

Short Communication

Molecular Evidence of the Dispersal of Lyme Disease *Borrelia* from the Asian Continent to Japan via Migratory Birds

Fubito Ishiguro*, Nobuhiro Takada¹ and Toshiyuki Masuzawa²

Fukui Prefectural Institute of Public Health and Environmental Science, Fukui 910-8551,

¹Department of Pathological Sciences, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193 and

²Department of Microbiology, Faculty of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan

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SUMMARY: Based on specific sequencing, we found that a *Borrelia garinii* strain from a rodent in Fukui Prefecture, Japan was highly similar to the unique *Borrelia* strains (pattern R'/R) isolated in northeastern China and Korea, and to strains from ticks feeding on migratory birds in Fukui Prefecture. These findings indicate that the *Borrelia* with this unique pattern may be locally naturalized to the epizootic transmission cycle in Japan, and that *Borrelia* is dispersed from the Asian Continent to Japan via migratory birds.

It is well known that migratory birds maintain Lyme disease-causing *Borrelia* spp. and play a role in the long-distance dispersal of these pathogens (1-3). The incidence of *Borrelia* spirochetes harbored in birds is usually estimated based on the *Borrelia* infection rate of bird-feeding immature ticks, regardless of whether or not the tick species is itself a vector of dispersal. When we classified *Borrelia* isolates from bird-feeding ticks in Mt. Nyu of Fukui Prefecture, central Japan by 5S-23S rRNA intergenic spacer restriction fragment length polymorphism (RFLP) analysis (4), two strains, Fi14f and Fi24f, were determined to have the unique R'/R pattern of *Borrelia garinii*, which had been recorded in northeastern China (Inner Mongolia) and Korea (6,7), while all of the *B. garinii* isolates from *Ixodes persulcatus* ticks and various rodents in Japan have been recorded as having the B'/B pattern (Eurasian type) or the C'/C pattern (Asian type) (5). This suggests that birds infected with the unique pattern *Borrelia* directly migrate from the Asian Continent into central Japan. We therefore investigated whether the unique R'/R pattern of *B. garinii* has been naturalized into the epizootic transmission cycle of *Borrelia* in Japan.

A total of 25 *Borrelia* isolates from field-collected *I. persulcatus* and 14 *Borrelia* isolates from field rodents in Hokkaido, Aomori, Fukushima and Fukui Prefectures, all of which were collected from 1993 to 1995 and tentatively identified as *B. garinii* or *Borrelia afzelii* by the previous method (8), were reexamined using 5S-23S rRNA intergenic spacer RFLP analysis (4,5,9). Of these, an isolate showing the unique pattern R'/R was subjected to the 5S-23S rRNA intergenic spacer sequence analysis (9), and compared with the same-pattern strains from the Asian Continent and migratory birds as described above (4).

When the 25 *Borrelia* isolates from *I. persulcatus* were tested by RFLP analysis, the isolate from Aomori Prefecture, northern Japan had the B'/B pattern of *B. garinii*, the 14 isolates from Fukushima Prefecture, eastern Japan had the B'/B ($n = 12$ cases) or C'/C ($n = 2$) patterns of *B. garinii*, and the

10 isolates from Fukui Prefecture had the B'/B ($n = 1$) or C'/C ($n = 9$) patterns of *B. garinii*. Among the 14 isolates from field rodents, the 10 from *Clethrionomys rufocanus bedfordiae* in Hokkaido, northernmost Japan had the C'/C pattern of *B. garinii* ($n = 9$) or the D'/D pattern of *B. afzelii* ($n = 1$), the 2 from *Apodemus speciosus* in Fukushima Prefecture had the C'/C pattern of *B. garinii*, the isolate (strain FiAE11) from *A. speciosus* on the peak of Mt. Heko (1,400 m above sea level), Fukui Prefecture (Fig. 1) had the Rv1'/Rv1 pattern of *B. garinii*, and the 2 isolates from *Eothenomys smithii* in Fukui Prefecture had the C'/C pattern of *B. garinii* ($n = 1$) or the D'/D pattern of *B. afzelii* ($n = 1$). The pattern Rv1'/Rv1 of the strain FiAE11 on Mt. Heko was the first recorded from a reservoir rodent in Japan (Table 1). Strain FiAE11 produced a 237-bp 5S-23S rRNA spacer amplicon that was similar in size to those of strains ChY13p (accession no. AB007450) from a tick in northeastern China, 935T (accession no. L39081) from a tick in Korea and Fi14f (accession no. AB015911) derived from a bird *Turdus pallidus* on Mt. Nyu, Fukui Prefecture, but strain Fi24f (accession no. AB015912) in Mt. Nyu produced a 240-bp 5S-23S amplicon. Strain FiAE11 showed three fragments (144, 52 and 41 bp in size)

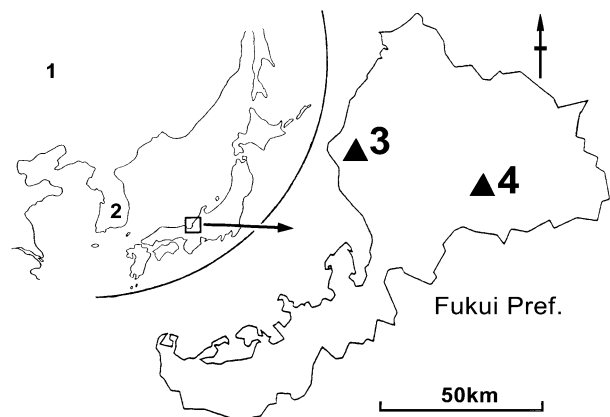


Fig. 1. A map showing the distribution of R'/R pattern strains of *B. garinii*. 1, strain ChY13p in northeastern China; 2, strain 935T in Korea; 3, strains Fi14f and Fi24f in Mt. Nyu, Fukui Prefecture; 4, the present strain FiAE11 in Mt. Heko, Fukui Prefecture.

*Corresponding author: Mailing address: Fukui Prefectural Institute of Public Health and Environmental Science, Haramecho 39-4, Fukui 910-8551, Japan. Tel: +81-776-54-5630, Fax: +81-776-52-6109, E-mail: ishigurofubito@fklab.fukui.fukui.jp

Table 1. RFLP analysis and sequence similarity of 5S-23S rRNA gene intergenic spacer

Locality	Source	Strain	<i>DraI</i>		<i>MseI</i>		Sequence similarity
			RFLP pattern	fragment size	RFLP pattern	fragment size	
Fukui, Japan							
Mt. Heko	<i>A. speciosus</i>	FiAE11	Rv1'	144,52,41	Rv1	107,51,41,38	–
Mt. Nyu	<i>H. flava</i> N	Fi14f	Rv1'	144,52,41	Rv1	107,51,41,38	100.0
	<i>H. flava</i> N	Fi24f	Rv2'	188,52	Rv2	107,82,51	95.0
Korea	<i>I. persulcatus</i>	935T	R'	185,52	R	107,79,51	99.6
Northeastern China	<i>I. persulcatus</i>	ChY13p	R'	185,52	R	107,79,51	95.0
France	<i>I. ricinus</i>	20047	B'	201,52	B	108,95,50	92.1

H. flava N, nymphal ticks feeding on the bird *T. pallidus*.

Strains other than strain FiAE11 in Mt. Heko were used as comparative reference strains.

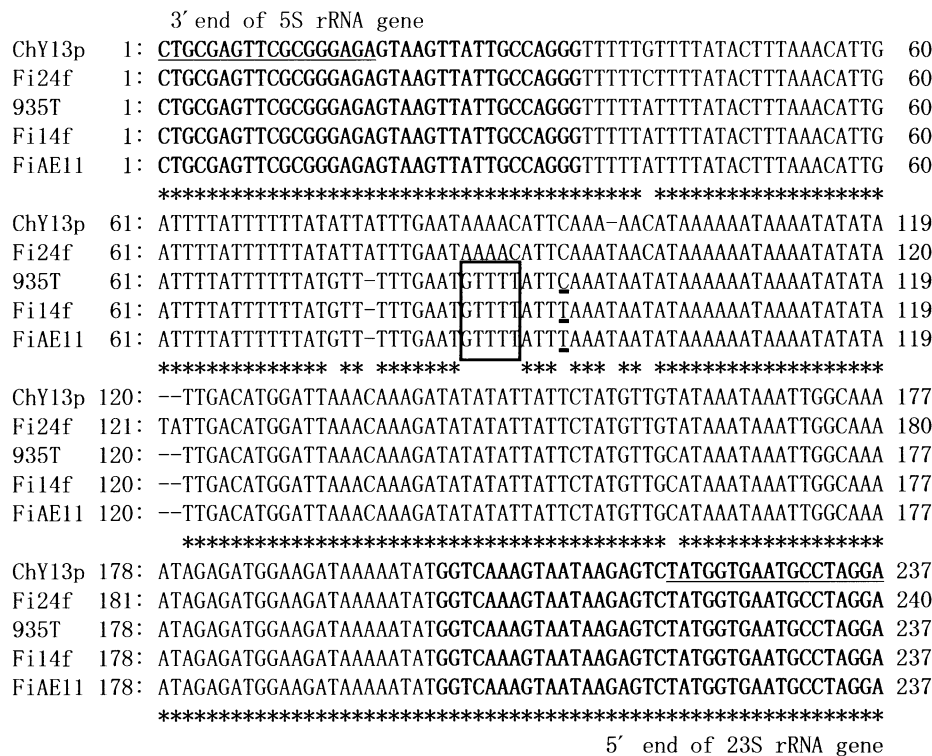


Fig. 2. Nucleotide sequence alignment of the 5S-23S rRNA intergenic spacer of FiAE11 and the reference *Borrelia* isolates. The 3' region of the 5S rRNA gene and the 5' region of the 23S rRNA gene are indicated in boldface type. The corresponding primers are underlined. The asterisks and a box indicate identical nucleotides and the specific sequence, respectively. Base substitutions are indicated by double underlining.

by digestion with *DraI* and four fragments (107, 51, 41 and 38 bp in size) by digestion with *MseI*, which were identical to those of strain Fi14f. Although the fragment sizes of FiAE11 differed from those of 935T, the nucleotide sequence showed only one base substitution between these strains: the nucleotide No. 94 of strain FiAE11 was T and that of strain 935T was C (Fig. 2). Strain FiAE11 had the nucleotide sequence GTTTT, which is common to those of strains 935T and Fi14f but is not found in strains Fi24f and ChY13p. On the other hand, we have already reported that the sequences of strains 935T and ChY13p were highly similar to those of strains Fi14f (99.6%) and Fi24f (97.9%), respectively (4). As shown in Table 1, the sequence of the present strain FiAE11 was highly similar to those of strains Fi14f (100%) and Fi24f (95.0%) derived from migratory birds in Fukui Prefecture, and also to those of strains 935T (99.6%) from Korea and ChY13p (95.0%) from China.

We previously indicated that migratory birds may disperse *Borrelia* from the Asian Continent to central Japan, based on

Borrelia isolations from immature *Haemaphysalis flava* // *I. persulcatus* ticks feeding on birds collected on Mt. Nyu, which is a low mountain that is *I. persulcatus*-free (4,10). Furthermore, we noted that *H. flava* occasionally transmits *B. garinii*. Despite such occasional cases, it is certain that *I. persulcatus* has the highest vectorial competence for *Borrelia* spp., as shown in many reports. We have confirmed that *I. persulcatus* is densely distributed on Mt. Heko, which is one of the highest mountains in Fukui (unpublished data). Therefore, the fact that the unique R'/R pattern of *B. garinii* (FiAE11) was isolated from a rodent on this mountain strongly suggests that an epizootic transmission cycle of R'/R pattern *Borrelia* exists there. In addition, Baba et al. (11) reported that a *Borrelia* strain isolated from a Lyme disease patient at the northern foot of Mt. Fuji, Yamanashi Prefecture demonstrated the R'/R pattern. This is the first and still the only human case infected with the R'/R pattern *Borrelia*, although many cases are known in northern and central Japan. Such findings in Fukui and Yamanashi Prefectures strongly sug-

gest that this unique pattern of *Borrelia* is locally scattered, but not yet common in Japan, and that it is introduced from the Asian Continent to Japan by infected migratory birds or ticks.

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REFERENCES

1. Olsen, B., Jaenson, T. G. T. and Bergstrom, S. (1995): Prevalence of *Borrelia burgdorferi* sensu lato-infected ticks on migrating birds. *Appl. Environ. Microbiol.*, 61, 3082-3087.
2. Smith, R. P., Jr., Rand, P. W., Lacombe, E. H., Morris, S. R., Holmes, D. W. and Caporale, D. A. (1996): Role of bird migration in the long-distance dispersal of *Ixodes dammini*, the vector of Lyme disease. *J. Infect. Dis.*, 174, 221-224.
3. Nicholls, T. H. and Callister, S. M. (1996): Lyme disease spirochetes in ticks collected from birds in midwestern United States. *J. Med. Entomol.*, 33, 379-384.
4. Ishiguro, F., Takada, N., Masuzawa, T. and Fukui, T. (2000): Prevalence of Lyme disease *Borrelia* spp. in ticks from migratory birds on the Japanese Mainland. *Appl. Environ. Microbiol.*, 66, 982-986.
5. Masuzawa, T., Komikado, T., Iwaki, A., Suzuki, H., Kaneda, K. and Yanagihara, Y. (1996): Characterization of *Borrelia* sp. isolated from *Ixodes tanuki*, *I. turdus*, and *I. columnae* in Japan by restriction fragment length polymorphism of rrf(5S)-rrl(23S) intergenic spacer amplicon. *FEMS. Microbiol. Lett.*, 142, 77-83.
6. Li, M., Masuzawa, T., Takada, N., Ishiguro, F., Fujita, H., Iwaki, A., Wang, H., Wnag, J., Kawabata, M. and Yanagihara, Y. (1998): Lyme disease *Borrelia* species in northeastern China resemble those isolated from far eastern Russia and Japan. *Appl. Environ. Microbiol.*, 64, 2705-2709.
7. Masuzawa, T., Fukui, T., Miyake, M., Oh, H. B., Cho, M. K., Chang, W. H., Imai, Y. and Yanagihara, Y. (1999): Determination of members of a *Borrelia afzelii*-related group isolated from *Ixodes nipponensis* in Korea as *Borrelia valaisiana*. *Int. J. Syst. Bacteriol.*, 49, 1409-1415.
8. Kawabata, H., Tashibu, H., Yamada, K., Masuzawa, T. and Yanagihara, Y. (1994): Polymerase chain reaction analysis of *Borrelia* species isolated in Japan. *Microbiol. Immunol.*, 38, 591-598.
9. Postic, D., Assous, M. V., Grimont, P. A. D. and Baranton, G. (1994): Diversity of *Borrelia burgdorferi* sensu lato evidenced by restriction fragment length polymorphism of rrf(5S)-rrl(23S) intergenic spacer amplicons. *Int. J. Syst. Bacteriol.*, 44, 743-752.
10. Ishiguro, F., Iida, H., Hatano, M., Yano, Y. and Takada, N. (1992): Tick fauna and the prevalence of Lyme *Borrelia* in Fukui Prefecture. *J. Acarol. Soc. Jpn.*, 1, 27-35 (in Japanese with English abstract).
11. Baba, S., Aoyama, T., Kinoshita, M., Suzuki, K., Koizumi, N. and Masuzawa, T. (2002): A case of Lyme disease diagnosed with *Borrelia*-positive erythema migrans. p. 710. *Proc. 101st Ann. Meet. Jpn. Dermatol. Assoc. Japanese Dermatological Association.*