Eur. J. Entomol. **106**: 171–178, 2009
http://www.eje.cz/scripts/viewabstract.php?abstract=1438
ISSN 1210-5759 (print), 1802-8829 (online)

Genetic and physiological variation among sexual and parthenogenetic populations of *Asobara japonica* (Hymenoptera: Braconidae), a larval parasitoid of drosophilid flies

YUSUKE MURATA[1], SHINSUKE IDEO[2], MASAYOSHI WATADA[2], HIDEYUKI MITSUI[1] and MASAHITO T. KIMURA[1]*

[1]Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan;
e-mail: mtk@ees.hokudai.ac.jp

[2]Department of Biology, Faculty of Science, Ehime University, Matsuyama, Ehime 790-8577, Japan

Key words. *Asobara japonica*, climatic adaptation, COI, cold tolerance, diapause, *Drosophila*, oviposition preference, parasitism, preimaginal period, genetic variation

Abstract. We studied variations in genetic, physiological, and ecological traits, and the phylogenetic relationship among sexual and parthenogenetic populations of *Asobara japonica*, a larval parasitoid of drosophilid flies, in order to understand how they adapt to local environments and have differentiated. The strain from Iriomote-jima (IR) differed from other Japanese strains in the nucleotide sequences of its cytochrome oxidase subunit I (COI) and in not undergoing diapause and having a shorter preimaginal period and a higher adult tolerance of cold. The strains other than IR showed a low level of nucleotide variation in COI but varied in their mode of reproduction; the strains from the Ryukyu Islands were sexual, whereas those from the main islands of Japan and Ogasawara were parthenogenetic. In addition, strains from higher latitudes generally showed a high incidence of diapause, although there were some exceptions. On the other hand, preimaginal period and adult cold tolerance varied little among the strains excluding IR, and pupal cold tolerance, oviposition preference and incidence of parasitism varied little among the strains including IR. Evolution and environmental adaptations in this species are discussed, particularly focusing on parthenogenetic populations.

INTRODUCTION

Parthenogenetic lineages are often considered as evolutionary dead ends, and their low potential to respond to rapid environmental changes is thought to be one of the factors leading to their extinction (Maynard Smith, 1978). However, recent studies have shown that parthenogenetic lineages of some organisms, such as aphids, are genetically more variable than expected (reviewed by Lushai & Loxdale, 2002) and have a substantial potential for rapid adaptive changes (Loxdale & Lushai, 2003; Lushai et al., 2003; Wilson et al., 2003; Castagnone-Sereno, 2006). Such genetic variability and adaptive potential could allow a parthenogenetic lineage to adapt to diverse environments following geographic and habitat expansion, resulting in geographic and ecological differentiation. However, genetic variability is not the only mechanism generating genetic differentiation in parthenogenetic populations. Repeated evolution of parthenogenesis in sexual populations would also result in the genetic differentiation among parthenogenetic populations (Cenis, 1993; Semblat et al., 1998; Castagnone-Sereno, 2006). It is possible to discriminate between these two processes of genetic differentiation by molecular phylogenetic analyses of parthenogenetic and sexual populations (Cenis, 1993; Semblat et al., 1998; Vorwerk & Fornech, 2007; Schwander & Crespi, 2009). Such phylogenetic analyses might also provide an estimate of when and where parthenogenesis evolved.

In this paper, we study variations in diapause traits, preimaginal period, cold tolerance, and host use among sexual and parthenogenetic populations of *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) in order to understand how they adapt to local environments. In addition, their phylogenetic relationship is analysed using nucleotide sequences of their mitochondrial cytochrome oxidase subunit I (COI) in order to understand how they have differentiated. The study species is a larval-pupal parasitoid of drosophilid flies (Diptera) occurring from Sapporo (cool-temperate region) to Iriomote-jima (subtropical region), and its populations on the main islands are parthenogenetic whereas those on the Ryukyu Islands are sexual (Mitsui et al., 2007). Thus, the parthenogenetic populations of this species are widely distributed unlike those of many other multicellular parthenogenetic organisms that are restricted to marginal environments (Peck et al., 1998). Widely distributed sexually-reproducing insects often show geographic variation in life-history traits, stress tolerance, or host use as a result of their adaptations to local environmental conditions (Danilevskii, 1965; Tauber et al., 1986; Danks, 1987; Roff, 1992; Kraaijeveld & van del Wel, 1994). In contrast, little is known about how widely distributed parthenogenetic populations vary geographically and adapt to local conditions.

In this study, some geographic strains were crossed in order to determine the extent to which they are geneti-

---

* Corresponding author

cally differentiated and whether they are reproductively isolated or not. Such crossing experiments cannot usually be done using parthenogenetic species. However, parthenogenetic populations of *A. japonica* often produce males when subjected to high-temperature or anti-biotic treatments (unpubl. data), which indicates that the parthenogenesis is a result of infection by *Wolbachia* (Stouthamer, 1997). These males, if fertile, can be used to cross parthenogenetic and sexual populations.

MATERIAL AND METHODS

Experimental strains

Strains of *Asobara japonica* were collected from thirteen localities in Japan (Fig. 1); Sapporo (SP: 43.0°N; collected in August, 2005), Hirosaki (HR: 40.1°N; July, 2007), Sendai (SE: 38.3°N; July, 2007), Fukui (FK: 36.1°N; September, 2005), Tokyo (TK: 35.7°N; June, 2005), Oshima (OS: 34.1°N; September, 1998), Matsuyama (MT: 33.8°N; June, 2007), Kagoshima (KG: 31.6°N; July, 2006), Amami-oshima (AM: 28.4°N; November, 2005), Okinoerabu-jima (OK: 27.4°N; April, 2005), Ogasawara (OG: 27.1°N; April, 2006), Kume-jima (KM: 26.3°N; March, 2001), Iriomote-jima (IR: 24.4°N; March, 2006). Three iso-female lines were established for each of the OK and OG populations (OK1-3 and OG1-3), and one iso- or mass-female line was established for each of the remaining populations. These experimental strains were maintained under 15L : 9D at 23°C in the laboratory for several months to a few years using *Drosophila simulans* Sturtevant as hosts. The AM, OK, KM, and IR strains reproduce sexually, whereas the other strains are parthenogenetic (Fig. 1). Table 1 lists the parasitoid strains used in the present analyses and experiments.

In the phylogenetic analysis, *Asobara rossica* Belokobylskij (from Sapporo), *A. pleuralis* (Ashmead) (from Iriomote-jima), and *A. tabida* Nees von Esenbeck (from Nagano, central Japan) were used for inter-specific comparison, and *Ganaspis xanthopoda* (Ashmead) (from Tokyo and Sendai) and *Leptopilina heterotoma* (Thompson) (from France) of the family Figitidae (Hymenoptera) were used as outgroups.

In the study of oviposition preference and incidence of parasitism, *Drosophila takahashii* Sturtevant, *D. bipectinata* Duda, *D. ficusphila* Kikkawa et Peng, *D. lacteicornis* Okada, *D. albomicans* Duda, and *D. ruberrima* de Meijere were used as potential hosts. These species are major fruit-feeding drosophilids in subtropical areas of Asia, including Iriomote-jima, and are likely to be frequently encountered by *A. japonica* in these areas (Hirai et al., 2001; Mitsui et al., 2007). The experimental strains of these *Drosophila* species were established from several females collected in Iriomote-jima and maintained on cornmeal-malt medium for two or three years in the laboratory.

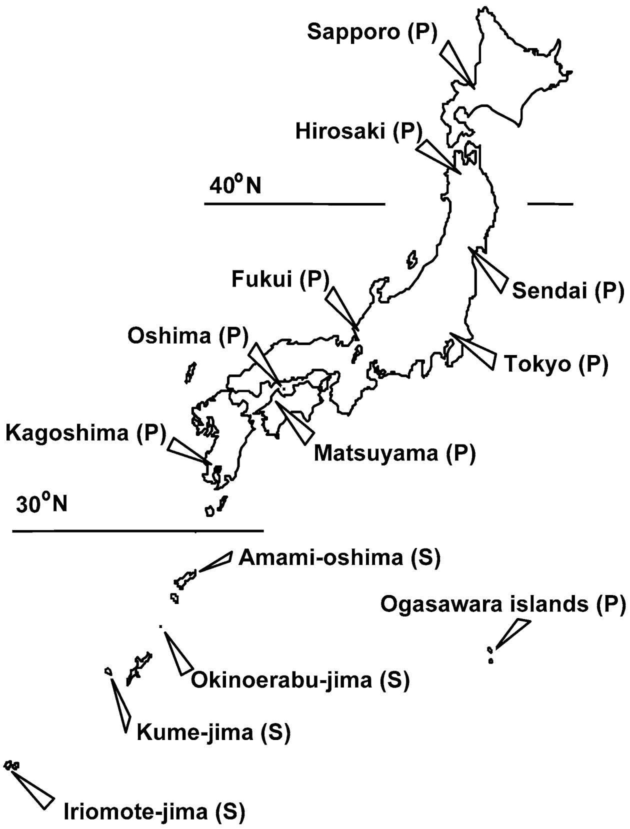

Fig. 1. Origins of the experimental strains of *Asobara japonica*. The mode of reproduction is given in parentheses (S – sexual; P – parthenogenetic).

Phylogenetic analysis using nucleotide sequences of COI

DNA was extracted from five females of each strain using DNAzol (Invitrogen Japan, Tokyo, Japan) and following the supplier's protocol. The primers LCO (5'-GGTCAA CAAATCATAAAGATATTGG-3') and HCO (5'-TAAACT TCAGGGTGACCAAAAAATCA-3') were used to amplify a region (645 bp) of COI (Folmer et al., 1994). PCR reaction mixtures (23 µL) contained 1.3 mM $MgCl_2$, 0.042 mM dNTPs, 2.6 µM primers, 0.042 U Ampli *Taq* DNA polymerase, and 2.4 µL 10× PCR buffer. Amplification was performed as follows: 10 min denaturation step at 94°C for one cycle; 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 50°C, and 1.5 min extension at 72°C; 12 min final extension at 72°C for one cycle. Amplified COI products were diluted to 1 ng/µL and used as sequencing templates after their sizes were determined. Sequencing of COI was done using the same primers used for PCR amplification. All sequence reactions were done using the Big Dye Terminator Cycle Sequencing Kit (ABI) and

TABLE 1. Strains of *Asobara japonica* used in the present analyses and experiments. See text for abbreviation of strains.

Experiment	Strains
Phylogenetic analysis	SP, HR, SE, FK, TK, OS, MT, KG, AM, OK, OG, KM, IR
Cross experiments	TK, AM, IR
Diapause incidence	SP, HR, SE, TK, MT, KG, AM, OG, IR
Preimaginal period	SP, HR, SE, TK, MT, KG, AM, OG, IR
Pupal cold tolerance	SP, SE, TK, KG, AM, IR
Adult cold tolerance	SP, HR, SE, TK, MT, KG, AM, OG, IR
Oviposition preference	TK, AM, IR
Incidence of parasitism	TK, AM, IR

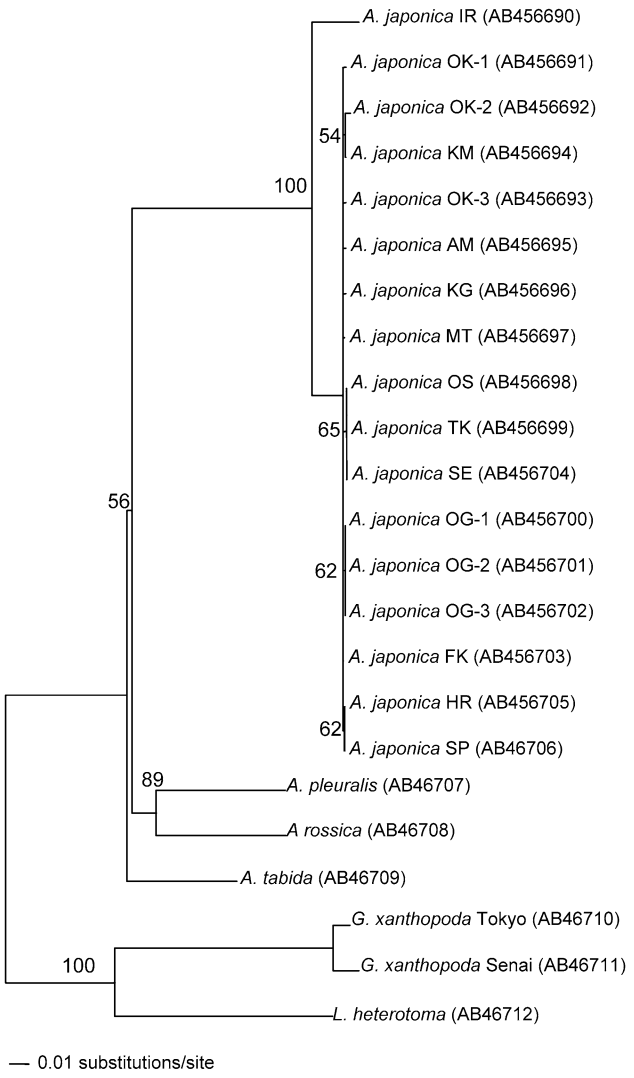

Fig. 2. Neighbour-joining tree based on COI gene sequences. Bootstrap values are indicated on the branches (values less than 50% are not shown). The accession number is given in parentheses.

sequencing was done with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems Japan, Tokyo, Japan). The 23 partial COI sequences obtained in this study were deposited in the DDBJ database (accession number AB456690–AB456712).

Phylogenetic analysis of the sequence data was performed using the PAUP 4.0b10 software (Swofford, 2002). COI sequences were aligned manually and aligned sequences were used to construct phylogenetic trees using the neighbour-joining (NJ) method (Saitou & Nei, 1987). The nucleotide distances used for the NJ trees were estimated by Kimura's two-parameter method (Kimura, 1980). Bootstrap values were obtained after 1000 replications.

Crossing experiments

In order to determine whether the strains are reproductively isolated the TK, AM, and IR strains were crossed. As previously indicated, males are produced in the parthenogenetic strains when they are reared at high temperatures or subjected to antibiotics. In the present study males of the TK strain were obtained by exposing larvae to 28°C for two days.

Unmated parasitoid females were collected by isolating them immediately after eclosion and then placed individually in vials or with two males. When F_1 females and males were obtained, they were used to produce F_2 and F_3 progenies. Experiments were made under 15L : 9D at 23°C using *D. simulans* larvae as hosts. There were eight to twenty replicates for each cross.

Incidence of diapause

In a preliminary study, individual parasitoids of the TK strain remained in the prepupal stage for a long period when reared under a short daylength (10L : 14D), and at a low temperature (15°C), which indicates the incidence of prepupal diapause. In this study, the incidence of prepupal diapause was determined in the SP, HR, SE, TK, MT, KG, AM, OG1, and IR strains.

Two parasitoid females were introduced into vials each with 20 two-day old host (*D. simulans*) larvae and kept at 15L : 9D and 23°C for a day. Vials with parasitized host larvae were then placed under various photoperiodic conditions at 15, 18, and 23°C. When the host larvae reached the third instar, tissue paper was placed on the surface of the Drosophila rearing medium to facilitate pupariation. When the adult parasitoids emerged, they were counted and sexed. Non-diapausing individuals usually emerged after 11–17, 25–35, and 40–70 days following oviposition at 23, 18, and 15°C, respectively. When emergence ceased (i.e., no parasitoid had emerged for 10 days) or emergence failed to occur after 60 days at 18°C or 90 days at 15°C, vials were transferred to diapause-averting conditions of 15L : 9D and 23°C, and the emergence of adult parasitoids was recorded. The measure of the incidence of diapause was the percentage of adult parasitoids that emerged after transfer to diapause-averting conditions. In addition, in order to determine whether this species enters diapause when reared at 15L : 9D and 23°C, vials kept under these conditions were exposed to 4°C (constant darkness) for 100 days, since diapause induced under a long daylength and a high temperature is often terminated by chilling (Tauber et al., 1986). These cold treated-vials were then again placed at 15L : 9D and 23°C, and the emergence of adult parasitoids was recorded. Usually, there were five vials (each with about 20 parasitized host larvae) in each treatment.

Preimaginal period

To determine the preimaginal (egg to eclosion) period, parasitized host larvae were reared as outlined above and then kept at 15L : 9D and 23°C, and the emergence of adult parasitoids was recorded daily. The preimaginal period was determined only for females since males were rarely produced.

Cold tolerance

Cold tolerance was determined at the pupal and adult stages. Parasitized host larvae were kept at 15L : 9D and 23°C, and tissue paper was placed on the rearing medium for their pupariation. To determine the cold tolerance of parasitoid pupae, host pupae were placed on wet paper in Petri dishes 10 (for those parasitized by the IR strain) or 11 days (for those parasitized by other strains) after being parasitized and exposed to 0°C (constant darkness) for 12 h to 4 days. After exposure to cold, host pupae were kept at 15L : 9D and 23°C, and the emergence of adult parasitoids was recorded. The results for twenty to 100 (usually 50) pupae in two to four Petri dishes were used for each datum point.

Adult cold tolerance was determined using 4- or 5-day-old females reared at 15L : 9D and 23°C. Parasitoid females were placed in vials with Drosophila medium (about 25 adults per vial) and exposed to 0°C (constant darkness) for 1 to 4 days. The Drosophila medium maintained a relative humidity in vials of about 90%. The vials were lined with filter paper, which prevented the parasitoids from being caught in water droplets forming on the walls. After exposure to cold they were placed at 23°C (15L : 9D) for 24 h, and the number surviving was

Table 2. The number of replicates of the crossing experiments with only male, only female or both male and female progeny. In each replicate, one female was kept with two males or without male.

Parental strain		No. of replicates with		
Female	Male	only male progeny	progenies of both sexes	only female progeny
TK	–	0	1*	8
TK	TK	0	2*	7
TK	AM	0	0	8
AM	TK	7	4	0
AM	–	12	0	0
IR	–	12	0	0
AM	AM	5	15	0
IR	IR	5	7	0
IR	AM	3	8	0
AM	IR	10	2	0

* Very few males emerged.

recorded. Those that were able to walk were designated as alive. The results for twenty-three to 100 (usually 50) individuals in two to four vials were used for each datum point.

Oviposition preference and incidence of parasitism

The incidence of parasitism and oviposition preference of the TK, AM, and IR strains were studied using *D. takahashii*, *D. bipectinata*, *D. ficusphila*, *D. lacteicornis*, *D. albomicans*, and *D. ruberrima* as potential hosts. To determine the oviposition preference, five two- or three-day old parasitoid females were placed with 30 two- or three-day-old drosophilid larvae in a small Petri dish containing a small amount of food medium and left for four hours at 23°C. Thereafter, the drosophilid larvae were dissected under a stereoscopic microscope, and the presence of parasitoid eggs was recorded.

To determine the incidence of parasitism two- or three-day-old parasitoid females were placed with a number of two- or three-day-old drosophilid larvae in a Petri dish containing a small amount of food medium. The oviposition behaviour of the parasitoids was then observed under a stereoscopic microscope. After parasitization the drosophilid larvae were transferred to vials containing food medium, and the emergence of drosophilids or parasitoids was recorded at 23°C.

RESULTS

Phylogenetic analysis

Fig. 2 shows the phylogenetic relationship of the different strains and species based on nucleotide sequences of COI (645 bp). The divergence in nucleotide sequences was, on average, 4.2% (3.9–4.3%) between the IR and other strains and 0 to 0.6% between all the strains other than IR. The divergence between the different species of *Asobara* was 13.6 to 20.1%.

Crossing experiments

Table 2 shows the results. TK females produced only female progeny in most replicates whether they were kept with or without males except for few replicates where very few males were produced. On the other hand, unmated AM and IR females always produced only male progeny. When AM females were placed with TK males, female and male progeny were produced in four of the 11 replicates, suggesting that TK males are capable of reproduction. In the experiments on the AM and IR strains, the frequency of replicates with both female and male progeny were significantly lower (χ^2 test, $P = 0.001$) in the cross between AM females and IR males than the cross in the opposite direction or within each strain (χ^2 test, $P < 0.01$). There may be weak asymmetrical sexual isolation.

Table 3. Incidence of diapause (%) in the nine geographic strains of *A. japonica* reared under 15L : 9D and 10L : 14D at 23 and 18°C.

Strain	23°C		18°C	
	15L : 9D	10L : 14D	15L : 9D	10L : 14D
Sapporo	0 (66)	0 (60)	0 (39)	0 (37)
Hirosaki	0 (55)	0 (60)	0 (66)	25.0 (28)
Sendai	0 (61)	0 (59)	0 (58)	100 (42)
Tokyo	0 (63)	0 (68)	0 (61)	100 (40)
Matsuyama	0 (67)	0 (66)	0 (20)	69.6 (46)
Kagoshima	0 (67)	0 (67)	0 (57)	71.1 (38)
Amami-oshima	0 (67)	0 (57)	0 (85)	40.0 (50)
Ogasawara	0 (60)	0 (53)	0 (24)	22.7 (22)
Iriomote-jima	0 (68)	0 (70)	0 (82)	0 (70)

The number in parentheses refers to the total number of individuals eclosed.

The crosses that produced F_1 females and males also produced F_2 and F_3 females and males (data not shown). These results indicate that these three strains are conspecific.

Incidence of diapause

At 23°C adult emergence occurred within 18–20 days of oviposition in all strains, whether reared under 15L : 9D or 10L : 14D conditions (Table 3). Thereafter, no emergence occurred even if the vials were kept at 0°C for 100 days (for those kept under 15L : 9D) or transferred to 15L : 9D (for those reared at 10L : 14D). Thus, this species does not enter diapause at 23°C.

When reared at 15L : 9D and 18°C no strain entered diapause (Table 3). At 10L : 14D and 18°C the incidence of diapause in the strains varied from 0 to 100% (Table 3). Except for the northernmost two strains (SP and HR) the incidence of diapause was generally higher in strains from higher latitudes.

Fig. 3 shows the photoperiodic responses recorded at 15°C. The IR strain did not enter diapause at any of the photoperiods used. The other strains showed a long-day response with the incidence of diapause higher when reared at short daylengths. Except for the SP and OG strains, the incidence of diapause was generally higher in strains from higher latitudes at every photoperiod used, and the HR strain showed a high incidence of diapause even at 15L : 9D. The SP strain showed a relatively low incidence of diapause and the OG strain a high incidence of diapause.

Preimaginal period

The females of the IR strain had significantly shorter preimaginal periods than those of all other strains, and those of the MT strain significantly longer ones than

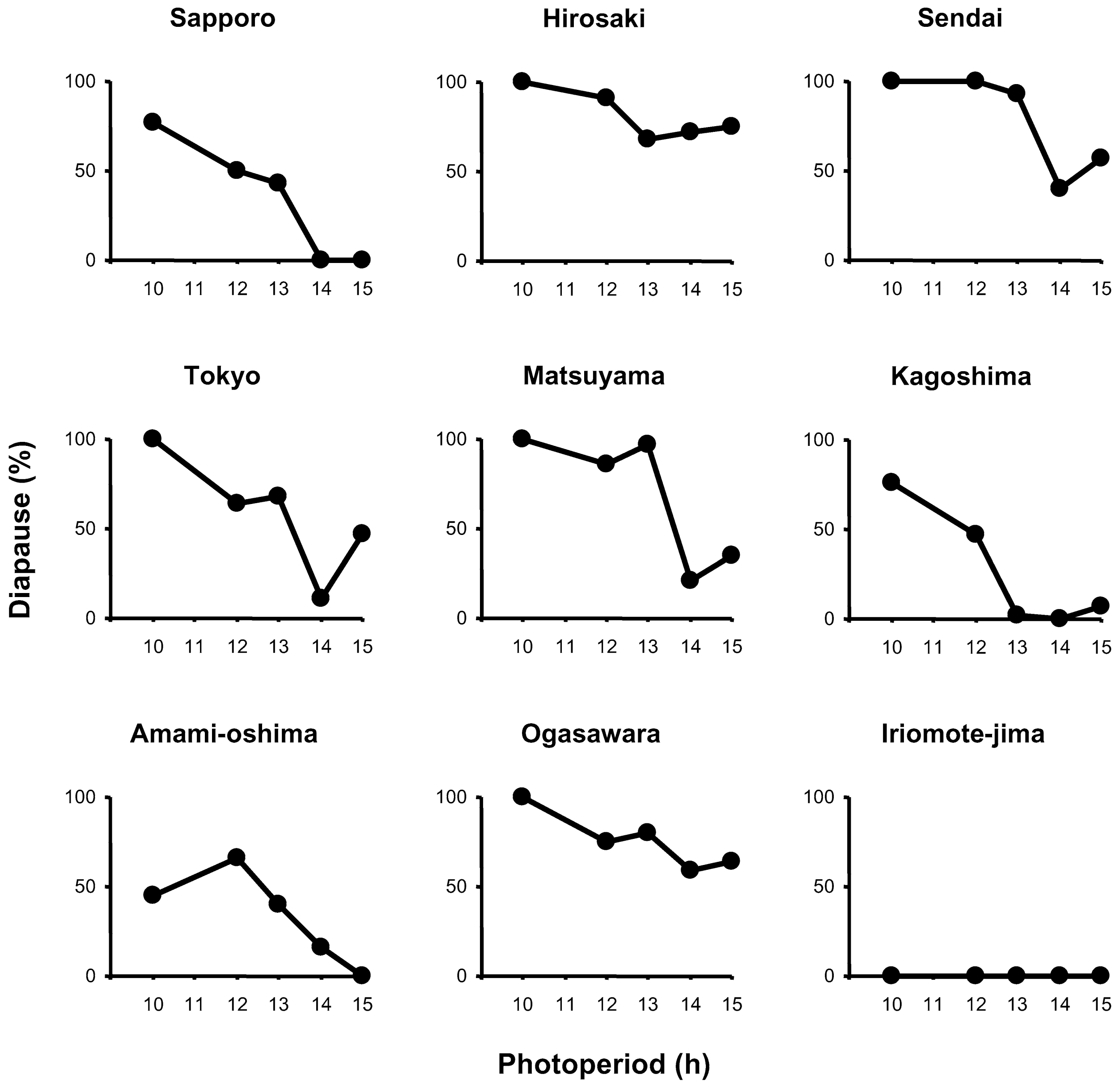

Fig. 3. Percentage diapause recorded for the different strains of *Asobara japonica* reared under different photoperiods at 15°C.

those of the SP, HR, and KG strains (Tukey's HSD test after ANOVA, $P < 0.05$; SP = 14.90 ± 0.14 (mean ± SD), HR = 14.92 ± 0.14, SE = 15.21 ± 0.14, TK = 15.00 ± 0.18, MT = 15.60 ± 0.12, KG = 14.91 ± 0.15, OG = 14.96 ± 0.18, AM = 15.19 ± 0.15, IR = 13.64 ± 0.26 days).

Cold tolerance

Fig. 4 shows the percentage of host pupae that produced adult parasitoids after exposure to 0°C for various periods. There was a slight difference in the cold tolerance of the strains with the percentage survival after one-day exposure significantly lower (χ^2 test, $P < 0.05$) in the SP and TK strains than the other four strains.

Fig. 5 shows the percentage survival of adult females after exposure to 0°C for various periods. The percentage survival after three-day exposure was significantly higher (χ^2 test, $P < 0.05$) in the IR strain than the other strains, and that after two-day exposure was significantly higher (χ^2 test, $P < 0.05$) in the SD, TK, and AM strains than the other five strains.

Oviposition preference and incidence of parasitism

The TK, AM, and IR strains readily oviposited in *D. takahashii*, *D. lacteicornis*, *D. bipectinata*, and *D. ficusphila* larvae, less so in *D. ruberrima* larvae, and not in *D. albomicans* larvae (Table 4). There was no significant difference (χ^2 test, $P > 0.05$) in the percentage of larvae oviposited by the TK, AM, and IR strains.

All strains successfully parasitized *D. takahashii* and *D. lacteicornis* larvae (Fig. 6) and there was no significant difference (χ^2 test, $P > 0.05$) in survival during the preimaginal period (i.e. from oviposition to adult eclosion) when they parasitized *D. takahashii* or *D. lacteicornis* larvae. On the other hand, none of the strains survived in *D. bipectinata* and *D. ficusphila* larvae (Fig. 6), even though they oviposited in these larvae (Table 4).

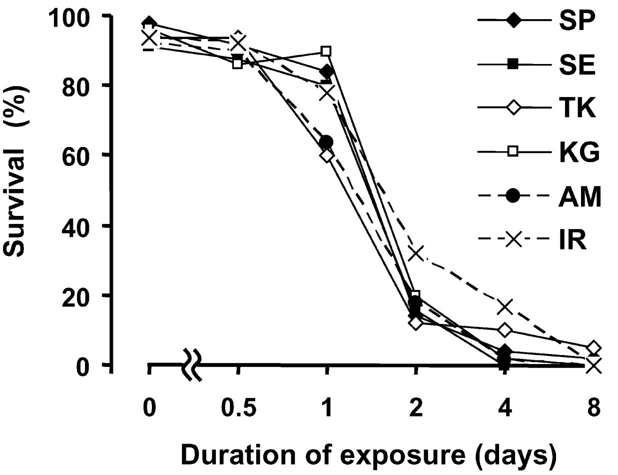

Fig. 4. Percentage survival of pupae of the different strains of *Asobara japonica* that were exposed to 0°C for various periods.

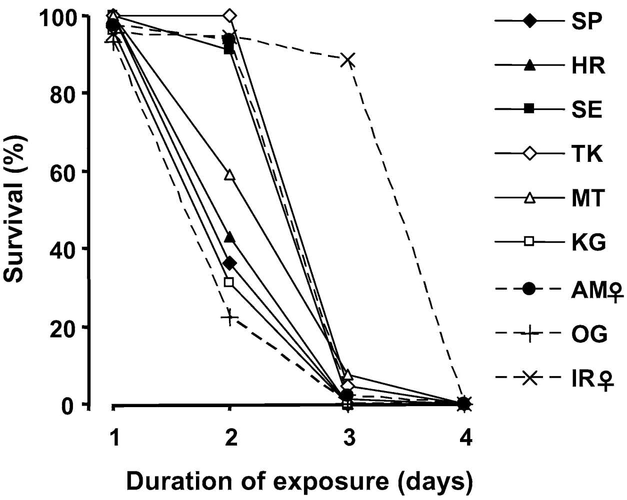

Fig. 5. Percentage survival of adult females of the different strains of *Asobara japonica* that were exposed to 0°C for various periods.

DISCUSSION

The Iriomote-jima (IR) population of *Asobara japonica* differs in its COI nucleotide sequence from northern populations. In addition, it does not diapause and has a faster preimaginal development, and its adults have a higher tolerance of cold. It is also reported that most populations and species of insects occurring in subtropical regions like the Iriomote-jima strain do not diapause or only weakly do so and develop faster (Masaki, 1961; Danilevskii, 1965; Tauber et al., 1986; Danks, 1987; Kimura, 1988; Shimizu & Kawasaki, 2001; Kato, 2005; Shiota & Kimura, 2007). However, it is unknown why the adults of the IR population have a higher tolerance of cold. It may be related to the lack of diapause. Since the IR population does not diapause, adults of this population could emerge in winter. A high tolerance of cold may lower its mortality in winter, even though temperatures on Iriomote-jima do not fall below 0°C (Hirai et al., 2000). In contrast, tolerance of cold in the adult is not necessary in the northern populations because they overwinter in prepupal diapause.

Furthermore, there seems to be weak asymmetrical sexual isolation between the IR and AM strains. The morphological and genetic distinction between populations from Iriomote-jima and Amami-oshima is also documented for a number of other animals and insects (Kimoto & Gressitt, 1966; Ohta, 1998). Such results suggest that these two regions have been geographically separated for a long time.

The *A. japonica* populations from the Ryukyu Islands, including Iriomote-jima, reproduce sexually whereas those from Ogasawara and the main islands of Japan are parthenogenetic (see Mitsui et al., 2007). However, there is no or very little differentiation in the COI nucleotide sequences of the sexually reproducing populations from the central Ryukyu Islands (Amami-oshima, Okinoerabu-jima and Kume-jima) and parthenogenetic populations from Ogasawara and the main islands of Japan. This suggests that parthenogenesis in this species has evolved recently.

The strains other than IR entered prepupal diapause when reared under short daylengths and low temperatures. As observed in many temperate insects (Danilevskii, 1965; Tauber et al., 1986; Danks, 1987) the incidence of diapause was usually higher in strains from higher latitudes, although there were exceptions (the SP and OG strains). Even so, it is unclear whether there is geographic variation in the critical daylength for diapause induction in this species, although it is commonly observed in temperate insects (Danilevskii, 1965; Tauber et al., 1986; Danks, 1987). Thus, *A. japonica* may adapt to local climatic conditions not by changing the critical daylength but the incidence of diapause.

TABLE 4. Percentages of drosophilid larvae oviposited by the TK, AM and IR strains of *Asobara japonica* in the oviposition preference test.

	TK		AM		IR		
	N	Oviposition (%)	N	Oviposition (%)	N	Oviposition (%)	χ^2-test
D. takahashii	22	81.8	25	96.0	30	93.3	$P = 0.202$
D. lacteicornis	21	85.7	28	85.7	30	86.7	$P = 0.317$
D. bipectinata	30	83.3	30	96.7	30	96.7	$P = 0.084$
D. ficusphila	30	86.7	30	86.7	30	80.0	$P = 0.713$
D. albomicans	27	0	30	0	30	0	–
D. ruberrima	26	19.2	24	12.5	30	3.3	$P = 0.167$

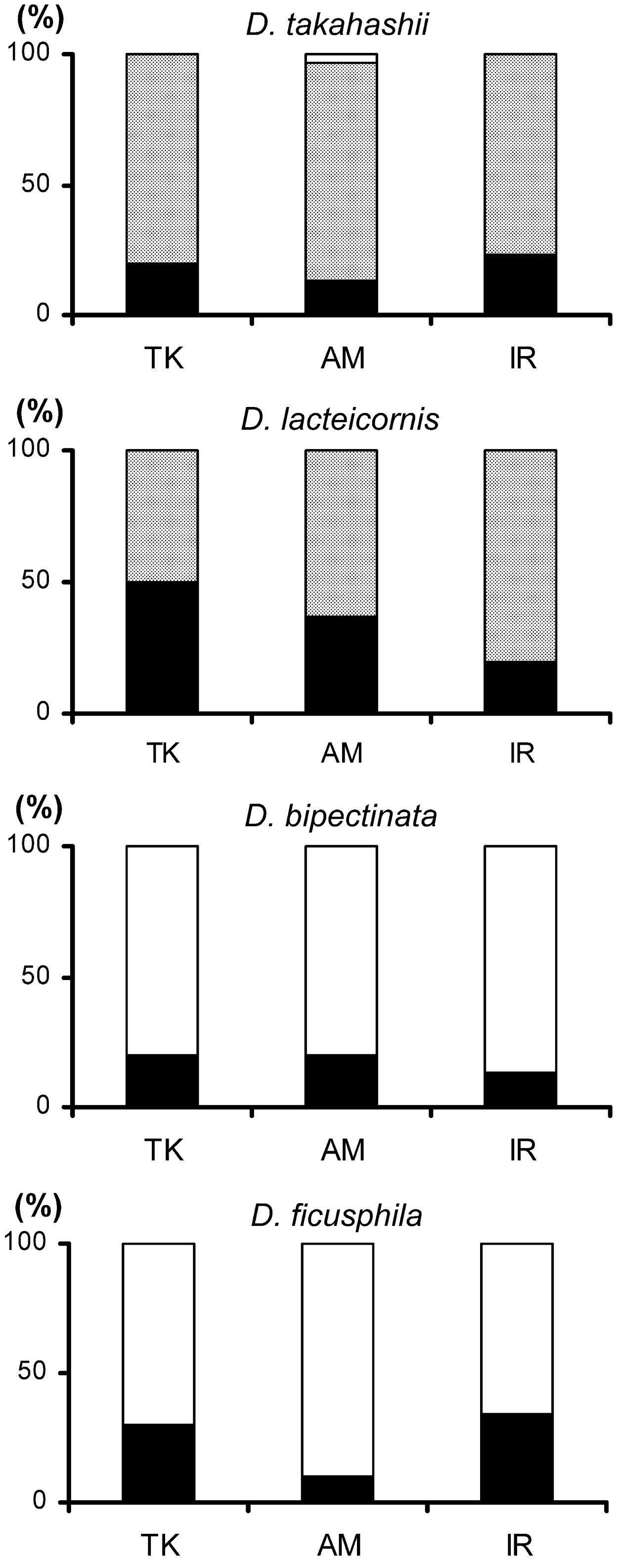

Fig. 6. Percentages of *Drosophila takahashii*, *D. lacteicornis*, *D. bipectinata*, and *D. ficusphila* larvae from which neither parasitoids or flies emerged (black), parasitoids emerged (grey), or flies emerged (white), after parasitization by the TK, AM, and IR strains of *Asobara japonica*. Thirty drosophilid larvae were used in each test.

The intensity of diapause in the SP and OG strains was relatively weak and strong, respectively. It is not known whether their responses are the result of adaptations to the specific conditions prevailing at Sapporo and Ogasawara. These populations may have been recently established by migrants from other localities.

Thus, diapause traits varied even among the parthenogenetic populations of *A. japonica*, and this variation is assumed to have arisen, at least to some extent, as a result of adaptations to local climatic conditions. There are processes for generating variation in parthenogenetic populations. First, adaptive changes may occur in a parthenogenetic lineage. Recently, it was suggested that parthenogenetic lineages have substantial potential for rapid adaptive changes (Loxdale & Lushai, 2003; Lushai et al., 2003; Wilson et al., 2003; Castagnone-Sereno, 2006). Second, parthenogenesis may have evolved independently in different sexually reproducing populations adapted to different climatic conditions. In nematodes, it is suggested that the transition from sexual to parthenogenetic reproduction has occurred many times (Cenis, 1993; Semblat et al., 1998; Castagnone-Sereno, 2006). The phylogenetic analysis using COI reported here failed to reveal how parthenogenetic *A. japonica* populations have evolved. More detailed genetic and molecular analyses are needed to answer this question.

There was only a low level of variation in the preimaginal period and tolerance of cold among the *A. japonica* strains studied, except for the IR strain, which has a shorter preimaginal period and higher adult tolerance of cold. Kimura et al. (1994) and Kimura (2004) also recorded no or little geographic variation in adult tolerance of cold in *Drosophila* species. These traits may be tightly linked by trade-offs with each other and/or with other adaptive traits, and may be subjected to evolutionary constraints (Kimura, 2004; Shiota & Kimura, 2007; Mori & Kimura, 2008).

In addition, there were no differences in oviposition preferences and incidence of parasitism at least among the TK, AM, and IR strains, although they use different host species in the field (Mitsui et al., 2007). For example, the IR population is likely to frequently encounter *D. lacteicornis*, *D. ruberrima*, *D. takahashii*, *D. bipectinata*, *D. ficusphila*, and *D. albomicans* but the TK population is unlikely to (Hirai et al., 2000; Beppu, 2006; Kondo & Kimura, 2008). However, the TK and IR strains showed almost the same responses to these drosophilid species. The incidence of parasitism and oviposition preferences of *A. japonica* may have evolved more slowly than diapause-related traits or tolerance of cold.

This study raises a question – why does *A. japonica* oviposit in the non-host drosophilid species *D. bipectinata* and *D. ficusphila*? There may not have been enough time for *A. japonica* to evolve an ability to discriminate between the larvae of *D. bipectinata* or *D. ficusphila* and host larvae. It is also possible that *A. japonica* is a kleptoparasitoid of *D. bipectinata* and *D. ficusphila*. That is, its larvae can develop in these species once they are parasitized by another parasitoid. Kraaijeveld (1999) reported kleptoparasitism by *A. tabida*. This parasitoid oviposits in *D. simulans* larvae even though its larvae are unable to develop when it is the only parasitoid present, but if the host is also parasitized by *Leptopilina boulardi* the larvae of *A. tabida* can develop in this host (Kraaijeveld, 1999). *A. tabida* increases its probability of surviving in *D. simulans* by taking advantage of the virulence of *L. boulardi*.

ACKNOWLEDGMENTS. We thank F. Vavre for providing us with the French strain of *Leptopilina heterotoma* and information on the mating behaviour of *Asobara japonica*. This work was supported by a Grant-in-Aid from Ministry of Education, Science, Sports and Culture of Japan (No. 19405013).

REFERENCES

BEPPU K. 2006: Seasonal change of drosophilid assemblage and adult age structure of the common drosophilid species in the Imperial Palace Grounds, Tokyo. *Mem. Nat. Sci. Mus.* **43**: 295–334 [in Japanese with English abstr.].

CASTAGNONE-SERENO P. 2006: Genetic variability and adaptive evolution in parthenogenetic root-knot nematodes. *Heredity* **96**: 282–289.

CENIS J.L. 1993: Identification of the four major Meloidogyne spp. by random amplified polymorphic DNA (RAPD-PCR). *Phytopathology* **83**: 76–80.

DANILEVSKII A.S. 1965: *Photoperiodism and Seasonal Development of Insects*. Oliver & Boyd, London, 283 pp.

DANKS H.V. 1987: *Insect Dormancy: an Ecological Perspective*. Biological Survey of Canada, Ottawa, 439 pp.

FOLMER O., BLACK M., HOEH W., LUTZ R. & VRIJENHOEK R. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Marine Biol. Biotech*. **3**: 294–299.

HIRAI Y., GOTO S.G., YOSHIDA T. & KIMURA M.T. 2000: Faunal and ecological surveys on drosophilid flies in Iriomote-jima, a subtropical island of Japan. *Entomol. Sci*. **3**: 273–284.

KATO Y. 2005: Geographic variation in photoperiodic response for the induction of pupal diapause in the Aristolochia-feeding butterfly Atrophaneura alcinous. *Appl. Entomol. Zool.* **40**: 347–350.

KIMOTO S. & GRESSITT J.L. 1966: The Chrysomelidae of the Ryukyu archipelago. *Pacif. Insects* **8**: 467–577.

KIMURA M. 1980: A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*. **16**: 111–120.

KIMURA M.T. 1988: Adaptations to temperate climates and evolution of overwintering strategies in the Drosophila melanogaster species group. *Evolution* **42**: 1288–1297.

KIMURA M.T. 2004: Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* **140**: 442–449.

KIMURA M.T, OHTSU T., YOSHIDA T., AWASAKI T. & LIN F.J. 1994: Climatic adaptations and distributions in the Drosophila takahashii species-subgroup (Diptera: Drosophilidae). *J. Nat. Hist.* **28**: 401–409.

KONDO M. & KIMURA M.T. 2008: Diversity of drosophilid flies in Kume-jima, a subtropical island: comparison with diversity in Iriomote-jima. *Entomol. Sci*. **11**: 7–15.

KRAAIJEVELD A.R. 1999: Kleptoparasitism as an explanation for paradoxical oviposition decisions of the parasitoid Asobara tabida. *J. Evol. Biol*. **12**: 129–133.

KRAAIJEVELD A.R. & VAN DEL WEL N.N. 1994: Geographic variation in reproductive success of the parasitoid Asobara tabida in larvae of several Drosophila species. *Ecol. Entomol*. **19**: 221–229.

LOXDALE H.D. & LUSHAI G. 2003: Rapid changes in clonal lines: the death of a 'sacred cow'. *Biol. J. Linn. Soc*. **79**: 3–16.

LUSHAI G. & LOXDALE H.D. 2002: The biological improbability of a clone. *Genet. Res.* **79**: 1–9.

LUSHAI G., LOXDALE H.D. & ALLEN J.A. 2003: The dynamic clonal genome and its adaptive potential. *Biol. J. Linn. Soc.* **79**: 193–208.

MASAKI S. 1961: Geographical variation of diapause in insect. *Bull. Fac. Agr. Hirosaki Univ*. **7**: 66–98.

MAYNARD SMITH J.S. 1978: *The Evolution of Sex*. Cambridge University Press, Cambridge, 222 pp.

MITSUI H., VAN ACHTERBERG K., NORDLANDER G. & KIMURA M.T. 2007: Geographical distributions and host association of larval parasitoids of frugivorous Drosophilidae in Japan. *J. Nat. Hist*. **41**: 1731–1738.

MORI N. & KIMURA M.T. 2008: Selection for rapid and slow recovery from chill- and heat-coma in Drosophila melanogaster. *Biol. J. Linn. Soc*. **95**: 72–80.

OTA H. 1998: Geographic patterns of endemism and speciation in amphibians and reptiles of the Ryukyu archipelago, Japan, with special reference to their paleogeographical implications. *Res. Popul. Ecol*. **40**: 189–204.

PECK J.R., YEARSLEY J.M. & WAXMAN D. 1998: Explaining the geographic distribution of sexual and asexual populations. *Nature* **391**: 889–892.

ROFF D.A. 1992: *The Evolution of Life Histories*. Chapman & Hall, New York, 535 pp.

SAITOU N. & NEI M. 1987: The neighbor-joining methods: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

SCHWANDER T. & CRESPI B.J. 2009: Multiple direct transitions from sexual reproduction to apomictic parthenogenesis in Timmen a stick insects. *Evolution* **63**: 84–103.

SEMBLAT J.P., WAJNBERG E., DALMASSO A., ABAD P. & CASTAGNONE-SERENO P. 1998: High resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. *Mol. Ecol*. **7**: 119–125.

SHIMIZU T. & KAWASAKI K. 2001: Geographic variability in diapause response of Japanese Orius species. *Entomol. Exp. Appl.* **98**: 303–316.

SHIOTA H. & KIMURA M.T. 2007: Evolutionary tradeoffs between thermal tolerance and locomotor and developmental performance in drosophilid flies. *Biol. J. Linn. Soc.* **90**: 375–380.

STOUTHAMER R. 1997: Wolbachia-induced parthenogenesis. In O'Neill S.L., Hoffman A.A. & Werren J.H. (eds): *Influential Passengers*. Oxford Univ. Press, Oxford, pp. 102–124.

SWOFFORD D.L. 2002: *PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4.0b10.* Sinauer, Sunderland.

TAUBER M.J., TAUBER C.A. & MASAKI S. 1986: *Seasonal Adaptations of Insects*. Oxford University Press, New York, 411 pp.

VORWERK S. & FORNECK A. 2007: Analysis of genetic variation within clonal lineages of grape phylloxera (Daktulosphaira vitifoliae Fitch) using AFLP fingerprinting and DNA sequencing. *Genome* **50**: 660–667.

WILSON A.C.C., SUNNUCKS P. & HALES D.F. 2003: Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biol. J. Linn. Soc*. **79**: 115–135.

Received February 2, 2009; revised and accepted March 13, 2009