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Detection of isozyme polymorphism and estimation of geographic variation in the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae)

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Abstract

Isozyme polymorphism in the brown planthopper, *Nilaparvata lugens* (Stål) was investigated using isoelectric focusing. Four of the 18 enzyme systems assayed were polymorphic. Allelic designations could be made for two enzyme systems (PGM and AK), but not for GPI and IDH, and GPI seemed to be sex-linked. Using the two highly polymorphic enzyme systems, GPI and PGM, geographic variation was estimated among several Asian (except Japanese) laboratory populations and several Japanese wild populations. Significant variation was observed among the Asian (except Japanese) populations, but the genetic structures of Japanese populations were very similar to each other. These results are suggestive of substantial differentiation among Asian populations and large panmictic structure of the migrant population.

Introduction

The brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), is a major rice pest, and is widely distributed from tropical to temperate areas of Asia and Australasia.

In eastern Asia, *N. lugens* is known to migrate northward every year (Cheng *et al.*, 1979); it migrates overseas to Japan in early summer and once there increases in numbers in paddy fields (Sogawa & Watanabe, 1991). However, the geographical origin of these long distance migrations and the extent of gene flow between the tropical populations of *N. lugens* is not precisely known.

The acoustic recognition signal of *N. lugens* is known to show marked geographic diversity; the populations from Australia and Solomon Islands, and those from Australia and Asia are especially distinct from one another (Claridge *et al.*, 1985). Consequently, laboratory hybridization between individuals from widely allopatric populations has not been very successful (Claridge *et al.*, 1984; 1985). These studies suggest the existence of substantial genetic variation and differentiation among natural populations of *N. lugens*, in spite of their long distance migration.

Genetic polymorphic markers, such as isozymes and nuclear or organellar DNA polymorphisms, have been developed for a variety of studies on population structure and subdivision (e.g., Hillis & Moritz, 1990; Avise, 1993). However, there have been few studies using biochemical techniques to examine genetic variation in populations of planthoppers (Bieman & Eggers-Schumacher, 1987), except for a few reports which were concerned with the relationship between insecticide resistance and esterases (e.g., Hasui & Ozaki, 1984).

In this study, isozyme polymorphism was investigated using isoelectric focusing (IEF), and the geographical variation among several populaions from Asia (except Japan) and those from Japan was estimated.

Materials and methods

Planthoppers

Fifteen populations of *N. lugens* were analysed (table 1, fig. 1). The Sri Lankan material was supplied from the Central Agricultural Research Institute, and the Alor Star

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Table 1. Collection sites of *Nilaparvata lugens* populations.

Population	Country	Collection data
Sri Lanka (Sr)	Sri Lanka	Unknown
Alor Star (Al)	Malaysia	Unknown
Bogor (Bo)	Indonesia	Dec 1984
Jatisari (Ja)	Indonesia	Dec 1983
Manila (Ma)	Philippines	Jan 1984
Taichung (Ta)	Taiwan	Sep 1983
Ishigaki Is. (Ig)	Japan	24, 27 Jun 1991
Omura (Om)	Japan	4 Jul 1991
Isahaya (Ih)	Japan	5 Jul 1991
Ureshino (Us)	Japan	6 Jul 1991
Chikushino (Ck)	Japan	7 Jul 1991
Fukuyama (Fk)	Japan	3 Aug 1991
Ishigaki Is. (2) (Ig(2))	Japan	late Sep 1991
Kanoya (Ky)	Japan	Unknown
Kaseda (Ks)	Japan	Unknown

(Malaysia) material from the Malaysia Agricultural Research and Development Institute (MARDI). These populations were maintained in the laboratory after collection.

Japanese populations, except those from Kanoya and Kaseda, were collected from paddy fields in 1991. Regarding the Ômura population, adults collected from paddy fields were analysed electrophoretically, but in other populations collected in 1991, adults of one or two generations raised in the laboratory after collection from paddy fields were analysed electrophoretically. The collection year was not known for the Kaseda and Kanoya populations, which were supplied by Ageo Research Laboratory, Nippon Kayaku Co., Ltd. The number of individuals at establishment of each colony collected in Japan in 1991 was more than fifty, except for the Fukuyama population. In the laboratory, *N. lugens* were reared at 25°C, on seedlings of the rice variety Nihonbare.

Sample preparation

Adults of *N. lugens* were examined by electrophoresis after collection, or, after storage in a deep freezer (−78°C). Each adult was ground using a glass pestle in a hollow (20 mm diam.), on a porcelain plate with cold 10 mM 2-mercaptoethanol solution (10 μl for males and 15 μl for females). The difference in homogenizing volume was based on the body size difference between sexes, and adjusted enzyme concentration per sample. The homogenate was absorbed with a piece of filter paper (6×1 mm) and applied to an electrophoretic gel after blotting excess moisture onto a paper towel. The best sample position on a gel was determined empirically for each enzyme system.

Electrophoresis

Isoelectric focusing was performed according to the recommendations of the ampholytes supplier (Anon., 1982) but with some modifications. The T5C3 polyacrylamide gel of 0.6-0.7 mm thickness containing 6.3% w/w Pharmalyte 3-10 (Pharmacia Fine Chemicals AB) was cast on a supporting film. Phosphoric acid (0.1 M) was used as anodal (+) electrode solution instead of 0.04 M aspartic acid. After prefocusing at 8 W constant power for 500 volt

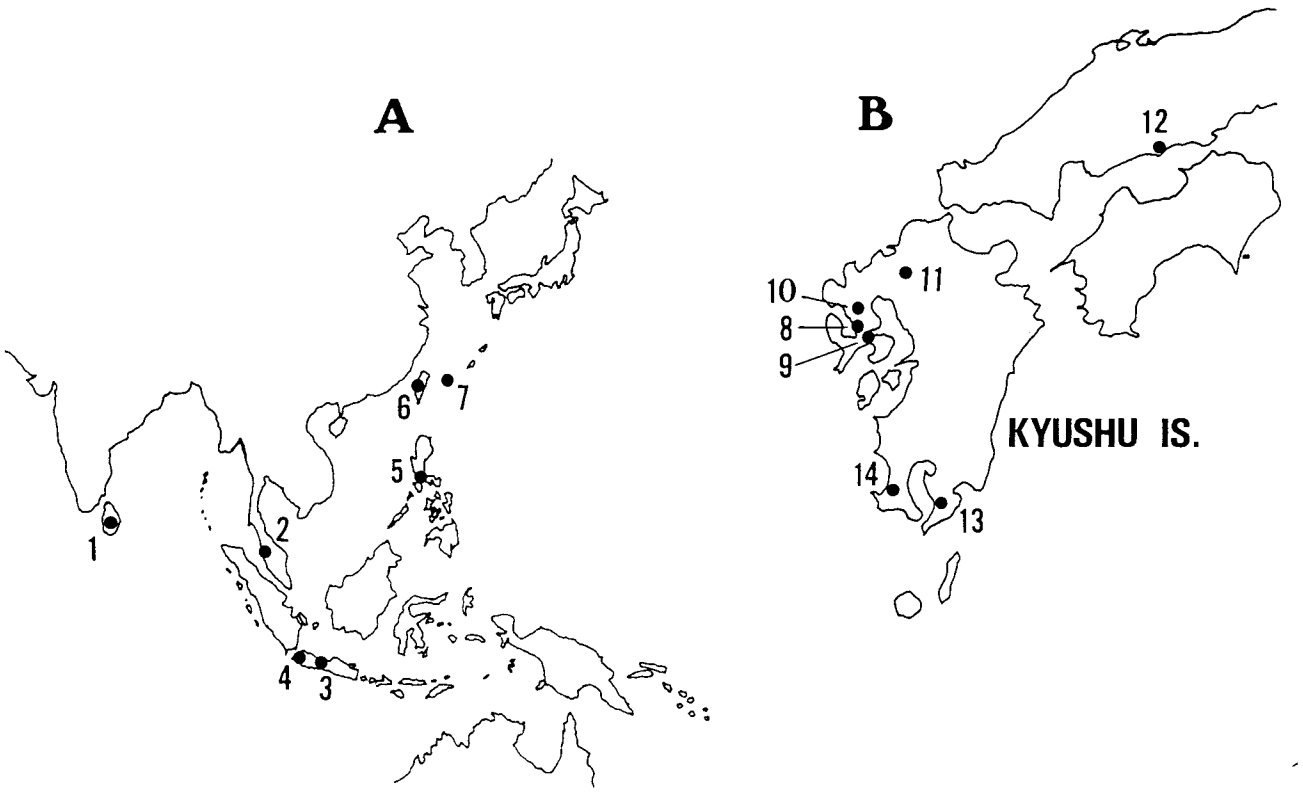

Fig. 1. Collection sites of *Nilaparvata lugens* populations. (A) Collection sites of six Asian populations and one Japanese population. 1: Sri Lanka, 2: Alor Star, 3: Jatisari, 4: Bogor, 5: Manila, 6: Taichung, 7: Ishigaki Is. (B) Collection sites of seven populations from western Japan. 8: Ômura, 9: Isahaya, 10: Ureshino, 11: Chikushino, 12: Fukuyama, 13: Kanoya, 14: Kaseda.

hours (Vh), samples were placed onto the gel and then run at 15 W for 3500 Vh. To prevent trailing of the zymogram, the filter papers were removed from the gel after 500 Vh.

Enzyme staining

Before staining, the gel was usually washed for several minutes with the same ice-cold buffer as that used for the staining mixture.

Eighteen enzymes were assayed: alcohol dehydrogenase (ADH, EC 1.1.1.1), lactate dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.40), isocitrate dehydrogenase (IDH, EC 1.1.1.42), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), xanthine dehydrogenase (XDH, EC 1.1.1), aldehyde oxidase (AOX, EC 1.2.3.1), hexokinase (HK, EC 2.7.1.1), phosphoglucomutase (PGM, EC 2.7.5.1), leucine aminopeptidase (LAP, EC 3.4.11.1) and glucose phosphate isomerase (GPI, EC 5.3.1.9) were stained according to Tsukamoto (1989). Sorbitol dehydrogenase (SDH, EC 1.1.1) and acid phosphatase (ACP, EC 3.1.3.2) were stained after Shaw & Prasad (1970). Alkaline phosphatase (ALP, EC 3.1.3.1), aldolase (ALD, EC 4.1.2) and α-glycerophosphate dehydrogenase (αGPD, EC 1.1.99.5) were stained following Tsukamoto (1984), Ayala *et al.* (1972) and Murai (1990), respectively. The staining mixture for adenylate kinase (AK, EC 2.7.4.3) comprised of 50 ml of 0.1 M Tris-HCl buffer (pH 8.0), 0.5 ml of 10% w/w $MgCl_2$ solution, 50 mg of glucose, 10 mg of adenosine-5′-diphosphate, sodium salt (ADP), 25 U of hexokinase, 10 U of glucose-6-phosphate dehydrogenase, 10 mg of nitro blue tetrazolium and a trace amount of phenazine methosulfate. The gel for AK was incubated at 37°C with the mixture until bands appeared.

After incubation, the gel was destained according to Ayala *et al.* (1972).

Analysis

Genetic differentiation among populations was assessed using the F statistics (Nei, 1987) and chi squared analysis.

Results and discussion

Enzyme polymorphism

No variation linked to wing polymorphism or to body colour variation was detected for any enzyme. The enzyme polymorphism was checked by analysing at least 20 individuals of several populations for each enzyme system (table 2). From the eighteen enzymes assayed, four enzymes, GPI, PGM, AK and IDH, showed more than one phenotype (table 2). In contrast with the high polymorphism of GPI and PGM, most of the populations seemed fixed to one common type with AK and IDH: AK was polymorphic only for the Kaseda and Kanoya populations, and IDH only for the Jatisari population.

A few studies have dealt with the enzyme polymorphism of *N. lugens* (Saxena & Mujer, 1984; den Hollander, 1989; Sawabe, 1991). Although the enzyme systems assayed differed slightly with each study, the enzymes which showed high polymorphism appeared to be PGM, GPI and esterase (EST). Results of this study were in agreement with those of previous studies. However, EST was not assayed in the present study, because EST is considered to be related to the development of insecticide resistance (Hasui & Ozaki, 1984). The intention of this study was to assess only natural variation and this enzyme was excluded. Due to small sample sizes and unknown technical problems in checking polymorphism, it is possible that additional polymorphic enzymes may have remained undetected, but it is at least certain that the major polymorphic enzymes are GPI and PGM.

Table 2. Polymorphism of 18 enzymes in *Nilaparvata lugens*.

Enzyme	N	Activity	No. of patterns
Polymorphic enzyme			
PGM : phosphoglucomutase	793	++	20
GPI : glucose phosphate isomerase	814	++	4
AK : adenylate kinase	99	+	3
IDH : isocitrate dehydrogenase	153	++	2
Monomorphic enzyme			
MDH : malate dehydrogenase	49	+	1
ME : malic enzyme	56	+	1
LDH : lactate dehydrogenase	50	±	1
HK : hexokinase	50	+	1
G6PD : glucose-6-phosphate dehydrogenase	75	+	1
ACP : acid phosphatase	35	±	1
ALP : alkaline phosphatase	31	−	0
LAP : leucine aminopeptidase	50	−	0
SDH : sorbitol dehydrogenase	24	−	0
ALD : aldolase	23	−	0
ADH : alcohol dehydrogenase	25	−	0
AOX : aldehyde oxidase	23	−	0
XDH : xanthine dehydrogenase	25	−	0
αGPD : α-glycerophosphate dehydrogenase	25	−	0

N: number of individuals tested.

Table 3. Type frequencies for GPI in male *Nilaparvata lugens*.

Phenotype	Sr (24)	Al (30)	Bo (25)	Ja (25)	Ma (30)	Ta (30)	Ig (27)	Ig(2) (25)	Om (21)	Ih (25)	Us (25)	Ck (25)	Fk (28)	Ky (28)	Ks (35)
I	0.250	–	–	–	–	–	0.222	0.080	0.048	0.120	0.320	0.240	–	–	–
II	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
III	0.750	0.167	1.000	1.000	0.967	1.000	0.704	0.840	0.810	0.720	0.640	0.760	0.929	0.929	0.600
IV	–	0.833	–	–	0.033	–	0.074	0.080	0.143	0.160	0.040	–	0.071	0.071	0.400

Figures in parenthesis are the numbers of individuals tested; see table 1 for abbreviations.

Table 4. Type frequencies for GPI in female *Nilaparvata lugens*.

Phenotype	Sr (30)	Al (39)	Bo (35)	Ja (25)	Ma (30)	Ta (30)	Ig (25)	Ig(2) (23)	Om (23)	Ih (25)	Us (24)	Ck (25)	Fk (19)	Ky (22)	Ks (30)
I	0.267	–	0.029	–	–	–	0.040	–	0.043	–	–	0.040	–	–	–
II	0.333	0.026	0.143	–	–	–	0.200	0.217	0.043	0.080	0.292	0.280	–	–	–
III	0.400	0.436	0.829	1.000	0.967	1.000	0.720	0.739	0.870	0.920	0.708	0.680	1.000	1.000	0.933
IV	–	0.538	–	–	0.033	–	0.040	0.043	0.043	–	–	–	–	–	0.067

Figures in parenthesis are the numbers of individuals tested; see table 1 for abbreviations.

Allelic designation

The inheritance of variants was examined preliminarily by cross testing for PGM, AK and GPI (data not shown). The putative alleles for PGM and AK were supported by cross-tests. Allelic interpretation of zymograms was possible for PGM and AK in the standard manner. From the banding patterns, the two enzymes were considered to be monomeric.

However, clear allelic interpretation was not similarly possible for GPI and IDH variants. For GPI, four types of zymogram (types I to IV) could be distinguished from the banding patterns, with the exception that some bands were observed to be specific to males. No zymogram type was considered as a heterozygote. No GPI zymogram of offspring from crossing between type III and type IV could be distinguished from the type III zymogram, although some of the offspring should have had heterozygotic GPI genotypes.

From cross-testing, one particular GPI zymogram type (type II) among the four was indicated as a heterozygotic genotype of types I and III. Furthermore, type II was recorded only for females (tables 3, 4). These indicate the sex relatedness of GPI; the genes coding for GPI are partly, or entirely, allocated on the X chromosome, because *N. lugens* harbours a XY sex determination system (Noda & Tatewaki, 1990).

Accordingly, GPI variants were classified only as types I to IV, not as genotypes. In population analysis, GPI type frequency was calculated for each sex. The frequency for males could be approximately considered as the allele frequency in the male subpopulation, because a male has only one X chromosome. On the other hand, there was no allelic information for females. In the analysis of PGM, the alleles were named alphabetically after their isoelectric points from cathode to anode.

Variation among Asian (except Japanese) populations

Variation among several Asian populations (except those of Japan) was analysed using the two highly polymorphic enzymes, GPI and PGM, as the genetic markers. Type frequency for GPI (tables 3, 4) and allele frequency for PGM (table 5) were calculated:

1. GPI. Type III was dominant for both sexes with the exception of the Alor Star (Malaysia) population in which type IV was most frequent. For females, the frequency f type II, the putative heterozygote of type I and III, seemed roughly congruent with the frequency expected from those of type I and III. In the Sri Lanka population, type I frequency was relatively high for both sexes.
2. PGM. Allele f was dominant, though variable in frequency, in all populations except for Bogor (Java, Indonesia). Allele c was secondly dominant except for Alor Star (Malaysia) and Jatisari (Java, Indonesia). In Bogor (Java, Indonesia), allele c was the most common allele, followed by e.

As inferred from the original frequency data, the result of F-statistics analysis for PGM suggested genetic differentiation among the Asian *N. lugens* populations (table 7). F_{IS}, which did not depart significantly from zero, indicated that these populations were under the Hardy-Weinberg equilibrium. The F_{ST} value could explain the large value of F_{IT}, which suggests departure in the total Asian (except Japanese) populations from the Hardy-Weinberg equilibrium. F_{ST}, an important index for genetic differentiation among populations (Nei, 1987), showed a statistically significant value of 0.100 ($P < 0.005$) among Asian (except Japanese) populations. This suggests the occurrence of

Table 5. Allele frequencies for PGM in Asian (except Japanese) populations.

Allele	Sr (55)	Al (50)	Bo (50)	Ja (45)	Ma (45)	Ta (50)
a	–	0.020	–	–	–	–
b	–	0.390	0.160	–	0.089	0.080
c	0.309	0.070	0.460	–	0.178	0.230
d	–	–	–	–	–	–
e	–	–	–	–	–	–
f	0.691	0.490	0.380	1.000	0.733	0.690
g	–	–	–	–	–	–
h	–	0.030	–	–	–	–
i	–	–	–	–	–	–

Figures in parentheses are the numbers of individuals tested; see table 1 for abbreviations.

Table 6. Allele frequencies for PGM in Japanese populations.

Allele	Ig (52)	Ig(2) (50)	Om (45)	Ih (50)	Us (50)	Ck (50)	Fk (51)	Ky (50)	Ks (50)
a	0.019	0.040	0.044	0.040	0.090	0.030	–	–	–
b	0.048	0.010	0.056	0.030	0.050	0.020	–	–	–
c	0.154	0.260	0.200	0.230	0.230	0.170	0.235	–	0.570
d	0.019	0.020	0.011	0.030	–	0.020	–	0.040	0.010
e	–	0.010	–	–	–	–	–	–	–
f	0.721	0.630	0.678	0.660	0.580	0.760	0.765	0.880	0.380
g	0.019	–	–	–	0.010	–	–	0.080	0.030
h	0.010	0.020	0.011	0.010	0.030	–	–	–	0.010
i	0.010	0.010	–	–	0.010	–	–	–	–

Figures in parenthesis are the numbers of individuals tested; see table 1 for abbreviations.

substantial geographic variation among these populations. The Jatisari population was excluded from the calculation because it showed monomorphism for the two enzymes, most likely due to a strong bottleneck effect during maintenance in the laboratories. Taichung was also excluded because *N. lugens* cannot overwinter in Taiwan.

den Hollander (1989) and Sawabe (1991) showed some electrophoretic data concerning geographic variation of *N. lugens*. However, detailed comparison is impossible because the three studies have adopted different allele designations. In addition, these studies, including this one, were conducted around laboratory populations. Therefore, crucial conclusions concerning the genetic relationship between Asian (except Japanese) geographic populations of *N. lugens* should await the survey of natural populations. However, this study has clearly shown the effectiveness of GPI and PGM as genetic markers. In future, it would be intriguing to compare the genetic structures of the Australian populations with those of the Asian ones in addition to the comparisons among the Asian populations alone.

Variation among Japanese populations

Little genetic differentiation was observed in allele frequency for the PGM locus among Japanese populations collected in the summer of 1991 (table 6). This was confirmed by F-statistics analysis (table 7). No significant departure from zero of F_{IS} value or the FST value indicated that the Japanese summer 1991 populations were under the Hardy-Weinberg equilibrium and that the Japanese summer 1991 populations were not differentiated genetically, at least for the PGM locus. The same conclusions could be drawn from the original GPI type frequency data (tables 3, 4). The Fukuyama population was excluded from the comparisons because of the very low number of individuals used for establishing the laboratory colony. Since the samples from Kyushu Island (summer 1991) were obtained during consecutive days from 4 to 7 July, when the large immigration of *N. lugens* was observed for the first time in 1991 (T. Teramoto, pers. comm.), most of the individuals collected were considered to be overseas immigrants. The Ishigaki Island population was also collected just after the large immigration. Therefore, the absence of variation among Japanese summer 1991 populations suggests that the *N. lugens* immigrants of summer 1991 were not composed of any subpopulations, but had a panmictic structure.

Table 7. F-statistics and χ^2 analysis for PGM locus.

	Japanese (1991)#	Asian#
F_{IS}	0.004	0.126
χ^2	0.004 (28)	3.185 (12)
F_{IT}	0.018	0.213
F_{ST}	0.014	0.100
χ^2	6.788* (4)	39.828** (3)

*$P < 0.25$, **$P < 0.005$. #Jpn (1991) and Asian represent the Japanese populations collected during 1991 and the Asian 4 populations, respectively (see text for details). Number in the parenthesis is degree of freedom. χ^2 for F_{IS} is calculated by Nei (1977) and for F_{ST} by Neel and Ward (1972).

This might be the case for the large population moving northward in China, since most of the Japanese immigrants are considered to come from southern China (Sogawa *et al.*, 1988). Some data seem to support this. Firstly, the Ishigaki Island (2) population, collected during the immigration of autumn 1991, showed very similar genetic structure to the Japanese summer 1991 populations (tables 3, 4, 6). This suggests the *N. lugens* population migrating in China did not change its genetic structure during the several generations throughout summer and autumn. Secondly, the populations collected at Shimane (Honshu Island, Japan) and on the eastern China Sea during summer 1991 showed a similar frequency pattern to this study at the PGM locus (T. Murai, pers. comm.).

As a panmictic structure was indicated for the Japanese 1991 immigrant population of *N. lugens*, the genetic characteristics of the population could be compared with those of other Asian populations. However, this was not done in this study because of the ambiguity of data for other Asian populations. Although analysis of more loci is desirable to understand the detailed genetic structure of *N. lugens* immigrants, continuous survey of allele frequency for PGM and GPI would be necessary to compare the genetic structure of immigrant populations year by year and to estimate their geographical origins.

The Kaseda population was distinct from other Japanese populations both for PGM and GPI (tables 3, 6). This could be due to several reasons such as a different migration origin of the Kaseda population, temporal change of genetic traits in the natural population, or alteration of genetic structure of the population during laboratory maintenance. These reasons could be verified after continuous monitoring of the temporal variability of the genetic characteristics of Japanese immigrant populations.

The occurrence of a peculiar *N. lugens* population has been reported in Japan. The typical Japanese immigrant population ordinarily consists of individuals of blackish body coloration which are mainly macropterous; however,

the unusual population consisted of yellowish coloured individuals many more of which were brachypterous (Sawabe, 1991). Sawabe (1991) suggested that this peculiar population came to Japan directly from the tropical areas of Asia. In the present study, the Japanese populations seem to include only the typical populations. It would be interesting to analyse the genetic structure and to estimate the geographical origin of the peculiar immigrant population.

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