuda, 2003) should assist in our understanding of the origins and processes of becoming stored-product pests in this genus.

References

Brown, J.M., Pellmyr, O., Thompson, J.N. & Harrison, R.G. (1994) Phylogeny of *Greya* (Lepidoptera: Prodoxidae) based on nucleotide sequences variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. *Molecular Biology and Evolution* **11**, 128–141.

Cognato, A.I. & Sperling, F.A.H. (2000) Phylogeny of *Ips* DeGeer species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I DNA sequence. *Molecular Phylogenetics and Evolution* **14**, 445–460.

Davies, N., Villablanca, F.X. & Roderick, G.K. (1999) Bioinvasions of the medfly *Ceratitis capitata*: source estimation using DNA sequences at multiple intron loci. *Genetics* **153**, 351–360.

Donnelly, P. & Tavare, S. (1986) The ages of alleles and a coalescent. *Advances in Applied Probability* **18**, 1–19.

Felsenstein, J. (2000) *Phylogeny inference package (Phylip), version 3.6a.* Department of Genetics, University of Washington, Seattle.

Funk, D.J., Futuyma, D.J., Orti, G. & Meyer, A. (1995) Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagous beetles (Chrysomelidae: *Ophraella*). *Molecular Biology and Evolution* **12**, 627–640.

Guglielmino, C.R. (1998) Genetic aspects of the worldwide colonization process of *Ceratitis capitata*. *Journal of Heredity* **89**, 501–507.

Harrell, J.C. & Holzapfel, E. (1966) Trapping of air-borne insects on ships in the Pacific, Part 6. *Pacific Insects* **8**, 33–42.

Holzapfel, E.P. & Perkins, B.D.Jr (1969) Trapping of air-borne insects on ships in the Pacific, Part 7. *Pacific Insects* **11**, 455–476.

Johnson, C.D. (1981) Seed beetle host specificity and the systematics of the Leguminosae. pp. 995–1027 *in* Polhill, R.M. & Raven, P.H. (*Eds*) *Advances in legume systematics Part 2*. Royal Botanical Gardens, Kew.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Kishimoto, R. (1975) Planthoppers migrating over sea. Chuo Koron, 233 pp. (in Japanese).

Kondo, N., Ijichi, N., Shimada, M. & Fukatsu, T. (2002) Prevailing triple infection with *Wolbachia* in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Molecular Ecology* **11**, 167–180.

Landry, B., Powell, J.A. & Sperling, F.A.H. (1999) Systematics of the *Argyrotaenia franciscana* (Lepidoptera: Tortricidae) species group: evidence from mitochondrial DNA. *Annals of the Entomological Society of America* **92**, 40–46.

Malacrida, A.R., Marinoni, F., Torti, C., Gomulski, L.M., Sebastiani, F., Bonvicini, C., Gasperi, G. & Guglielmino, C.R. (1998) Genetic aspects of the worldwide colonization process of *Ceratitis capitata*. *Journal of Heredity* **89**, 501–507.

Moore, W.S. (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49**, 718–726.

Mun, J.H., Song, Y.H., Heong, K.L. & Roderick, G.K. (1999) Genetic variation among Asian populations of rice planthoppers, *Nilaparvata lugens* and *Sogatella furcifera* (Hemiptera: Delphacidae): mitochondrial DNA sequences. *Bulletin of Entomological Research* **89**, 245–253.

Nahdy, M.S., Ellis, R.H., Silim, S.N. & Smith, J. (1998) Field infestation of pigeonpea (*Cajanus cajan* (L.) Millsp.) by *Callosobruchus chinensis* (L.) in Uganda. *Journal of Stored Products Research* **34**, 207–216.

Nahdy, M.S., Silim, S.N. & Ellis, R.H. (1999) Effect of field infestations of immature pigeonpea (*Cajanus cajan* (L.) Millsp.) pods on production of active (flight) and sedentary (flightless) morphs of *Callosobruchus chinensis* (L.). *Journal of Stored Products Research* **35**, 339–354.

Navajas, M., Lagnel, J. & Gutierrez, J. (1998) Species-wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. *Heredity* **80**, 742–752.

Okajima, H. & Shida, Y. (1986) *Hansho no sho*. Tokyo, Nosongyoson Bunka Kyokai (translated into Japanese).

Ouedraogo, P.A., Monge, J.P. & Huignard, J. (1991) Importance of temperature and seed water content on the induction of imaginal polymorphism in *Callosobruchus maculatus*. *Entomologia Experimentalis et Applicata* **59**, 59–66.

Plant Quarantine Office (1964) *Plant Quarantine Statistics*, **29**.

Plant Quarantine Office (1965) *Plant Quarantine Statistics*, **30**.

Plant Quarantine Office (1966) *Plant Quarantine Statistics*, **31**.

Plant Quarantine Office (1967) *Plant Quarantine Statistics*, **32**.

Plant Quarantine Office (1973) *Plant Quarantine Statistics*, **40**.

Plant Quarantine Office (1974) *Plant Quarantine Statistics*, **41**.

Plant Quarantine Office (1993) *Plant Quarantine Statistics*, **60**.

Plant Quarantine Office (2000) *Plant Quarantine Statistics*, **67**.

Shinoda, K. & Yoshida, T. (1990) The life history of the azuki bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), in the field. pp. 149–159 *in* Fujii, K., Gatehouse, A.M.R., Johnson, C.D., Mitchel, R. & Yoshida, T. (*Eds*) *Bruchids and legumes: economics, ecology and coevolution*. Dordrecht, Kluwer.

Shinoda, K., Yoshida, T. & Okamoto, T. (1991) Two wild leguminous host plants of the azuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). *Applied Entomology and Zoology* **26**, 91–98.

Shufran, K.A., Burd, J.D., Anstead, J.A. & Lusha, G. (2000) Mitochondrial DNA sequence divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host-adapted races. *Insect Molecular Biology* **9**, 179–184.

Southgate, B.J. (1979) Biology of the Bruchidae. *Annual Reviews of Entomology* **24**, 449–473.

Sperling, F.A.H. & Hickey, D.A. (1994) Mitochondrial-DNA sequence variation in the spruce budworm species complex (*Choristoneura*, Lepidoptera). *Molecular Biology and Evolution* **11**, 656–665.

Tanaka, M. (1998) Insects depicted in Edo Period atlases. *Insectarium* **35**, 12–20 (in Japanese).

Tomooka, N., Kashiwaba, K., Vaughan, D.A., Ishimoto, M. & Egawa, Y. (2000) The effectiveness of evaluating wild species: searching for sources of resistance to bruchid beetles in the genus *Vigna* subgenus *Ceratotropis*. *Euphytica* **115**, 27–41.

Tsutsui, N.D., Suarez, A.V., Holway, D.A. & Case, T.J. (2001) Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. *Molecular Ecology* **10**, 2151–2161.

Tuda, M. (2003) A new species of *Callosobruchus* (Coleoptera: Bruchidae) feeding on seeds of *Dunbaria* (Fabaceae), a closely related species to a stored-bean pest, *C. chinensis*. *Applied Entomology and Zoology* **38**, 197–201.

Tuda, M., Fukatsu, T. & Shimada, M. (1995) Species differentiation of bruchid beetles (Coleoptera: Bruchidae) analyzed by mitochondrial DNA polymorphism. *Applied Entomology and Zoology* **30**, 377–380.

Tuda, M., Shima, K., Johnson, C.D. & Morimoto, K. (2001) Establishment of *Acanthoscelides pallidipennis* (Coleoptera: Bruchidae) feeding in seeds of the introduced legume *Amorpha fruticosa*, with a new record of its *Eupelmus* parasitoid in Japan. *Applied Entomology and Zoology* **36**, 269–276.

Tuda, M., Chou, L.-Y., Niyomdham, C., Buranapanichpan, S. & Tateishi, Y. Ecological factors associated with pest status in *Callosobruchus* (Coleoptera: Bruchidae): high host specificity of non-pests to Cajaninae (Fabaceae). *Journal of Stored Products Research,* in press.

Udayagiri, S. & Wadhi, S.R. (1989) *Catalog of Bruchidae*. American Entomological Institute, Gainesville.

Watanabe, T. (2000) *Cyclopedia of beans*. Tokyo, Saiwai-shobo (in Japanese).

Watterson, G.A. & Guess, H.A. (1977) Is the most frequent allele the oldest? *Theoretical Population Biology* **11**, 141–160.

Yoshimoto, C.M. & Gressitt, J.L. (1959) Trapping of air-borne insects on ships on the Pacific, Part 2. *Proceedings of the Hawaiian Entomological Society* **17**, 150–155.

Zar, J.H. (1996) *Biostatistical analysis*, 3rd edn. New Jersey, Prentice Hall.