Genetic diversity of *Torymus* species among islands off Kyushu

Midori Tuda,[1*] Kazunori Matsuo,[1] Kaori Yara,[2] Kumiko Kagoshima,[1] Yôzô Murakami,[3] Nobuo Ohkubo [4] and Seiichi Moriya[5]

[1]Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

[2]National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba 305-8604, Japan

[3]1-28-28 Minamikatae, Jyonan-ku, Fukuoka 814-0143, Japan

[4] 1-94-3 Uematsu, Omura, Nagasaki 856-0027, Japan

[5] National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan

[*]tuda@grt.kyushu-u.ac.jp

Introduction

Torymus sinensis Kamijo (Hymenoptera: Torymidae) was introduced from China to Japan in 1975 to control the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a serious pest of chestnuts that originated in China. The distribution and biology of native, congeneric *T. beneficus* Yasumatsu et Kamijo, which is indistinguishable from *T. sinensis* except for its short ovipositor, was less well known at the time of *T. sinensis* introduction. Later, two emergence types of *T. beneficus* were found. One of them was recently shown by molecular analysis to be more closely related to *T. sinensis* (Yara, 2004). Past surveys revealed that *T. beneficus* is distributed sporadically in various parts of Japan, including small islands off the main island.

Indigenous *T. sinensis* inhabits the Tsushima Islands, which are located in the Tsushima Straits between Korea and Kyushu (the southernmost big island of Japan) (Ohkubo, 1992). An attempt to introduce Chinese *T. sinensis* for control in 1989 was made without recognizing the native conspecific populations (Murakami, 1997), and whether the introduced population was successfully established on the island has been unknown to date. The seasonal prevalence of the Tsushima population was shown to be similar to that of the South Korean populations (Murakami *et al*., 1993).

In this study our goal was to understand divergence and similarity of the *Torymus* fauna within/among the islands off Kyushu, Korea and China, with respect to genetic and species diversity.

Materials and Methods

Dry chestnut galls were collected from islands off Kyushu and Korea, namely, Tsushima, Oki, Goto and Jeju, in either February 2002 or February 2009 (Fig. 1). For comparison, chestnut galls were also collected from Kyushu (Ozu in 2000 and 2001), Gyeongsangnam-do (southern part of the Republic of Korea in 1992) and Liaoning Province (northeastern part of China in 1993), which may serve as natural source populations of *T. sinensis*. Furthermore, to test a possible effect of

geographic distance from the source populations, galls were collected from Oshima Island off Sagami Bay near the main island of Japan. Emerging adults were collected and frozen until use.

Fig. 1. Collection sites of chestnut galls for *Torymus* spp., parasitoids of chestnut gall wasps.

Crude DNA was extracted from the adults with a DNeasy tissue kit (Qiagen). Mitochondrial cytochrome oxidase subunit I (COI) and nuclear internal transcribed spacer 2 (ITS2) were sequenced, using the primers designed by Yara (2004, 2006) and Tuda *et al*. (2004). Newly designed primers were also used when these failed to amplify. A parsimony haplotype network (Templeton *et al*., 1992) was estimated, using TCS 1.21 (Clement *et al*., 2000).

To compare the key morphological traits separating the two *Torymus* species, we measured the length of the ovipositor sheath for females from these populations.

Results and Discussion

DNA sequences of 101 individuals, including a single *T. koreanus*, and 30 additional sequences from Genbank were analyzed. The parsimony network of COI identified three major clades (subgroups 1-3 *sensu* Yara, 2004) for *T. sinensis sensu lato* (Fig. 2). Two of these were *T. sinensis*, and the other was previously called the late emergence type of '*T. beneficus*' (Fig. 2). The early emergence type of *T. beneficus* was clustered in a single clade (Fig. 2). These clusters were as suggested by Yara (2004).

One female from Korea was *T. koreanus*. Both COI and ITS2 successfully distinguished this species from two other *Torymus* species. This individual was closer to *T. beneficus* than to *T. sinensis*, judging from the base substitution rates of COI.

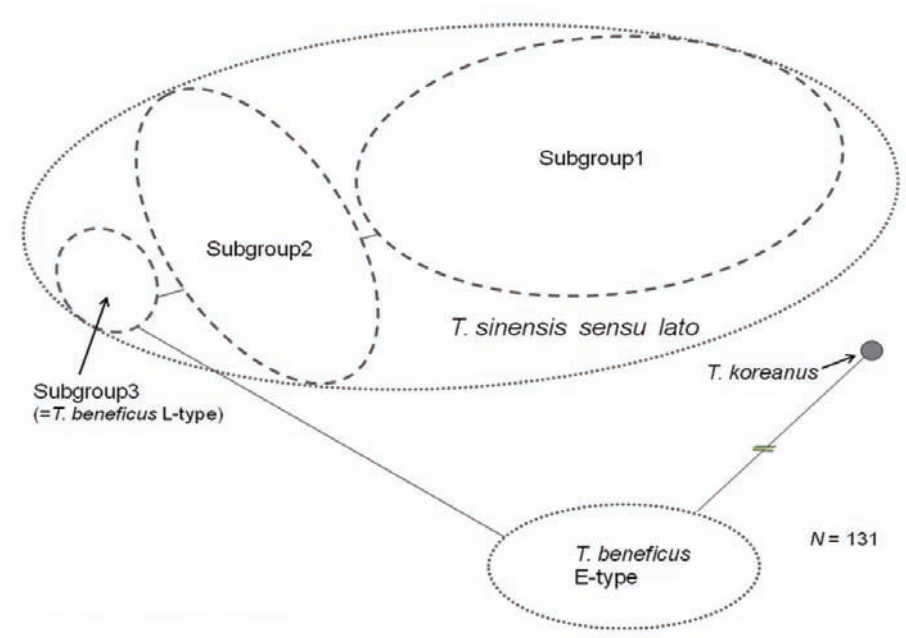

Fig. 2. Parsimony network of COI haplotypes of *Torymus* spp.

The Tsushima population was similar to the Korean population, in that the two populations shared many haplotypes that belonged only to subgroup 2 (Fig. 2). This genetic evidence supports the similarity between the two populations in terms of their ecology (i.e., seasonal prevalence, Murakami *et al*., 1993). By contrast, there were no shared haplotypes between the Tsushima and Chinese populations, indicating that the attempt to introduce a Chinese population was probably unsuccessful.

The Goto and Iki populations had not only unique haplotypes but also haplotypes identical to those from nearby islands and Korea. Interestingly, a single *T. sinensis* female possessed a mitochondrial haplotype and nuclear genotype of different emergence types of '*T. beneficus*'. This provides good evidence that these coexisting *Torymus* species are undergoing introgression and/or hybridization, although such incidence may be infrequent and/or the descendants of an introgressed line or hybrids may have low fitness.

The length of the ovipositor sheath was shorter for the Chinese *Torymus* species population than that of other populations. This probably reflects selection and/or maternal effect through chestnut gall size at the time of parasitization by *Torymus*.

Acknowledgments

This study was supported in part by a Grant-in-Aid (B) (19380037) from JSPS.

References

Clement, M., D. Posada and K. A. Crandall (2000) TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9: 1657–1659.

Murakami, Y. (1997) *Natural enemies of the chestnut gall wasp.* Kyushu University Press, Fukuoka. 308 pp. (In Japanese)

Murakami, Y., N. Ohkubo and Y. Gyoutoku (1993) Origin of *Torymus* (*Syntomaspis*) *sinensis* native to Tsushima Islands. *Proc. Assoc. Pl. Prot. Kyushu* 39: 124-126. (In Japanese with English summary)

Murakami, Y., N. Ohkubo, S. Moriya, Y. Gyoutoku, C.-H. Kim and J.-K. Kim (1995) Parasitoids of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in South Korea with particular reference to ecologically different types of *Torymus* (*Syntomaspis*) *sinensis* (Hymenoptera: Torymidae). *Appl. Entomol. Zool.* 30: 277-284.

Ohkubo, N. (1992) Distribution of parasitoids of chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu in Nagasaki Prefecture. *Proc. Assoc. Pl. Prot. Kyushu* 38: 190-192. (In Japanese)

Templeton, A. R., K. A. Crandall and C. F. Sing (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619–633.

Tuda, M., N. Wasano, N. Kondo, S.-B. Horng, L.-Y. Chou and Y. Tateishi (2004) Habitat-related mtDNA polymorphism in a stored-bean pest *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Bull. Entomol. Res*. 94: 75-80.

Yara, K. (2004) Relationship between the introduced and indigenous parasitoids *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae) as inferred from mt-DNA (COI) sequences. *Appl. Entomol. Zool.* 39: 427–433.

Yara, K. (2006) Identification of *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae), introduced and indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), using the ribosomal ITS2 region. *Biol. Control* 36: 15-21.