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The influence of prior experience on preference and performance of a cryptoparasitoid *Scleroderma guani* (Hymenoptera: Bethylidae) on beetle hosts

LI LI,[1,2] DANIEL R. MILLER[3] and JIANGHUA SUN[1] [1]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, [2]Graduate University, Chinese Academy of Sciences, Beijing 100049, China and [3]USDA Forest Service, Southern Research Station, Athens, Georgia, U.S.A.

Abstract. 1. Numerous studies have reported the effects of learning or experience on parasitoid host preference and location. However, the integration of pre-imaginal and adult experiences on the subsequent host preference and adult/offspring performance has been rarely tested in host–parasite interactions.

2. We present direct evidence that theses two kinds of experiences affect host preference and related fitness in the polyphagous parasitoid, *Scleroderma guani*. Two colonies of parasitoids were reared on *Monochamus alternatus* and *Saperda populnea* (Cerambycidae: Lamiinae). Individuals from the two colonies were given host-switching experience for one generation (pre-imaginal experience) while other individuals were given prior ovipositing experience on the two species, respectively (adult experience).

3. *Scleroderma guani* females demonstrated that their experiences determined adult behavioural responses and their subsequent performance to hosts. Females maximised both adult fitness (fecundity and longevity) and offspring fitness (survival and sex ratio) when they encountered hosts similar to their maternal hosts. Behavioural plasticity in host choice was affected by adult experience, resulting in improved adult feeding and ovipositing behaviour and further modifying adult fecundity and the offspring sex ratio. There was a positive correlation between oviposition preference and adult fecundity.

4. The results indicated that *S. guani* exhibited positive preference–performance correlations. This is most likely due to an adaptation to maternal hosts over multiple generations. However, foraging potential of adults to available cues from hosts may be driven quickly by an experience-induced learning process rather than by natural selection processes shaped over many generations.

Key words. Behavioural plasticity, bethylidae, host–parasite interactions, host selection, optimality theory, preference–performance correlations, prior experience.

Introduction

Host preference by adult parasitoids and subsequent performance of their offspring on those hosts are central to understanding the biology, ecology, and evolution of the complex interactions in host–parasite associations (Godfray, 1994). A vast amount of research has attempted to explain host preference based on the role of learning from prior foraging experience of parasitoids in modulating behavioural decisions. Pre-imaginal experience (Tully *et al*., 1994; Rietdorf & Steidle, 2002; Gandolfi *et al*., 2003; Gutiérrez-Ibáñez *et al*., 2007) and/or imaginal experience (Barron & Corbet, 1999; van Emden *et al*., 2002) have already been reported to influence subsequent host preference. As a result of pre-imaginal

Correspondence: Jianghua Sun, Institute of Zoology, Chinese Academy of Sciences, 1 Beichen West Road, Chaoyang District, Beijing 100101, China. E-mail: sunjh@ioz.ac.cn

experiences, parasitoids in the immature stage can learn from their host or environment. This learned experience can be transferred from the pre-imaginal stage to the adult, i.e. the so-called Hopkins' host selection principle (HHSP). Since its first proposal in 1916, the principle and meaning has changed significantly (Barron, 2001; Rietdorf & Steidle, 2002). Barron (2001) stated that the HHSP is the observation that adult insects may show an enhanced preference for the host species on which they carried out their larval development, when compared to the general population. There are, however, some disagreements on how the HHSP should be formulated, especially with respect to its potential mechanism (van Emden *et al*., 1996; Janz *et al*., 2009).

Most adult parasitoids with prior experience of a particular host species or novel odour, may show a biased preference towards the same host species or odour, in the future (Papaj & Prokopy, 1989; Szentesi & Jermy, 1990; Takasu & Lewis, 2003). Such preferences may result from adaptive learning which allows parasitoids to focus on the most reliable and available cues; learned mechanisms which include an increase in the number of stimuli eliciting a response, development, and retention of a preference (Vet & Papaj, 1992; Prokopy *et al*., 1993), and a decrease in the variability of a parasitoid's response to a stimulus (Vet *et al*., 1990; Turlings *et al*., 1993). Some recent studies have focused on the effects of learning on population-level patterns of host use by parasitoids in field populations (Langley *et al*., 2006). Indeed, most evidence stressed the importance of prior experience on behavioural responses during foraging for host species and habitation. Current hypotheses propose that learning may be advantageous, not only in adult host selection behaviour through improved recognition, accuracy or selectivity in foraging, but also in subsequent adult/offspring performance.

The ectoparasitoid, *Scleroderma guani* (Hymenoptera: Bethylidae) usually locates its host by walking on the bark. Once detected, females paralyse the larvae or pupae by injecting venom into it before laying eggs on the host. The eggs complete development on a single host paralysed by maternal females. *Scleroderma guani* is of particular interest to researchers in China, primarily for its wide use in biological control of *Monochamus alternatus*, the most important vector of the pinewood nematode, *Bursaphelenchus xylophilus* Steiner et Buhrer in Japan and China (Kobayashi *et al*., 1984; Ding *et al*., 2001). Based on previous biological research of the *S. guani* parasitoid, the Cerambycidae–Bethylidae system has several favourable attributes for the effect of learning on behavioural preference and subsequent adult/offspring fitness. *S. guani* is a synovigenic parasitoid and must continue to feed to ensure continuation of oogenesis throughout its reproductive lifetime (Koch, 1973; Lauzière *et al*., 2000). In contrast to proovigenic parasitoids that do not feed during the adult stage (Scheirs & De Bruyn, 2002), host selection by *S. guani* must be directed to mediate foraging host and habitation. Since immature stages of *S. guani* have limited mobility to relocate in relation to a host, there is a strong selection pressure for the adult to oviposit on hosts that will favour offspring development. Lastly, *S. guani* likely require effective searching tactics in finding their hosts, which tend to be solitary wood-boring insects in cryptic situations (wattle, trunk, wood, and seed) (Evans, 1964; Gauld & Hanson, 1995; Zhang, 2004).

The present study demonstrates that both preference and performance of *S. guani* parasitoids were influenced by prior experience (pre-imaginal and imaginal experiences). Our aim was to evaluate the host-selection adaptabilities of *S. guani* with respect to the effects their experiences had on feeding and oviposition preference by female *S. guani* and on the performance of adults and offspring. We hypothesise that *S. guani* can adaptively integrate foraging preference with adult performance (fecundity and longevity) and offspring performance (survival and sex ratio), depending on those recent experiences.

Materials and methods

We conducted eight experiments looking at the effects of pre-imaginal and imaginal experiences on: (1) feeding and ovipositioning choices by female *S. guani*; and (2) fitness performance by offspring and adult females. Base stocks of *S. guani* for all experiments were obtained from two laboratory colonies. Single-host colony of *S. guani* was maintained separately on the larvae of two different hosts for 11 successive generations. One colony was reared solely on the larvae of *M. alternatus* whereas the other colony was reared solely on the larvae of *Saperda populnea*. *Saperda populnea* (Cerambycidae: Laminae) was used generally as a substitute host in mass rearing of *S. guani* (Wang *et al*., 2006). Final instar larvae of *M. alternatus* were collected from Zhejiang province, whereas larvae of *S. populnea* were provided by the Xishan Forest Factory. All larvae were stored at 8–10°C prior to use in parasitoid rearing.

For both colonies, individual *S. guani* were reared in glass pipes (7.5 cm in height × 1.2 cm in diameter), each blocked with a tampon on the port and kept at 25 ± 5°C, 70% RH under a LD 14:10 h. Mated female *S. guani* were fed on 10% honey for 5–6 days to ensure oogenesis and were then presented with host larvae in each pipe for subsequent ovipositioning/feeding. For the colony reared on *M. alternatus*, the larvae were presented at a ratio of 3:1 parasitoid : host, whereas a ratio of 1:1 parasitoid: host was used for those reared on *S. populnea*. The difference in ratios was due to the differences in larval size (Zhang, 2004). Generation times for *S. guani* was approximately 35 days on *M. alternatus* and 25 days on *S. populnea*.

Effect of pre-imaginal experience on feeding and ovipositioning preferences

To test the effect of pre-imaginal experience on subsequent host choices by females (Experiments 1 and 2, respectively), we used four types of *S. guani*, based on their rearing histories. For two types of individuals, *S. guani* were reared successively on the same host species as their mothers. One reared from the larvae of *M. alternatus* was used as MA_{MA} colony. The other reared from larvae of *S. populnea* was used as SP_{SP} colony. The two remaining treatment groups *S. guani* were obtained by switching their host species from that used by their mothers. Female wasps from F_{11} of MA_{MA}colony were allowed to feed and reproduce on the larvae of *S. populnea*, producing offspring with a brood history on *S. populnea* (SP_{MA}). Conversely, female wasps from F_{11} of SP_{SP} colony were allowed to feed and reproduce on the larvae of *M. alternatus*, producing offspring with a brood history on *M. alternatus* (MA_{SP}).

In Experiment 1, we determined the feeding preferences of the four treatment groups (based on rearing history): (1) MA_{MA}; (2) SP_{SP}; (3) SP_{MA}; and (4) MA_{SP}. Using a two-choice bioassay, two larvae (one of each species) were put simultaneously in a glass Petri dish of a diameter of 12 cm. One female wasp was put in the middle of each dish with a fine brush. For each of the four treatment groups, five females were tested simultaneously per dish with five replicates (a total of 25 females). Choice tests were conducted at 25–26°C under a lamp (100 Lx) hanging approximately 0.5 m above the roof of the experimental arena.

Feeding preference was expressed as the successful host-selecting rate (SSR) of female *S. guani* to two hosts at the following intervals: 10 min, 30 min, 1, 2, 4, 6, 12, and 24 h, respectively. In a successful host selection, female *S. guani* walk, search, and probe throughout the arena, generally not changing positions for 24 h after making their selection. SSR of *S. guani* was defined as the proportion of females that attacked hosts with simultaneous probing, stinging and feeding behaviours for up to 5 min [SSR = (number of females attacking on each host/total number of females) × 100%].

In Experiment 2, the oviposition preferences of individuals from the four treatment groups (as described above) were determined by the same type of two-choice arenas as in Experiment 1. New female individuals from the four treatment groups were used in the test. Oviposition preference of parasitoids was expressed as the number of eggs per female adult on hosts per weight (mg), to control for differences in host size among hosts. The choice tests for ovipositing lasted 2 weeks until females did not lay any eggs. For each of the four treatment groups, 100 females were tested simultaneously with 20 replicates.

Effect of adult experience on feeding and ovipositioning preferences

To test the effect of adult experience (prior oviposition experience) on subsequent host selection choices for feeding and ovipositing by female *S. guani* (Experiments 3 and 4, respectively), we allowed 50 mated females (1–2 days in age) from both colonies to lay two to three eggs on their respective hosts over a time span of 4–5 days (MA_{EOMA} and SP_{EOSP}, respectively). The subscripts EOMA and EOSP refer to experienced female wasps ovipositing on *M. alternatus* and *S. populnea*, respectively. The procedure was repeated with alternate hosts (MA_{EOSP} and SP_{EOMA}, respectively). Simultaneously, inexperienced female wasps from the colonies on *M. alternatus* and *S. populnea* were used as control groups (MA_{NE} and SP_{NE}, respectively). All females with oviposition experience were then removed from their hosts and placed in new separate pipes, kept at 8–10°C, and used for experiments within 1 week. Newly emerged and mated females from each colony kept apart from hosts prior to testing, were fed on 10% honey for 5–6 days, and served as inexperienced controls.

In Experiment 3, we determined the feeding preferences of six treatment groups: (1–2) MA_{EOMA} and MA_{EOSP}: female *S. guani* from the colony reared on *M. alternatus* with prior oviposition experience on *M. alternatus* and *S. populnea*, respectively; (3–4) SP_{EOMA} and SP_{EOSP}: female *S. guani* from the colony reared on *S. populnea* with prior oviposition experience on *M. alternatus* and *S. populnea*, respectively; and (5–6) MA_{NE} and SP_{NE}: inexperienced female from both colonies (as described above). The same experimental protocols were used with Experiment 1 and in total 125 females were tested with 25 replicates for each of the six treatment groups.

In Experiment 4, the oviposition preferences of individuals from each of the six treatment groups (based on oviposition experience) were determined using the same protocols as with Experiment 2. One hundred females were tested simultaneously with 20 replicates too, for each of the four treatment groups.

Effect of pre-imaginal experience on performance of female Scleroderma guani *and offspring*

In Experiments 5 and 6, we determined the effects of host switching on the performance of female *S. guani* and offspring, respectively. We used 20 *S. guani* of each of the four treatment groups (based on rearing history, as used in Experiment 1): (1) MA_{MA}; (2) SP_{SP}; (3) SP_{MA}; and (4) MA_{SP}. Each female adult was kept at 8–10°C. Each adult was deprived of food for 12 h before testing and used only once. No-choice tests were carried out in a glass pipe (7.5 cm in height × 1.2 cm in diameter), blocked with a tampon on the port. Active female adults of 5–6 days in age were used in bioassays. All tests were tested at 25–26°C and LD 14:10 h daylight regime.

In Experiment 5, we determined female adult performance from all four treatment groups. We used a no-choice bioassay in which a host was offered a newly emerged and mated female, at a ratio of 3:1 and 1:1 for larvae of *M. alternatus* and *S. populnea*, respectively. Each treatment was replicated 20 times (a total of 320 females). Female fecundity (number of eggs per female) and longevity (days), pre-oviposition and oviposition period (days) on the two hosts were observed and recorded. For evaluating female realised longevity, surviving maternal females were moved into new pipes after their offspring had become cocoons. They were provided with 10% honey until they died. The realised longevity was recorded.

In Experiment 6, offspring performance was determined by no-choice experiments. Females of the four treatment groups were allowed to oviposit on *M. alternatus* and *S. populnea*, respectively. Twenty newly laid eggs were left on their original hosts, and offspring performance was determined by parameters of survival, sex ratio, and total development time. Each treatment was replicated 20 times (a total of 400 eggs). The deteriorating hosts were replaced and mortality was checked daily.

Effect of adult experience on performance of female Scleroderma guani *and offspring*

In Experiments 7 and 8, we determined the effects of prior oviposition experience on the performance of female *S. guani* and offspring, respectively (Fig. 2). We used 20 female *S. guani* for each of the six treatment groups (MA_{EOMA}, MA_{EOSP}, SP_{EOMA}, SP_{EOSP}, MA_{NE}, SP_{NE}) as described in Experiment 3. We used the same protocols noted in Experiments 5 and 6, respectively (as described above).

Data analysis and statistics

Statistical analyses for this study were performed using SPSS 13.0 for Windows. Analysis of feeding and oviposition preference was based on females from two populations. Chi-square tests were used to compare feeding preference (the success host-selection rate) to two host species among parasitoids. Differences in oviposition preference between individuals with and without oviposition experience were analysed with an independent sample t-test. One-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparisons were performed to assess the differences in adult performance (realised fecundity and longevity, pre-oviposition and oviposition period) and offspring performance (total development time, survival and sex ratio) of *S. guani* with different experiences. Pre-oviposition period, oviposition period and total development time were transformed by a square root transformation prior to the analysis. The percentage-based data (survival and sex ratio) were analysed following an arcsine square root transformation. Pearson's correlation analyses were used to determine the relationships between preference and performance. Only those performance characteristics that differed significantly among hosts were included in the analyses.

Results

The effect of pre-imaginal experience on host preference

Female *S. guani* from *M. alternatus* and *S. populnea* colonies showed significant preference to their original hosts (Fig. 1). In two-choice treatments, a higher proportion of MA_{MA} *S. guani* landed on *M. alternatus* than on *S. populnea* after 6 h (Fig. 1a; at 6 h, $\chi_1^2 = 3.857$, $P < 0.05$). Similarly, SP_{SP} females had a higher rate of successful selection to *S. populnea* over *M. alternatus* after 30 min (Fig. 1b; in 30 min; $\chi_1^2 = 8.333$, $P < 0.01$). However, after switching brood hosts of the two colonies for one generation, SP_{MA} parasitoids preferred to attack *S. populnea* after 30 min (Fig. 1a; in 30 min; $\chi_1^2 = 4.500$, $P < 0.05$), which was opposite to the preferences of MA_{MA} females. Compared to SP_{SP} *S. guani*, MA_{SP} parasitoids prefer to feed on *M. alternatus* than *S. populnea* after 6 h (Fig. 1b; in 6 h; $\chi_1^2 = 4.765$, $P < 0.05$).

Females from MA_{MA} and SP_{SP} colonies oviposited more frequently on their maternal hosts than on the alternate host (Fig. 2; t-test: MA_{MA}, $t_{38} = 13.139$, $P < 0.001$; SP_{SP}, $t_{38} = 13.103$, $P < 0.001$). The mean number of eggs laid by individual MA_{MA} females on *M. alternatus* per weight (mg) was 0.481 ± 0.030, and by SP_{SP} females was 0.140 ± 0.009. On average, SP_{SP} females laid 3.094 times more eggs on *S. populnea* than MA_{MA} females. Females from host switches, MA_{SP} and SP_{MA}, significantly changed their oviposition preference to their alternative host than their maternal host (Fig. 2; t-test: SP_{MA}, $t_{38} = 10.775$, $P < 0.001$; MA_{SP}, $t_{38} = 10.657$, $P < 0.001$). The number of eggs laid by SP_{MA} females on *M. alternatus* was 0.155 ± 0.013, and decreased 3.105 times comparative to MA_{MA} females, but increased 2.842 times on *S. populnea* (0.486 ± 0.016). Moreover, the number of eggs laid by MA_{SP} females on *M. alternatus* was 0.454 ± 0.013, and increased by 3.240 times over SP_{SP} females, but was 0.249 ± 0.013 on *S. populnea*.

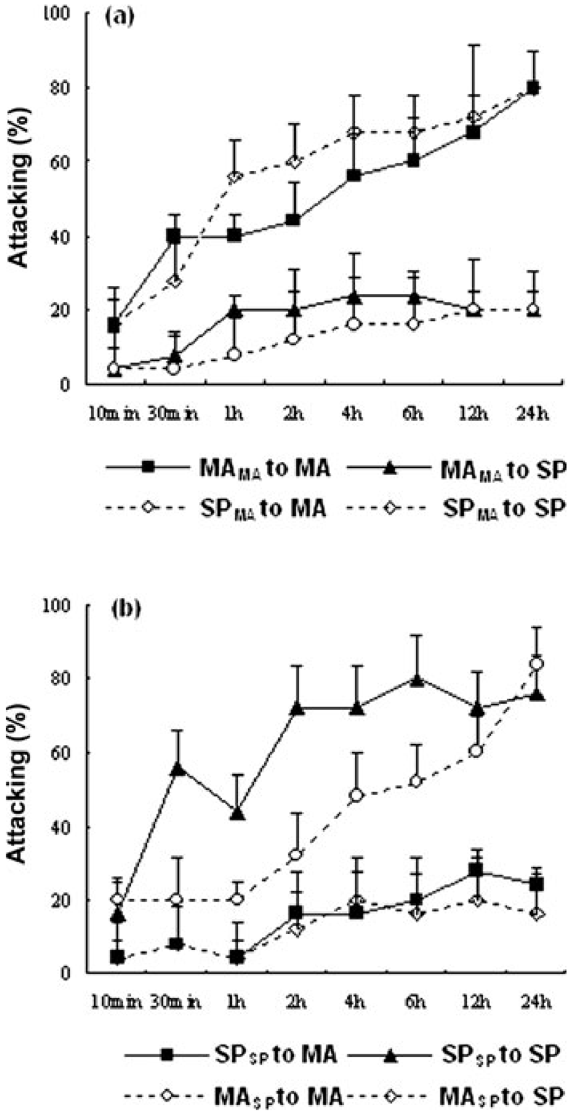

Fig. 1. The effect of pre-imaginal experience on feeding preference of *Scleroderma guani* from different culturing systems to host species. (a) The feeding selection of female MA_{MA} and SP_{MA} to two host species, (b) The feeding selection of female SP_{SP} and MA_{SP} to two host species. Abbreviations used: MA, larvae of *Monochamus alternatus*; SP, larvae of *Saperda populnea*; MA_{MA}, parasitoids from MA reared colony; SP_{SP}, parasitoids from SP reared colony; SP_{MA}, parasitoids from the MA_{MA} colony but reared by SP for one generation; MA_{SP}, parasitoids from the SP_{SP} colony but reared by MA for one generation (mean ± SE, $n = 25$ in each treatment).

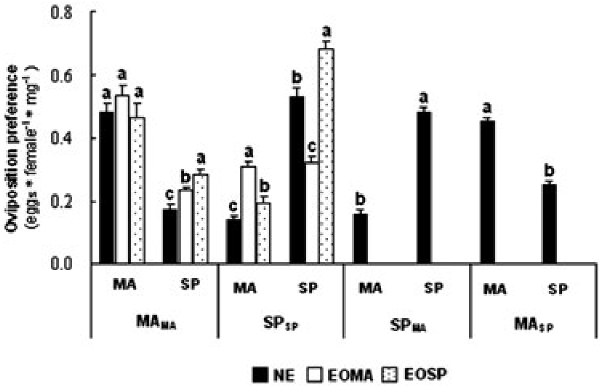

Fig. 2. The effect of pre-imaginal and adult experience on oviposition preference of *Scleroderma guani* to host species. Preference here is shown as the number of eggs laid by each female on per milligram of hosts. Same letters on bars indicate no significant differences ($P < 0.05$). Abbreviations used: MA, larvae of *Monochamus alternatus*; SP, larvae of *Saperda populnea*; EOMA, females with previous oviposition experience on MA; EOSP, $\mathrm{MA_{MA}}$ or $\mathrm{SP_{SP}}$ females with previous oviposition experience on SP; NE, $\mathrm{MA_{MA}}$ or $\mathrm{SP_{SP}}$ females without experience (m*ean* ± SE, $n = 20$ in each treatment).

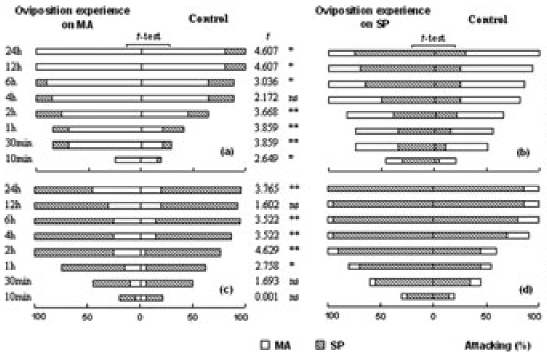

Fig. 3. The effect of adult experience on feeding preference of *Scleroderma guani* to host species. Asterisks indicate significant difference ($^{**}P < 0.01$, $^{*}P < 0.05$, ns, non-significant). (a,b) The feeding preference of $\mathrm{MA_{MA}}$ females with oviposition experience on MA and SP, respectively; (c,d) the feeding preference of $\mathrm{SP_{SP}}$ females with oviposition experience on MA and SP, respectively; control, the feeding preference of $\mathrm{MA_{MA}}$ or $\mathrm{SP_{SP}}$ females without experience. Abbreviations used: MA, larvae of *Monochamus alternatus*; SP, larvae of *Saperda populnea* (mean ± SE, $n = 25$ in each treatment).

The effect of adult experience on host preference

The $\mathrm{MA_{MA}}$ *S. guani* with adult experience on *M. alternatus* ($\mathrm{MA_{EOMA}}$) were quicker and more efficient in landing and feeding on *M. alternatus* than those without prior experience on *M. alternatus* ($\mathrm{MA_{NE}}$) (Fig. 3a; at 24 h; *t*-test: $t_7 = 4.607$, $P < 0.05$), except at 4 h (Fig. 3a; *t*-test: $t_7 = 2.172$, $P = 0.087$). Surprisingly, prior oviposition experience of $\mathrm{MA_{MA}}$ *S. guani* on *S. populnea* ($\mathrm{MA_{EOSP}}$) induced a complete shift in feeding preference within 24 h (Fig. 3b; *t*-test: at 24 h, $t_7 = 8.577$, $P < 0.001$).

Similarly, $\mathrm{SP_{SP}}$ females with prior oviposition experience of *S. populnea* ($\mathrm{SP_{EOSP}}$), had a higher proportion of landing on *S. populnea* than those without prior experience ($\mathrm{SP_{NE}}$); up to 75% in 24 h (Fig. 3d; *t*-test: in 24 h, $t_7 = 8.193$, $P < 0.001$). $\mathrm{SP_{SP}}$ females with prior oviposition experience of *M. alternatus* ($\mathrm{SP_{EOMA}}$), had a higher proportion of landing on *M. alternatus* compared to controls ($\mathrm{SP_{NE}}$), although they still were inclined to choose *S. populnea* over *M. alternatus* as a whole (Fig. 3c).

The adult experience by females also improved their subsequent oviposition preference to host species. Comparing oviposition preference of $\mathrm{MA_{MA}}$ and $\mathrm{SP_{SP}}$ females with different experiences EOMA, EOSP, and NE, the oviposition preference of the females *S. guani* ranked as follows: (1) for the oviposition rate on host *M. alternatus*, $\mathrm{SP_{EOMA}} > \mathrm{SP_{EOSP}} > \mathrm{SP_{NE}}$ (Fig. 2; ANOVA; $F_{2,57} = 26.753$, $P < 0.001$); but $\mathrm{MA_{EOMA}} = \mathrm{MA_{EOSP}} = \mathrm{MA_{NE}}$ (Fig. 2; ANOVA; $F_{2,57} = 1.132$, $P = 0.329$); (2) for the oviposition rate on host *S. populnea*, $\mathrm{SP_{EOSP}} > \mathrm{SP_{NE}} > \mathrm{SP_{EOMA}}$ (Fig. 2; ANOVA; $F_{2,57} = 48.560$, $P < 0.001$); $\mathrm{MA_{EOSP}} > \mathrm{MA_{EOMA}} > \mathrm{MA_{NE}}$ (Fig. 2; ANOVA; $F_{2,57} = 23.518$, $P < 0.001$).

The effect of pre-imaginal experience on adult and offspring performance

Adult performance of females varied significantly between host species. In the no-choice tests, female fecundity and longevity of $\mathrm{MA_{MA}}$ was much higher on *M. alternatus* than on *S. populnea* (Fig. 4a,b, *t*-test: fecundity: $t_{38} = 8.210$, $P < 0.001$; longevity: $t_{38} = 6.619$, $P < 0.001$). However, when it was reared for only one generation (pre-imaginal experience) on the other host *S. populnea*, then performance of its progeny ($\mathrm{SP_{MA}}$) completely switched towards this host (Fig. 4a,b, *t*-test: $\mathrm{SP_{MA}}$; fecundity: $t_{38} = -7.210$, $P < 0.001$; longevity: $t_{38} = 5.651$, $P < 0.001$). Likewise, fecundity and longevity of $\mathrm{SP_{SP}}$ were clearly higher on *S. populnea* than on *M. alternatus* (Fig. 4a,b, *t*-test: $\mathrm{SP_{SP}}$; fecundity: $t_{38} = 3.450$, $P < 0.001$; longevity: $t_{38} = 5.013$, $P < 0.001$), whereas those of $\mathrm{MA_{SP}}$ perform better on *M. alternatus* (Fig. 4a,b, *t*-test: fecundity: $t_{38} = 5.646$, $P < 0.001$; longevity: $t_{38} = 7.360$, $P < 0.001$).

The pre-oviposition periods on *S. populnea* were shorter than those on *M. alternatus*, regardless of the source colony (Fig. 4c). However, pre-oviposition periods of $\mathrm{SP_{SP}}$ females on *M. alternatus* were significantly shortened by approximately 1.900 days compared with those of the $\mathrm{MA_{SP}}$ females (Fig. 4c). With switching the brood host of $\mathrm{MA_{MA}}$ females to *S. populnea*, its progeny females ($\mathrm{SP_{MA}}$) significantly prolonged ovipositing on *M. alternatus* by approximately 2.305 days (Fig. 4c).

Offspring of $\mathrm{MA_{MA}}$ and $\mathrm{MA_{SP}}$ females always had longer total development time on *M. alternatus* than on *S. populnea* (Fig. 4d; *t*-test: $\mathrm{MA_{MA}}$; $t_{38} = 19.047$, $P < 0.001$; $\mathrm{MA_{SP}}$; $t_{38} = 21.121$, $P < 0.001$). Offspring survival of $\mathrm{MA_{MA}}$ colony did not differ between the two host species (Fig. 4e; *t*-test: $t_{38} = 1.809$, $P = 0.078$). However, offspring of $\mathrm{MA_{SP}}$ had higher survival on *M. alternatus* than on *S. populnea* (Fig. 4e; *t*-test: $t_{38} = 3.560$, $P < 0.001$). Female-biased sex ratios of progeny were maintained in both the original colonies and those with pre-imaginal experience (Fig. 4f).

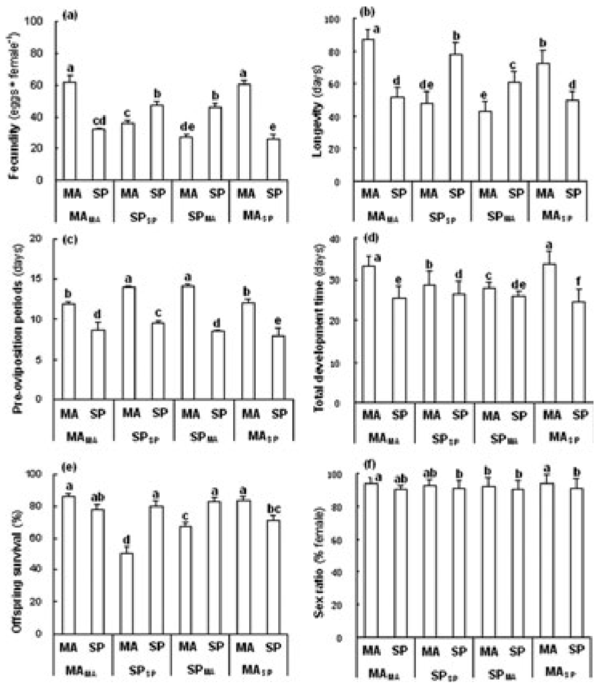

Fig. 4. The effect of pre-imaginal experience on performance of *Scleroderma guani* from different culturing systems to host species. (a,b) Female fecundity and longevity; (c,d) pre-oviposition period of adult females and total development of female offspring; (e,f) offspring survival and sex ratios (proportion of females). Same alphabet letters on bars indicate no significant differences ($p < 0.05$). For abbreviations see Fig. 1 (mean ± SE, $n = 20$ in each treatment).

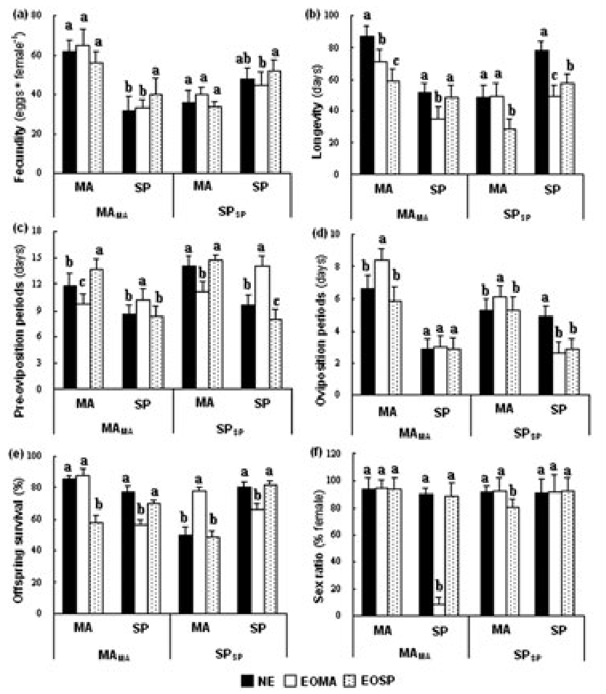

Fig. 5. The effect of adult experience on performance (mean ± SE) of *Scleroderma guani* from different culturing systems to host species. (a,b) Female fecundity and longevity; (c,d) pre-oviposition and oviposition period of adult females; (e,f) offspring survival and sex ratios (proportion of females). Same alphabet letters on bars indicate no significant differences ($p < 0.05$). For abbreviations see Fig. 2 (mean ± SE, $n = 20$ in each treatment).

In contrast, offspring of SP$_{\mathrm{SP}}$ and SP$_{\mathrm{MA}}$ *S. guani* had better performance on *S. populnea* than on *M. alternatus*, including a shorter development time (Fig. 4d; t-test: SP$_{\mathrm{SP}}$; $t_{38} = -7.011$, $P < 0.001$; SP$_{\mathrm{MA}}$; $t_{38} = 4.662$, $P < 0.001$), and higher offspring survival (Fig. 4e; t-test: SP$_{\mathrm{SP}}$; $t_{38} = 3.536$, $P < 0.001$; SP$_{\mathrm{MA}}$; $t_{38} = -4.598$, $P < 0.001$). There were no significant differences in sex ratio between host species (Fig. 4f).

The effect of adult experience on adult and offspring performance

Prior oviposition experience of female *S. guani* affected the variation of adult and offspring performance, mainly with respect to adult realised fecundity, the period of pre-oviposition and oviposition, offspring survival and sex ratio (Fig. 5).

In comparing adult females of the MA$_{\mathrm{MA}}$ colony with prior oviposition experiences on *M. alternatus* (MA$_{\mathrm{EOMA}}$) and on *S. populnea* (MA$_{\mathrm{EOSP}}$) with those with no prior experience (MA$_{\mathrm{NE}}$), we found that the pre-oviposition period of MA$_{\mathrm{EOMA}}$ on *M. alternatus* clearly decreased, whereas the oviposition period was longer (Fig. 5c,d, ANOVA; $F_{2,57} = 57.676$, $P < 0.001$; $F_{2,57} = 13.74$, $P < 0.001$). There were no significant differences in adult realised fecundity, although longevity was shortened (Fig. 5a,b,ANOVA; fecundity, $F_{2,57} = 1.715$, $P = 0.189$; longevity, $F_{2,57} = 12.146$, $P < 0.001$). Both MA$_{\mathrm{EOMA}}$ and MA$_{\mathrm{NE}}$ *S. guani* had higher offspring survival than MA$_{\mathrm{EOSP}}$ on *M. alternatus* (Fig. 5e; ANOVA; $F_{2,57} = 35.228$, $P < 0.001$). There were no differences in offspring sex ratios among the treatments (Fig. 5f; ANOVA; $F_{2,57} = 0.869$, $P = 0.425$).

In contrast, adult fecundity and longevity on *S. populnea* were lower for MA$_{\mathrm{EOMA}}$ females than those with EOSP (MA$_{\mathrm{EOSP}}$) (Fig. 5a,b; ANOVA; fecundity, $F_{2,57} = 11.612$, $P < 0.001$; longevity, $F_{2,57} = 43.481$, $P < 0.001$). The pre-oviposition period of MA$_{\mathrm{EOMA}}$ on *S. populnea* was prolonged among the treatments (Fig. 5c; ANOVA; $F_{2,57} = 27.829$, $P < 0.001$). Remarkably, we observed highly significant differences in offspring survival, declining to 56.777%, and sex ratios at only 7.993% (Fig. 5e,f).

Adult performance (fecundity and longevity) of SP$_{\mathrm{SP}}$ females developed on *S. populnea* (SP$_{\mathrm{EOSP}}$) were significantly higher than those with EOMA (SP$_{\mathrm{EOMA}}$). The pre-oviposition period was shorter for SP$_{\mathrm{EOSP}}$ than for SP$_{\mathrm{EOMA}}$ and controls (Fig. 5c). Offspring survival was in accordance with controls, but were higher than SP$_{\mathrm{EOMA}}$ (Fig. 5e; ANOVA; $F_{2,57} = 8.251$, $P < 0.001$). There were no differences in sex ratios among the treatments (Fig. 5f; ANOVA; $F_{2,57} = 2.323$, $P = 0.107$).

However, offspring survival for SP$_{\mathrm{SP}}$ females with EOSP or EOMA (SP$_{\mathrm{EOSP}}$ or SP$_{\mathrm{EOMA}}$) developed on *M. alternatus* were 49.141% and 78.185%, respectively. Sex ratios of SP$_{\mathrm{EOSP}}$ declined slightly to 80.457% females, whereas SP$_{\mathrm{EOMA}}$ was similar to controls with up to 92.0% females (Fig. 5f). In addition, SP_{EOSP} had a lower fecundity than SP_{EOMA} and the controls (Fig. 5a; ANOVA; $F_{2,57} = 3.067$, $P < 0.001$), and had a longer period of pre-oviposition than SP_{EOMA} parasitoids (Fig. 5c; $F_{2,57} = 13.025$, $P < 0.001$).

Preference–performance correlations

For the two colonies reared on *M. alternatus* (MA_{MA}) and *S. populnea* (SP_{SP}), there was nearly perfect correlation between oviposition preference and female realised fecundity on *M. alternatus* and *S. populnea*, respectively (Table 1). Oviposition preference of the MA_{MA} colony was partly correlated with female realised longevity ($r = 0.833$, $P < 0.001$). The correlations between host preference and the offspring performance measures (survival and sex ratio) were always weaker than the host preference–realised fecundity relationship.

With pre-imaginal and adult experience, oviposition preference was significantly correlated with female realised fecundity (Table 2). Adult longevity and offspring performance did not correlate with oviposition preference and the relationship was not significant.

Discussion

Female *S. guani* from both colonies (MA_{MA} and SP_{SP}) demonstrated higher preference and performance on maternal hosts, which was likely to be an adaptation to a specific host over multiple generations. However, the pre-imaginal and adult experience of *S. guani* induced varieties not only in adult behavioural preference through improved recognition, accuracy, or selectivity in foraging, but also in subsequent adult/offspring performance.

When the parasitoid switched brood host and was reared for only one generation (pre-imaginal experience), there were remarkable changes in host preference and fitness consequences of progeny to host species. We presumed that *S. guani* females were likely to adapt to the physiological conditioning through incorporation of chemicals, unique to one species, into the new adult. Our results provided compelling evidence for a behavioural preference that might be quickly driven by a learning process from the current generation rather than by genetic variation shaped over generations. This phenomenon suggested that adult host-choice behaviour could be influenced by hosts of immature developmental experience in some instances. An explanation for this phenomenon might be based on the neo-Hopkins principle (Jaenike, 1983; Storeck *et al*., 2000; Rietdorf & Steidle, 2002) or chemical legacy hypothesis (Corbet, 1985). The former hypothesis stressed that host preference might also be determined by experience gained in early adult stages (during or shortly after emergence from the pupa) (Jaenike, 1983; Rietdorf & Steidle, 2002) when compared with the Hopkins host-selection principle. It is essential to manage early adult experience to distinguish between the Hopkins host-selection principle and the neo-Hopkins principle (Rietdorf *et al*., 2002). To date, the latter hypothesis is still the most comprehensive theory on the induction of host preferences (Corbet, 1985). It postulated that effects of larval chemosensory

Table 1. Correlation matrix between preference and performance of the two colonies with pre-imaginal experience on the two host species (Pearson's correlation analyses).

	Oviposition preference															
	MA_{MA}				SP_{SP}				SP_{MA}				MA_{SP}			
Parasitoid	MA		SP		MA		SP		MA		SP		MA		SP	
Host	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Adult performance																
Adult fecundity	0.900	<0.001	0.859	<0.001	0.364	0.115	0.780	<0.001	0.995	<0.001	0.740	<0.001	0.861	<0.001	0.937	<0.001
Adult longevity	0.874	<0.001	−0.361	0.118	0.133	0.576	−0.011	0.962	0.029	0.903	−0.164	0.489	−0.373	0.105	0.291	0.213
Pre-oviposition period	−0.014	0.955	−0.007	0.978	0.251	0.286	0.159	0.504	−0.073	0.758	−0.393	0.087	0.017	0.942	0.053	0.824
Oviposition period	0.000	0.999	−0.478	0.033	0.253	0.281	−0.267	0.255	0.005	0.983	−0.017	0.943	0.236	0.316	−0.220	0.352
Offspring performance																
Total development time	−0.299	0.201	0.407	0.075	0.022	0.926	−0.678	0.001	−0.106	0.658	−0.107	0.655	−0.004	0.988	−0.068	0.777
Offspring survival	0.177	0.454	−0.252	0.283	−0.177	0.455	0.032	0.892	0.411	0.072	−0.178	0.452	−0.371	0.107	−0.117	0.623
Sex ratio (% female)	−0.244	0.301	−0.184	0.437	−0.187	0.429	0.622	0.003	0.621	0.003	0.067	0.780	0.221	0.350	0.291	0.214

MA, larvae of *Monochamus alternatus*; SP, larvae of *Saperda populnea*; MA_{MA}, parasitoids from MA reared colony; SP_{SP}, parasitoids from SP reared colony; SP_{MA}, parasitoids from the MA_{MA} colony but reared by SP for one generation; MA_{SP}, parasitoids from the SP_{SP} colony but reared by MA for one generation.

Table 2. Correlation matrix between preference and performance of the two colonies with adult experience on the two host species (Pearson's correlation analyses). For abbreviations see Fig. 2.

	Oviposition preference															
Parasitoid	MA_{EOMA}				MA_{EOSP}				SP_{EOMA}				SP_{EOSP}			
Host	MA		SP		MA		SP		MA		SP		MA		SP	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Adult performance																
Adult fecundity	0.980	<0.001	0.637	0.003	0.377	0.101	0.721	<0.001	0.718	<0.001	0.670	0.001	0.915	<0.001	0.509	0.022
Adult longevity	0.022	0.927	0.614	0.004	−0.077	0.745	0.044	0.854	0.323	0.164	−0.246	0.296	−0.219	0.353	−0.035	0.884
Pre-oviposition period	0.005	0.982	0.040	0.868	0.098	0.681	0.310	0.184	0.056	0.814	−0.398	0.082	−0.002	0.992	−0.147	0.537
Oviposition period	0.184	0.437	−0.391	0.088	0.031	0.897	0.439	0.053	0.337	0.147	−0.445	0.049	−0.094	0.694	−0.007	0.976
Offspring performance																
Total development time	−0.076	0.751	−0.479	0.033	−0.038	0.874	−0.357	0.122	−0.145	0.541	−0.198	0.403	0.037	0.877	−0.208	0.380
Offspring survival	0.238	0.312	0.403	0.078	−0.382	0.096	−0.419	0.066	−0.442	0.051	0.097	0.685	−0.527	0.017	−0.207	0.382
Sex ratio (% female)	−0.093	0.696	−0.095	0.692	0.004	0.985	0.097	0.683	0.562	0.010	−0.040	0.866	0.141	0.554	0.383	0.095

environment on adult chemosensory responsiveness depend not (or not only) on persistent neural changes ('memory'), but on traces of chemical cues, inside or outside the insect's body, which influence adult behavior (Corbet, 1985). The induction may happen at any developmental stage by contact with traces of chemicals transferred from an earlier stage, resulting in the central nervous system (CNS). To some extent, learning or experience of parasitoid *S. guani* might happen before, during, or shortly after its adult emergence. Since both the parasitoid colonies learned to prefer their maternal hosts over other hosts and yield higher reproductive output on the maternal hosts than other hosts, the learning may be adaptive for *S. guani*.

Evidently, *S. guani* females do not immediately perceive host quality, but need prior feeding and oviposition experience on the hosts to adjust their preference. With the adult experience, we considered that preconditioning of female *S. guani* affected their behavioural choices as well as adult/offspring performance. Consequently, the adult experience of the parasitoid influenced its host preference and performance was adapted to alternate hosts. The initial exposure provided available information about host species or quality to the female wasp. As a result, their decisions on subsequent choices were biased that way until they encountered other hosts, and the performance of adult/offspring was also significantly improved by the prior experience. Females with prior oviposition experience selected more reliable or suitable hosts than those that had never experienced hosts, thereby maximising their adult fecundity.

We also found that offspring sex ratio was changed based on the oviposition experience of *S. guani*. When the female parasitoids, reared on *M. alternatus*, with previous oviposition experience on *M. alternatus* completed development on *S. populnea*, sex ratios of their offspring sharply declined to 7.9% females. Similarly, for parasitoids, reared on *S. populnea* with prior oviposition experience on *S. populnea*, their offspring had a lower percentage of females (80.5%) emerging from *M. alternatus*. We concluded that the reasons for such patterns may result from the determination of ovipositing females to the quality varieties of the hosts *M. alternatus* and *S. populnea*. Further research is clearly needed to determine how host quality can regulate the sex ratio of *S. guani*.

There were positive correlations between oviposition preference and adult realised fecundity. Thus, the relatively higher preference of *S. guani* for the colony host may be the result of an increase in adult fecundity on the colony host. This predicted that optimal foraging might shape host preference of *S. guani*. Our results were consistent with previous works in which optimisation of adult performance determined host selection of phytophagous insects (Scheirs *et al*., 2000; Scheirs & De Bruyn, 2002; Scheirs *et al*., 2004). These previous studies found that host preference of a polyphagous leafminer *Liriomyza trifolii* was determined by optimal foraging, as this was the best strategy in order to maximise female fitness. Female realised fecundity correlated almost perfectly with host preference of *L. trifolii*. However, the relationships between host preference and the offspring performance measures were always weaker. Surprisingly, our study suggested that the prior experience of *S. guani* females significantly influenced preference–performance relationships in the polyphagous parasitoid. With the pre-imaginal experience and adult experience, oviposition preference was almost perfectly and significantly correlated with female realised fecundity. This predicted that female *S. guani* from the two colonies with different prior experiences still behaved according to the predictions of the optimal foraging hypothesis.

In a host–parasitoid system, host selection closely interacts with the performance of adult and offspring on hosts, especially for idiobiont parasitoids (Vinson, 1998), since development of their offspring closely depends on the abilities of female adults to forage and incapacitate suitable hosts. In our experiments, we observed that the immature stage of *S. guani* was not nearly as dependent on adult performance. For example, offspring performance in both $\mathrm{MA}_{\mathrm{MA}}$ and $\mathrm{SP}_{\mathrm{SP}}$ colonies did not sharply decline after switching hosts. Their offspring still maintained a moderate survival rate and higher female-biased sex ratios. These results suggested that when needed, larvae of *S. guani* can quickly adapt and utilise all or most hosts selected by their adults to survive, even though their preference and performance temporarily decreases on never experiencing hosts. The population could thus increase with substantial development on new hosts.

Our experimental treatments omitted much of the complexity of parasitoid natural population and host species. For example, the adaptive learning experiments were limited to two host species and two parasitoid colonies. However, this study indicated that both preference and performance in host–parasitoid were determined by adaptive learning in pre-imaginal and adult stage. Although we focused on behavioural and biological comparison to elucidate the host selection of *S. guani*, the potential mechanisms of behavioural alternation were not involved. Future work should focus on distinguishing the underlying influence of larval and early adult experience on the behavioural response, extrinsic selection pressures on adult and offspring performance, as well as the physiological state of parasitoids in order to reveal the conditions suitable for shaping host preference patterns.

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