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Reproductive compatibility and genetic variation between two strains of *Aphelinus albipodus* (Hymenoptera: Aphelinidae), a parasitoid of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae)

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Abstract

Aphelinus albipodus Hayat and Fatima is a potential biological control agent of the soybean aphid, *Aphis glycines* Matsumura, which is a newly introduced soybean pest in the United States. We compared the reproductive compatibility and molecular genetic variation between two geographic strains of *A. albipodus*. One strain was collected from soybean aphids in Japan and the other recovered from Russian wheat aphid, *Diuraphis noxia* (Mordvilko), in the western U.S., populations of which were established with parasitoids imported from Eurasia. We present results of crossing experiments between the two strains, genetic differences based on RAPD-PCR markers, rDNA ITS1 and ITS2 gene sequences, and presence of *Wolbachia* in the two strains using PCR amplification of the *wsp* gene. We found no reduction in the production of females in reciprocal crosses between strains, but a significant reduction in fecundity when F_1 females stemming from one of the reciprocal crosses were backcrossed to males from either source. The two strains differed by 3.4% in the rDNA ITS1 sequence and by presence/absence of one RAPD-PCR marker from a total of 20 RAPD primers screened, but their rDNA ITS2 sequences were identical. We used restriction enzyme analysis to separate the strains by differential digestion of the ITS1 PCR product. *Wolbachia* was present in 100% of males and females of both strains of *A. albipodus*.

Keywords: *Aphelinus albipodus*; *Aphis glycines*; Reproductive compatibility; PCR; RAPD-PCR; Internal transcribed spacer; *Wolbachia*; Aphid parasitoid; Biological control

1. Introduction

The soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), is native to Asia but has recently invaded North America (Ragsdale et al., 2004; Venette and Ragsdale, 2004; Wu et al., 2004). It was first detected in the United States in the summer of 2000, and has become an important pest throughout soybean-growing areas of the Midwest. Initial biological control efforts directed at the soybean aphid have resulted in the importation of several aphid parasitoids and predators from China, Japan and South Korea into quarantine, including a strain of the aphelinid parasitoid, *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae) from Japan in 2001 (Heimpel et al., 2004). Additionally, a population of *A. albipodus* that had been released against the Russian wheat

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aphid, *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae), was found to attack the soybean aphid as well. This strain had been introduced to the western U.S. from Eurasia in the early 1990s and successfully established (Elliott et al., 1995; Hopper et al., 1998; Prokrym et al., 1998), eventually becoming the dominant parasitoid of the Russian wheat aphid in Colorado and Wyoming (Brewer et al., 2001; Burd et al., 2001). A collection of *D. noxia* parasitoids in Wyoming in 2001 yielded *A. albipodus* that attacked and developed on the soybean aphid, and a culture stemming from this collection (henceforth the 'Wyoming' strain) was established at the USDA-APHIS Invasive Pests Management Laboratory in Niles, Michigan.

Populations from different geographic regions often display various levels of reproductive incompatibility and exhibit different biological traits and genetic variability because they have been geographically isolated, experienced different selection pressures, and/or adapted to various, local environments (Diehl and Bush, 1984; Hopper et al., 1993). For instance, a bewildering array of substantial and complete reproductive incompatibility leading to various combinations of sympatric and allopatric strains and cryptic species have been identified in a complex of entities encompassing *Trichogramma* parasitoids (Pinto et al., 1986, 1991, 1992, 2003; Pinto and Stouthamer, 1994; Stouthamer et al., 1996). The status of these entities can have important implications for biological control both because of differences in biology and because of the production of inviable offspring resulting from crosses between cryptic species (Stouthamer et al., 2000). Levels of complexity similar to that found in *Trichogramma* are being found within the genus *Aphelinus*. Kazmer et al. (1996) conducted mating studies among seven cultures of *D. noxia* parasitoids that were all identified as *A. asychis* Walker but stemmed from various geographic locations. A range of partial and complete reproductive isolation was identified in these studies, revealing three differentiated groups (one Chinese, one Kazakhstani, and one Mediterranean) that could arguably be labeled cryptic species. Similar work on *A. varipes*, *A. albipodus*, and *A. hordei* has revealed the possibility of cryptic species as well as hybridization in this complex as well (Hopper et al., 1998), while a phylogenetic study using mitochondrial 16S rDNA sequence data suggested an *A. varipes* complex containing the three *Aphelinus* species (Chen et al., 2002).

Our aim in this research was to evaluate two strains of *A. albipodus* within this complex. We crossed and backcrossed these strains to evaluate reproductive compatibility and estimated molecular genetic differentiation between the two strains. We also analyzed both strains for *Wolbachia* endosymbionts, which have been shown to cause reproductive incompatibility in other insects (Werren, 1997).

2. Materials and methods

2.1. Aphids and parasitoids

The Japanese strain of *A. albipodus* was initiated from 258 mummies collected in Japan by R.J.O. and D.J.V. in July 2001 from five sites on the island of Honshu near the cities of: Tskuba City, Ibariki (N36°01′ E140°06′), Utsunomiya, Tochigi (N36°32′/E139°54′), Morioka, Iwate (N39°37′/E141°08″), Yamagata, Yamagata (N38°15/E140°14′), and Furukawa, Miyagi (N38°34′/E140°56′). Progeny from these mummies were maintained at the USDA-ARS Beneficial Insect Introductions Research Laboratory, Newark, Delaware, for host-range evaluation. The 'Wyoming' strain was initially recovered from *D. noxia* on oats, barley, and wheat by John Larson and Boone Herring in Goshen and Laramie counties, Wyoming, in October 2001. A total of 21 females and 17 males of *A. albipodus* were produced from the mummies collected, and then the colony was reared at the USDA-APHIS Invasive Pests Management Laboratory, Niles, Michigan. Both strains were maintained on soybean aphids before they were shipped to Minnesota for our study. The two strains were reared separately on soybean aphids under the conditions of 25 °C, 75% R.H., and 16L:8D. Soybean aphids were originally collected from a soybean field in St. Paul, Minnesota, and aphid colonies were maintained on soybeans in a separate chamber under the same conditions.

2.2. Crossing experiments

Reciprocal crosses and backcrosses were conducted to determine reproductive compatibility. Matings were observed in gelatin capsules (size 1, SPI Supplies, PA) using a dissecting microscope to determine the mating time and time spent in copula. As in other *Aphelinus* species, two distinct phases of physical contact could be easily distinguished (Kazmer et al., 1996). In the first, males mounted the dorsum of females, and in the second, genital contact occurred while the male repositioned himself by backing up to hang onto the female's venter. For this study, we defined mating time as the sum of mounting and copulation. After a 3-h exposure to females, males were removed and frozen at −80 °C. Females were allowed to oviposit on soybean aphids growing in plastic cylindrical rearing cages (11 cm diameter × 21 cm height) for 48 h, and then were removed and frozen at −80 °C. Fifty second instar aphids were placed on the plants for oviposition. Second instars were used because *Aphelinus* spp. typically prefer to lay eggs in younger nymphs (Cate et al., 1977; Tang and Yokomi, 1996). Cages were kept in a growth chamber at 16L:8D, 21 °C, and 75% R.H. Aphids were monitored daily and newly developed mummies were individually placed into gelatin capsules that were provisioned with

a drop of undiluted honey on the inner wall of capsules. Emerged parasitoids were sexed using a dissecting microscope. For backcrosses, virgin daughters from the above reciprocal crosses were crossed with virgin males from either strain. We also compared the mating time, copulation duration, number of mummies, number of adult offspring per female, wasp emergence rate, and sex ratio stemming from all of these crosses. Additionally, to confirm arrhenotoky, individual virgin females from both strains were allowed to oviposit for 48 h on 50 second instar aphids in the cylindrical cages described above. Mummies produced per female, and the number and sex of adult offspring were recorded. The virgin female assays were not run concurrently with the reproductive compatibility experiment and so are not included in analyses of this experiment.

2.3. DNA extraction

Parasitoid DNA was extracted using Chelex (Bio-Rad Laboratories, Richmond, CA)/ProteinaseK (Fischer Scientific, Fair Lawn, NJ) extraction from whole insects. Individual parasitoids were ground in 0.5 ml microcentrifuge tubes with pestles on ice, and 200 µl of 10% Chelex solution and 8 µl of 2.5 mg/ml ProteinaseK solution were added to the tubes. These solutions were incubated overnight at 56 °C and heated to 95 °C for 10 min, and were then centrifuged for 2 min at 14,000 rpm. The resultant supernatant was used for PCR.

2.4. PCR amplification

For RAPD-PCR, we used the protocol outlined by Kazmer et al. (1995) on 20 10-base primers (OPC 1–20, Operon Technologies, Alameda, CA). PCR was conducted in 25 µl reaction mixtures containing the following ingredients: 2.5 µl of 10× buffer (Promega), 1.5 µl of 25 mM $MgCl_2$, 2 µl dNTPS (10 mM each), 0.5 µl primer, 17.75 µl dd H_2O, 0.25 µl *Taq* polymerase (1.25 U), and 0.5 µl template DNA. PCR cycling conditions were 5 min at 95 °C followed by a 'hot-start' at 80 °C during which time the *Taq* was added, 45 cycles (1 min at 92 °C, 1 min at 35 °C, and 2 min at 72 °C with 1 °C increase every 8 s from 35 °C) and 7 min at 72 °C. For the RAPD-PCR test, 60 wasps (50 females and 10 males) from the 'Wyoming' strain and 60 wasps (51 females and nine males) from the Japanese strain were individually tested. All samples were tested at least twice to confirm the consistency of results. While there was some within-strain individual variation from some of the RAPD primers, we differentiated between strains by using banding patterns which were found to be unique for each strain and present in all individuals from the same strain.

PCR amplification of a fragment of ITS1 rDNA was conducted using forward primer ITS5 (5′ GGAAG TAAAA GTCGT AACAA GG) and reverse primer RNA2 (5′ CACGA GCCGA GTGAT CCACC GCTAA GAGT) (Chang et al., 2001). The fragment length expected from these particular primers was not clear from the literature, but the ITS1 region is typically 500- to 600-bp long in insects (e.g., Chang et al., 2001; Hoogendoorn and Heimpel, 2001). Reactions were conducted in a 25 µl volume containing 2.5 µl of 10× buffer (Promega), 1.5 µl of 25 mM $MgCl_2$, 1 µl dNTPS (10 mM each), 2.5 µl 10 µM mixed forward and reverse primers, 15.25 µl dd H_2O, 0.25 µl *Taq* polymerase (1.25 U), and 2 µl template DNA. PCR amplification was performed using the following conditions: 3 min at 94 °C, 31 cycles (45 s at 94, 50, and 72 °C each), and 3 min at 72 °C.

A fragment of ITS2 rDNA was amplified using the forward primer 58S (5′ TGTGA ACTGC AGGAC ACATG AAC) and reverse primer Aalv (5′ GTAAT TTATT TCGTA CACAC). A PCR product of approximately 530 bp was expected using these primers (Zhu and Greenstone, 1999). The PCR recipe for ITS2 reaction was the same as that for ITS1. PCR conditions were 3 min at 94 °C and 50 cycles (30 s at 94 and 50 °C each, and 1 min at 72 °C). We tested 17 females and three males individually from both strains for ITS1 and for ITS2.

To detect *Wolbachia*, general *wsp* primers 81F (5′ TGGTC CAATA AGTGA TGAAG AAAC) and 691R (5′ AAAAA TTAAA CGCTA CTCCA) were used. The PCR amplification protocol followed Braig et al. (1998) and Zhou et al. (1998). PCR was performed in a 20 µl reaction volume containing 2 µl of 10× buffer (Promega), 2 µl of 25 mM $MgCl_2$, 0.5 µl dNTPs (10 mM each), 0.5 µl of 20 µM forward and reverse primers, 0.5 µl *Taq* DNA polymerase (Promega), 13.5 µl dd H_2O, and 1 µl template DNA. The PCR amplification protocol was modified from Zhou et al. (1998): 40 cycles each consisting of 1 min at 94, 58, and 72 °C each. A DNA fragment of 590–632 bp was expected depending on the *Wolbachia* strain (Zhou et al., 1998). Of the parasitoids screened individually for *Wolbachia*, 73 (35 females and 38 males) were from the 'Wyoming' strain and 71 (37 females and 34 males) from the Japanese strain.

All PCRs were performed in a PTC-100 Programmable Thermal Controller (MJ Research, Watertown, MA). PCR products of RAPD, ITS1, ITS2, and the *wsp* gene of *Wolbachia* were electrophoresed on 1.2% agarose gels, stained with ethidium bromide (1 mg/ml) in 0.5× TBE buffer, and visualized using UV light. PCR products of ITS1 and ITS2 were purified using MinElute PCR Purification Kit (Qiagen, Valencia, CA) prior to sequencing. Restriction sites for ITS1 were determined using WebGene (Yin, 2002). Restriction enzymes *Bsa*JI (New England BioLabs, Beverly, MA) and *Bse*DI (MBI Fermentas, Hanover, MD) were used to digest the ITS1 PCR products following the manufacturers' instructions. The digestion was run for 1 h at 60 °C for *Bsa*JI and at 55 °C for *Bse*DI, respectively.

2.5. Data analysis

For the crossing experiments, we used analysis of variance to compare the mating time, copulation duration, number of mummies, number of adult offspring produced per female, wasp emergence rate, and sex ratio between the two strains. A square transformation was used to equalize variances for the mummies and offspring produced per female, but assumptions of ANOVA were met for all other variables. The fractions of all-male families and families producing no mummies were compared using goodness-of-fit tests. Families not producing any mummies were excluded in the analysis of mummy and adult wasp data. Small families (<5 progeny) were not included in the analysis of wasp emergence rate, sex ratio or the fraction of all-male families, and all-male families were also omitted from the analysis of sex ratio. DNA banding patterns of RAPD-PCR amplification were visually counted and compared. Sequences of ITS1 and ITS2 of the two strains were aligned and compared using ClustalX 1.83.

3. Results

3.1. Reproductive compatibility between the two strains

Mummy and offspring production did not differ significantly between females mated to males from their own strain compared to females mated to males from the other strain (Table 1). In the backcrosses, however, F_1 females with a 'Wyoming' mother produced fewer mummies and offspring than all of the pure-strain female crosses and F_1 females with a Japanese mother (Table 1; mummies: $F = 3.07$, $df = 7$, $p < 0.05$; offspring: $F = 2.66$, $df = 7$, $p = 0.013$). The adult emergence rate (the fraction of mummies producing adults) was uniformly high (89–98%) but slightly and significantly higher in backcross families (Table 1; $F = 5.55$, $df = 7$, $p < 0.001$). Sex ratios varied between 0.38 and 0.56 (proportion females) but did not differ significantly among crosses (Table 1). Similarly, no significant difference was found in the fraction of all-male families ($\chi^2 = 3.33$, $df = 7$, $p > 0.1$) among the crosses, which ranged from 0.13 to 0.30. At least some females from all but two of the backcrosses (JW × W and WJ × W) produced no mummies at all, but this occurred at a low rate (<16%) without a significant difference among the crosses ($\chi^2 = 10.62$, $df = 7$, $p > 0.1$). Finally, there was no significant difference in the mating time ($F = 1.60$, $df = 7$, $p > 0.1$) among all crosses, but the copulation duration for the backcrosses was slightly but significantly longer than that in crosses involving pure cultures (Table 1; $F = 3.77$, $df = 7$, $p < 0.001$).

Only male offspring were produced by virgin females in both strains and there was no significant difference in the number of mummies produced (13.8 ± 1.7 for Japanese and 11.6 ± 1.6 for 'Wyoming' females; $t = 0.92$, $df = 27$, $p = 0.37$), or the wasp emergence rate (0.90 ± 0.03 for Japanese and 0.94 ± 0.02 for 'Wyoming' females; $t = -1.25$, $df = 27$, $p = 0.22$) between the two strains.

3.2. Molecular genetic variation between the two strains

Among 20 RAPD-PCR primers, only OPC 4 (5′ CCGCATCTAC) produced consistent and strain-specific DNA products (Fig. 1). The 'Wyoming' strain differed from the Japanese strain by the presence of a fragment that was approximately 1350 bp in length. Other primers either produced inconsistent products or ones that did not differ between the strains.

A single 920-bp fragment was obtained from PCR amplification of the rDNA ITS1 region in both strains (Fig. 2), within which a 740-bp section gave interpretable sequence data (GenBank Accession No.: Japanese: AY603663; 'Wyoming': AY603664). The length of this fragment was greater than would be expected from ITS1 alone, which indicates that some of the 18S and/or 5.8S rDNA genes, which flank the ITS1 region, were amplified along with ITS1 (Chang et al., 2001). There was 3.4% variation (a total 25 bp over 13 sites) within this 740-bp portion of the sequence between the two strains,

Table 1
Outcome of crosses and backcrosses involving two strains of *Aphelinus albipodus*

Cross		*n*	Mummies produced	Wasps produced	Wasp emergence rate	Sex ratio (proportion females)	Mating time (s)	Copulation time (s)
♀	♂							
J	J	19	20.89 ± 2.51	18.78 ± 2.45	0.89 ± 0.02	0.56 ± 0.05	146.13 ± 15.83	4.5 ± 0.4
J	W	26	20.05 ± 2.27	18 ± 2.21	0.89 ± 0.02	0.4 ± 0.06	123.48 ± 13.81	4.29 ± 0.35
W	J	21	21.79 ± 2.44	19.74 ± 2.38	0.91 ± 0.02	0.38 ± 0.07	123.9 ± 14.52	4.58 ± 0.37
W	W	19	19.19 ± 2.66	17.31 ± 2.59	0.89 ± 0.02	0.5 ± 0.08	104 ± 14.92	4.22 ± 0.38
JW	J	20	17.89 ± 2.44	17.58 ± 2.38	0.98 ± 0.02	0.42 ± 0.06	144.3 ± 14.16	5.65 ± 0.36
JW	W	23	19.87 ± 2.22	19.39 ± 2.16	0.98 ± 0.01	0.46 ± 0.06	134.04 ± 13.2	6.04 ± 0.34
WJ	J	20	9.67 ± 2.51	9.17 ± 2.45	0.96 ± 0.02	0.39 ± 0.07	160.3 ± 14.16	5.5 ± 0.36
WJ	W	20	10.4 ± 2.38	9.85 ± 2.32	0.96 ± 0.02	0.44 ± 0.07	115.2 ± 14.16	5.25 ± 0.36

J, Japanese strain; W, 'Wyoming' strain; and JW and WJ refer to the F_1 hybrid daughters from crosses between the Japanese and 'Wyoming' strains in which the first letter represents mother and the second father. Values are means ± SE.

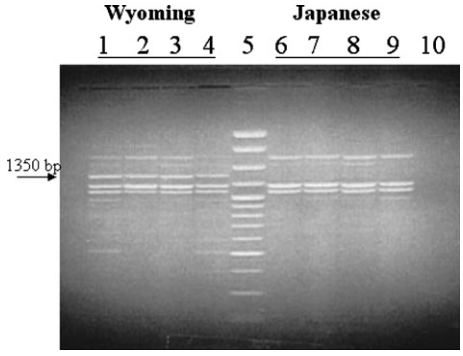

Fig. 1. Agarose gel electrophoresis of RAPD by primer OPC 4 of *Aphelinus albipodus*. Lanes 1–4, 'Wyoming' strain; lane 5, 100-bp DNA ladder; lanes 6–9, Japanese strain; and lane 10, negative control.

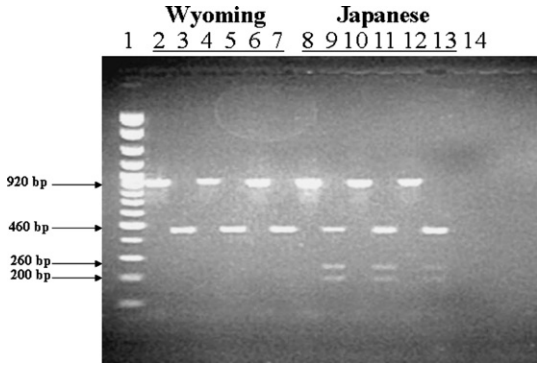

Fig. 2. PCR amplification of ITS1 region and digestion patterns of *Aphelinus albipodus*. Lane 1, 100-bp DNA ladder; lanes 2–7, 'Wyoming' strain (2, 4, and 6, before digestion; 3, 5, and 7, after digestion); lanes 8–13, Japanese strain (8, 10, and 12, before digestion; 9, 11, and 13, after digestion); and lane 14, negative control.

including 19 bp of deletions/insertions and 6 bp of substitutions (Fig. 3). We found no individual variation within the strains using five high-quality sequences per strain for analysis. After digestion by the restriction enzymes *Bsa*JI or *Bse*DI, this DNA fragment in the 'Wyoming' strain was cut in half, producing a single evident band, while three bands (approximately 200, 260, and 460 bp) were obtained in the Japanese strain (Fig. 2).

A single fragment of 490 bp was amplified from the ITS2 region instead of ca. 530 bp found by Zhu and Greenstone (1999), with no difference found in an interpretable 428-bp portion of sequence between the two strains (GenBank Accession No.: Japanese: AY603665; 'Wyoming': AY603666). Fourteen and 15 sequences, respectively, from the Japanese and 'Wyoming' strains were compared.

3.3. Detection and prevalence of Wolbachia

An approximately 615-bp fragment of the *Wolbachia wsp* gene was amplified from all samples screened from both strains.

4. Discussion

In this study, *A. albipodus* females used in all pure-strain crosses, and hybrid females that were the offspring of Japanese mothers and 'Wyoming' fathers produced 18–22 mummies on average in a 48-h exposure period. This daily oviposition rate is similar to that found in some other *Aphelinus* spp. (e.g., Bai and Mackauer, 1990; Couty and Poppy, 2001; Holler and Haardt, 1993; Mackauer, 1982) including *A. albipodus* attacking *D. noxia* and other hosts at temperatures similar to the one we used (Bernal et al., 1997). Females that were the offspring of 'Wyoming' mothers and Japanese fathers, however, produced approximately half as many mummies as any other females in the study, regardless of the strain of the male they were mated with. This indicates some level of outcrossing depression between these populations. In the haplodiploid mite *Tetranychus urticae* Koch, a mode of outcrossing depression has been identified in which hybrid females are themselves viable and fertile but have reduced fitness (De Boer, 1985). In this case, classified as a form of hybrid breakdown, the viability of haploid offspring (sons) of affected hybrid females is reduced (Vala et al., 2000, 2003). This pattern is not consistent with our data, however, since sex ratios produced by WJ were similar to those produced by JW females. The reduction in fecundity in the WJ females is more reminiscent of generalized outbreeding depression, in which one or more fitness-related traits decline with increasing geographic distance (Alstad and Edmunds, 1983; Armbruster et al., 1997; Aspi, 2000; Blows, 1993; Lynch, 1991; Thornhill, 1993). Outbreeding depression can represent an initial step in the speciation process (Shields, 1982) and our data are therefore consistent with incipient allopatric speciation between these two geographic strains of *A. albipodus*.

The outbreeding depression uncovered in our study is non-reciprocal: hybrid crosses composed of a 'Wyoming' mother and a Japanese father have reduced fecundity while the reciprocal cross does not. The fact that the fecundity decline is seen in daughters of only one of the reciprocal crosses suggests a negative interaction between nuclear and cytoplasmically inherited genes, as was found in hybrid crosses between two *Nasonia* species (Breeuwer and Werren, 1995). In particular, the asymmetry noted in our study suggests negative interactions between Japanese nuclear genes and 'Wyoming' cytoplasmically inherited genes. As expected under this hypothesis, the genotype of the male mate of the hybrid female had no effect on fecundity. In other work on the *A. varipes/albipodus* complex of which these strains are members, both complete reproductive incompatibility and partial compatibility have been found (K.R.H., unpublished data). Collections from the same region but different hosts are in some

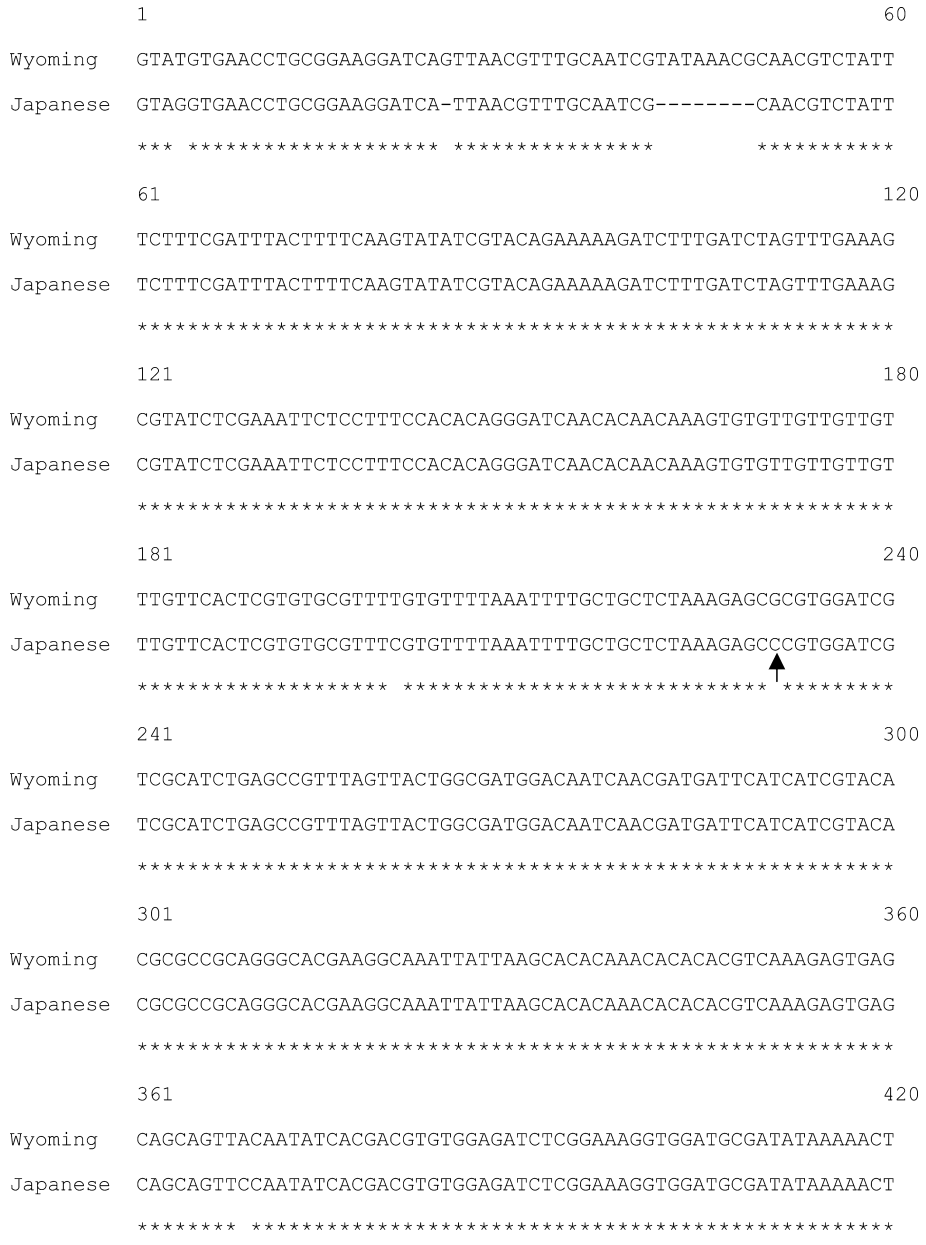

Fig. 3. A portion (740 bp) of sequences of ITS1 region in the two strains of *Aphelinus albipodus* was aligned. Asterisk, same nucleotide; dash, depletion or insertion. Arrow, the place cut by restriction enzymes (*Bsa*JI or *Bse*DI).

cases completely incompatible, but those from the same hosts but different regions are in some cases partially compatible.

Our genetic analyses revealed considerable sequence divergence between the strains. Both RAPD-PCR and ITS1 sequence analysis showed genetic divergence, and both of these methods can be used to distinguish the strains. Restriction enzyme analysis can also be used in lieu of sequencing to identify differences in ITS1 rDNA. The level of differentiation in ITS1 sequence between these two strains is greater than that found between completely reproductively isolated sibling species in the *A. varipes/albipodus* complex (K.R.H., unpublished data).

As far as we are aware, our finding of *Wolbachia* in these populations of *A. albipodus* represents the first documentation of *Wolbachia* infecting an aphid parasitoid. West et al. (1998) found no evidence of *Wolbachia* when they screened 19 species of primary aphid parasitoids including *Aphelinus abdominalis* (Dalman), although the number of individuals tested from each species was too small (<3) to rule out the possibility of a low frequency variant. A previous report of *Wolbachia*-induced thelytoky in three *Lysiphlebus* spp. (Hymenoptera: Braconidae) (Stouthamer et al., personal communication to Stary, 1999) turned out to be incorrect (R. Stouthamer, personal communication). While a number of aphelinid species are known to har-

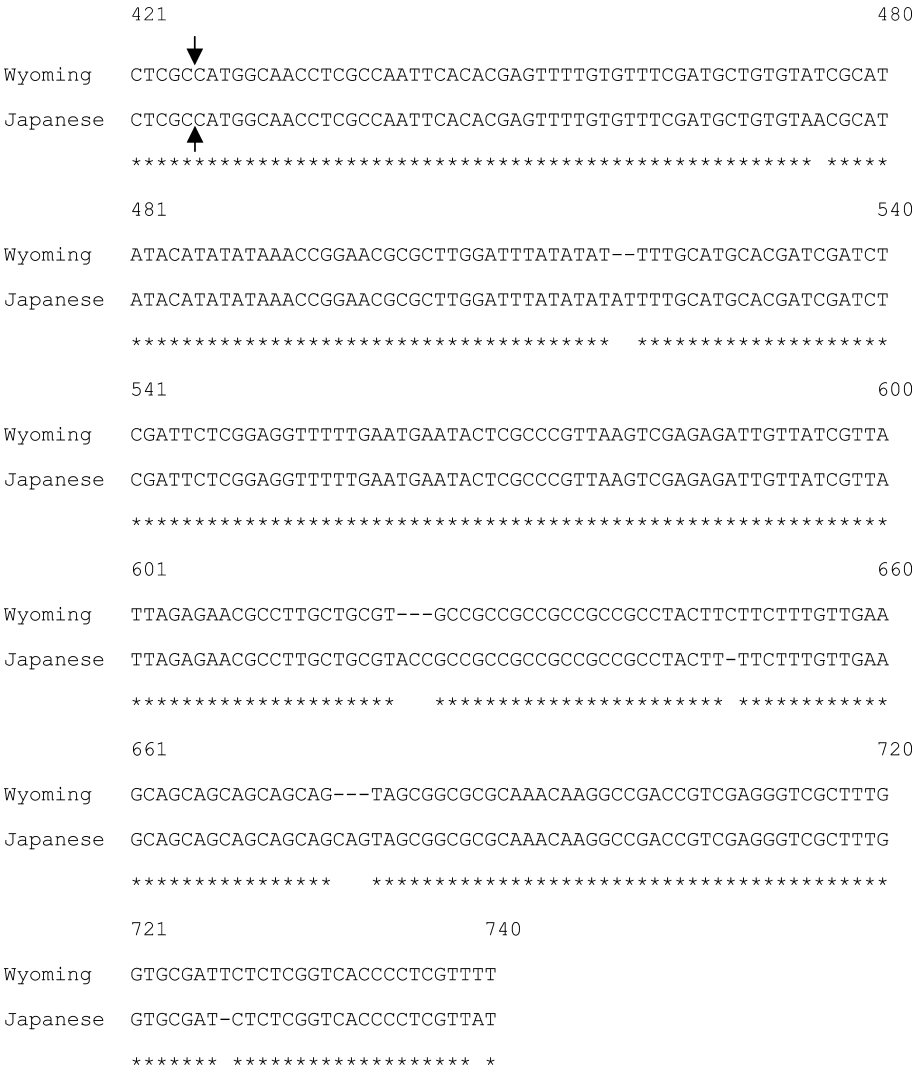

Fig 3. (*continued*)

bor parthenogenesis-inducing *Wolbachia* (Cook and Butcher, 1999), this report represents the first record of *Wolbachia* from an *Aphelinus* species (all of which are aphid parasitoids). We have not yet determined the phenotypic effect of this *Wolbachia* endosymbiont, but we have excluded thelytoky induction since infected virgin females did not produce females in our trials. Preliminary evidence from other species in the *A. varipes/albipodus* complex indicates they harbor a cytoplasmic-incompatibility *Wolbachia* (K.R.H., unpublished data). If indeed the *Wolbachia* carried by the populations studied here cause cytoplasmic incompatibility, our finding of female production from all reciprocal crosses between populations would suggest that the two *A. albipodus* populations carry the same strain of *Wolbachia*.

In conclusion, we have found that two geographically isolated strains of *A. albipodus* show almost complete reproductive compatibility despite molecular divergence and the presence of *Wolbachia*. Reproductive compatibility was not complete, however, and a non-reciprocal halving of fecundity was found in hybrid females. This outbreeding depression could have implications for biological control if both species were to be released against *A. glycines* in North America (see Heimpel et al., 2004). The fecundity of half of the hybrid females would in principle be reduced by one-half. Releasing both strains together could therefore lead to a lower level of biological control than releasing either of the strains alone. Similar conclusions were drawn for more severe cases of non-reciprocal incompatibility mediated by *Wolbachia* in *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) (Mochiah et al., 2002), and for cryptic species of *Trichogramma* (Hymenoptera: Trichogrammatidae) that engage in incompatible matings (Stouthamer et al., 2000).

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