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Is host selection influenced by natal and adult experience in the parasitoid *Necremnus tutae* (Hymenoptera: Eulophidae)?

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Abstract

Host selection in parasitoids can be influenced by learning cues obtained during natal and/or adult experience. We investigated the effects of natal and adult host experience on host selection by the indigenous parasitoid *Necremnus tutae* (Hymenoptera: Eulophidae), with an exotic host, the invasive tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), and a native host, *Cosmopterix pulchrimella* (Lepidoptera: Cosmopterigidae). Colonies of the parasitoid *N. tutae* were reared on the exotic host *T. absoluta* and the native host *C. pulchrimella*, infesting tomato and upright pellitory plants, respectively. Six groups were obtained based on their rearing histories (*T. absoluta* or *C. pulchrimella* larvae) and adult experience (no exposure to host larvae or oviposition experience on the two host species). Parasitoid females of the six groups were tested for behavioural responses to the two host-plant complexes (HPCs) in Y-tube olfactometer assays and in two-choice assays in test arenas. The results suggest that host experience during both natal and adult life may affect the HPC preference of this parasitoid species. In particular, adult experience proved to influence the host preference in both olfactometer and two-choice assays. By contrast, natal experience showed a significant influence on host choice only in naïve parasitoid females in the olfactometer bioassays. In general, the exotic host *T. absoluta*-tomato complex was the odour source preferred by the parasitoid *N. tutae*. The role of natal and adult experience in host-shifting dynamics and how these experiences can combine in adapting mechanisms to a new exotic insect of a generalist parasitoid species are also discussed.

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Host selection of insect parasitoids, usually determined by habitat preferences and foraging behaviours, has profound effects on behaviour and fitness of an individual and its progeny (Duan,

Messing, & Dukas, 2000; Stamps & Davis, 2006; Vinson, 1998). Besides a heritable component of habitat preference, accumulated experience may affect the subsequent choice of host-habitat complex (Segura, Viscarret, Carabajal Paladino, Ovruski, & Cladera, 2007). Natal experience, i.e. the experience accumulated during the early stages of life before the dispersal phase, can change subsequent habitat preferences of the organism during the dispersal phase. This effect is described by the natal habitat preference induction (NHPI) theory (Davis, 2008; Davis & Stamps, 2004; Ryan & Bidart-Bouzat, 2014). Similarly, the experience gained during the adult stage may provide mechanisms by which individuals are more likely to select cues related to previous experiences, possibly because they are indicative of the presence of suitable habitat or host food (Davis & Stamps, 2004; Mousseau & Fox, 1998; Ousterhout, Luhring, & Semlitsch, 2014; Papaj & Prokopy, 1989).

Learning from previous experience is one of the main processes involved in habitat choice of a broad range of animals, both vertebrates and invertebrates (Dukas, 2008; Moore, 2004). Among invertebrates, the influence of learning on habitat choice has been demonstrated on *Drosophila melanogaster* over 23-46 generations, highlighting also the importance of learning mechanisms in an evolutionary perspective (Mery & Kawecki, 2004). Learning, if adaptive (i.e. learning that improves the fitness), can affect phenotypic plasticity and in the last instance, drive the evolution of the genetic basis of phenotypic traits (Baldwin effect theory: see Dukas, 2013; Kawecki, 2010; Sznajder, Sabelis, & Egas, 2012). Although its possible influence on adaptation and evolution is widely acknowledged (Dukas, 2013), the role of learning in adaptive responses of natural enemies to new invasive species and in the consequent shift among native and exotic prey/host species has rarely been investigated (Berthon, 2015).

Insect parasitoids represent good model systems to study behavioural ecology and its consequences for evolution, because their search/attack behaviour is strictly related to their fitness, since the number of parasitized hosts is directly proportional to the number of offspring produced (Godfray, 1994; Hoedjes, Kraleman, van Vugt, Vet, & Smid, 2014; Le Lann et al., 2008; Paur & Gray, 2011; Petitt, Turlings, & Wolf, 1992; Takemoto, Powell, Pickett, Kainoh, & Takabayashi, 2009).

Moreover, since the parasitoid offspring are compelled to adapt to the host-habitat complex environmental conditions determined by the mother's choice, the parasitoid female is committed to maximizing her chances of finding and exploiting the host (Godfray, 1994). Therefore, the ability of parasitoid females to learn cues previously experienced can increase the chances of selecting an optimal host, with direct effect on the progeny's fitness (Liu, Xu, Li, & Sun, 2011; Mousseau & Fox, 1998).

Many studies have focused on the precise timing of preference induction during the life stages of insect parasitoids. But if adult learning capabilities have been clearly demonstrated (Hoedjes et al., 2014; Petitt et al., 1992; Takemoto et al., 2009), the effects and especially the timing of the influence of natal experience on adult choices are still debated (Barron, 2001; Colazza & Wajnberg, 2013; Gandolfi, Mattiacci, & Dorn, 2003a; Van Alphen & Bernstein, 2008; Villagra, Pennacchio, & Niemeyer, 2007). Here, we use the broad definition of natal experience of holometabolous insects given by Davis and Stamps (2004), that is the total amount of stimuli received during both preimaginal (i.e. the egg and larval stages) and early adult stage (i.e. recently emerged adult). Therefore, this research focused on the study of the occurrence of preference induction during the predispersal phase (i.e. NHPI).

The behavioural flexibility due to induction/learning capabilities permits the parasitoid to better cope with changes in the environment and community structure (Guerrieri, Pennacchio, & Tremblay, 1997), and can be particularly important when the parasitoid is faced with the arrival of new invasive species (Strauss, Lau, & Carroll, 2006). Although behavioural adaptations play a key role in a successful exploitation of exotic hosts (Wang, Hogg, Levy, & Daane, 2013), the mechanisms that regulate switching from a native to an exotic host by indigenous parasitoids are still unclear (Beckage, 2005; Jones, Bilton, Mak, & Sait, 2015; Strauss et al., 2006) and poorly investigated (Chabaane, Laplanche, Turlings, & Desurmont, 2015; Hassell, 2000; Schönrogge, Stone, & Crawley, 1996).

In this study, we focused on the effects of natal and adult host experience on host selection by an indigenous parasitoid in the Mediterranean area, *Necremnus tutae* (Hymenoptera: Eulophidae), with an exotic host, the invasive tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae) and a native host, *Cosmopterix pulchrimella* (Lepidoptera: Cosmopterigidae). *Necremnus tutae* is a solitary idiobiont parasitoid and the most abundant larval parasitoid associated with *T. absoluta* in the newly invaded areas of the Mediterranean basin (Gabarra et al., 2014; Zappalà et al., 2013). Currently, native hosts of *N. tutae* are not yet known, since this species (formerly reported as *N. sp. nr. artynes*) has just recently been described (Gebiola, Bernardo, Ribes, & Gibson, 2015). However, *C. pulchrimella*, a leafminer of upright pellitory, *Parietaria diffusa* (Urticaceae), is reported to be a natural host of the related species *Necremnus artynes* and *Necremnus cosmopterix* (Gebiola et al., 2015; Noyes, 2014). Moreover, individuals of *N. tutae* collected on *T. absoluta* had successfully parasitized and developed on *C. pulchrimella* larvae, thus confirming this species as a possible indigenous host of *N. tutae* (N. Bodino, C. Ferracini & L. Tavella, personal observation). Previous studies have already found a strong preference of *N. tutae* females for volatiles coming from tomato leaves (either healthy or infested by *T. absoluta* larvae), compared with upright

pellitory leaves (either healthy or infested by *C. pulchrimella* larvae; Ferracini et al., 2012). However, *N. tutae* individuals used by Ferracini et al. (2012) were provided by insectaries of a private company (Bioplanet s.c.a., Cesena, Italy), where the parasitoid population was reared on the host *T. absoluta* for several generations, with the possibility of a shift in its natural host preference due to transgenerational NHPI-mediated behaviour (Belda & Riudavets, 2012; Davis & Stamps, 2004; Gandolfi et al., 2003a; Saadat, Seraj, Goldansaz, & Karimzadeh, 2014). Therefore, we analysed the preference and foraging behaviours on the exotic and native host species by *N. tutae* reared and exposed to one of these two hosts during its natal and adult life in olfactometer and two-choice arena experiments. The study aimed to assess whether natal and adult experience can affect the host preference of *N. tutae* towards the host species previously encountered, regardless of whether they are native or exotic. We predicted that both natal and adult host experience could influence the habitat choice (i.e. HPC), increasing the acceptance of the HPCs previously encountered, especially when exposed to the same HPC during both natal and adult stages. In the light of the results obtained, we also discuss the role of host experience in host selection dynamics of a native parasitoid, and how it can influence switching behaviour to a new exotic host.

METHODS

Insect Rearing and Plant Cultivation

Colonies of the exotic host *T. absoluta* were established starting from individuals provided by Bioplanet laboratories (Bioplanet s.c.a., Cesena, Italy). Continuous mass rearing was maintained on tomato plants in cages with an insect-proof net (mesh 680 mm; BugDorm: 60×60×60 cm, MegaView Science Co., Taichung, Taiwan) for at least 10 generations before the experiments. Tomato seedlings were initially sown in plastic pots (diameter 20 cm), and plants were transplanted individually in pots (diameter 10 cm) after 3 weeks. Tomato plants of the Marmande variety (Green Paradise s.r.l., Milano, Italy) were used for both mass rearing and laboratory trials. Plants used in the experiments had four or five true leaves (14-15 BBCH-scale) and were approximately 60 days old.

Colonies of the native host *C. pulchrimella* were established starting from initial cultures collected on upright pellitory in Piedmont (northwestern Italy), and maintained in screen cages (BugDorm: 47.5×47.5×47.5 cm) for at least six generations before the experiments. Upright pellitory plants were collected in wastelands, and cuttings were taken to obtain new plants for *C. pulchrimella* rearing. Both *T. absoluta* and *C. pulchrimella* mass rearings were kept in an open-sided greenhouse at 25±3 °C, 50±20% relative humidity and 16:8 h light:dark.

Adults of the parasitoid *N. tutae* were received from Bioplanet, where colonies were established starting from individuals collected on tomato in Sardinia (Italy) in 2009-2010, and then mass-reared on *T. absoluta*-tomato host-plant complex (HPC). Parasitoid mass rearings were conducted on both HPCs, *T. absoluta*-tomato (AT) and *C. pulchrimella*-upright pellitory (AC), and maintained for four to seven generations before starting the experiments. To obtain the adult parasitoids used in the bioassays, additional rearings were set up as follows: about 10 adults (sex ratio ca. 1♀:1♂), from one of the two mass rearings (AT or AC), were released into a Plexiglas cage (40×40 cm and 50 cm high) containing the same HPC on which they were reared. After 4 days, parasitoids were removed and plants were transferred into a screen cage (BugDorm: 47.5×47.5×47.5 cm) for 6 days, allowing parasitoid larvae to conclude their larval development. Plants were then cut and placed in clean Plexiglas cages (35×35×35 cm) with drops of honey on cardboard, awaiting parasitoid emergence. All parasitoid rearing occurred in a climatic chamber (25±1 °C, 60±5% relative humidity and 16:8 h light:dark). Newly emerged adult parasitoids were transferred daily from the rearing cages to glass vials (24×120 mm) supplied with small honey drops, and placed in a climatic chamber (15±1 °C, 60±5% relative humidity and 16:8 h light:dark). Parasitoid adults were removed from emergence cages as soon as they emerged to avoid any direct experience of the adult on the host larvae. All insect rearing and experiments were conducted in accordance with the legislation and guidelines of the European Union for the protection of animals used for scientific purposes (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). All experimental protocols using insects were approved by the ad hoc Committee of DISAFA of the University of Torino.

Natal and Adult Learning Experience of N. tutae

To test the effects of natal and adult experience on host preference of *N. tutae* females (=A), six groups [two rearing conditions (T = *T. absoluta*; C = *C. pulchrimella*) × three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*)] based on their natal and adult histories on different host species were obtained as follows. Newly emerged parasitoid females (1-2 days old) from both colonies AT and AC were assigned randomly to three different adult experiences: (1) no adult experience on host larvae (naïve) (=ATn and ACn groups); (2) experience on the exotic host *T. absoluta* (=ATt and ACt groups); and (3) experience on the native host *C. pulchrimella* (=ATc and ACc groups). Female wasps assigned to the groups exposed to host larvae were placed in plastic cages (24×15 cm and 12 cm high) together with males (sex ratio ca. 1♀:1♂) and provided with two or three host larvae per parasitoid female (larvae of *T. absoluta* on tomato leaflets or *C. pulchrimella* on upright pellitory leaves) and with honey drops on cardboard. Larvae of similar size (i.e. third-instar larvae of the exotic host *T. absoluta*, and third- to fourth-instar larvae of the native

host *C. pulchrimella*) were used, in order to avoid any influence of host size on parasitoid choice (Ameri, Rasekh, & Michaud, 2014; Liu et al., 2011). Cages were kept in climatic chambers (25±1 °C, 60±5% relative humidity and 16:8 h light:dark), allowing parasitoid females to mate and oviposit and/or feed on host larvae for 24 h. Naïve females were maintained in the same conditions described above but without any host larvae; therefore, they could mate but had no oviposition or feeding experience on the host. Female wasps were then individually isolated in glass tubes (24×120 mm) with honey, and maintained in a climatic chamber (25±1 °C, 60±5% relative humidity and 16:8 h light:dark) for a further 24 h before the experiments. To assess the occurrence of oviposition and/or host feeding, host larvae exposed to parasitoid females were examined under a stereomicroscope (magnification 25×).

Olfactometer Bioassays

To evaluate the effects of natal and adult experience on subsequent odour choices, *N. tutae* females (4-6 days old) of the six experience groups (ATn, ACn, ATt, ATc, ACt, ACC) were tested in an olfactometer to assess their responses to the odours of the exotic and native HPCs (*T. absoluta*-tomato versus *C. pulchrimella*-upright pellitory). The two HPCs tested as odour sources consisted of two infested leaves of tomato and five infested leaves of upright pellitory, in order to use a similar total leaf surface. The two tomato leaves and the five upright pellitory leaves were infested with 15 third-instar larvae of the exotic host *T. absoluta* and third- to fourth-instar larvae of the native host *C. pulchrimella*, respectively. The leaf stalks were inserted in plastic tubes (Micro tube 1.5 ml, Sarstedt AG & CO, Nümbrecht, Germany) filled with water and sealed with Parafilm 'M' (Bemis, Neenah WI, U.S.A.). This procedure maintained the leaves' turgidity throughout all of the bioassays.

The olfactometer set-up consisted of a Y-shaped Pyrex tube (internal diameter 12 mm), with a 115 mm central stem and two 100 mm side arms set at a 70° angle. The olfactometer tube was placed with a 35° tilt angle and isolated from external stimuli by enclosing it in a white polystyrene box (45×30 cm and 24 cm high). A hole in the top of the holding box allowed even illumination by one fluorescent bulb, positioned 60 cm above the set-up, with a plastic screen used to obtain diffused light. Air was pumped through silicon tubing by an air pump (Air 275R, Sera, Heinsberg, Germany), purified by an activated CO₂ filter, humidified by bubbling through distilled water and then passed through a flowmeter (EK-2NRK, Comer, Bologna, Italy) at a flow rate of 1.5 litres/min. The air flow was then split with a plastic Y-connector and passed through two round modified beakers (250 ml volume capacity, diameter 9 cm), each containing the odour source, and then through the arms of the Y-tube.

In each test, a single parasitoid female was released at the entrance of the central stem of the Y-tube. Just prior to the beginning of experiments, the wasps were kept in a climatic chamber at 14 ± 1 °C and $50\pm 10\%$ relative humidity for 1 h; this increased their responsiveness to odours as assessed in preliminary trials. In the olfactometer set-up, walking of the tested wasp beyond the decision line (2 cm up either side arms) for a minimum of 20 s was recorded as a response, while not making any choice within 10 min was considered as no response. Each parasitoid female was tested only once to avoid any conditioned behaviour, and different females were tested until 30 responses were recorded for each group. To compensate for spatial asymmetry in the set-up, we switched the odour sources after testing five wasps. The Y-tube and beakers were cleaned with mild soap and alcohol (70% v) and sterilized in an autoclave at 120 °C for 20 min. Olfactory bioassays were conducted at 24 ± 1 °C, $45\pm 10\%$ relative humidity and 150 ± 2 lx.

Two-choice Assays

Parasitoid females (4-6 days old) of the six experience groups (ATn, ACn, ATt, ATc, ACt, ACc) were tested in two-choice assays to assess the effect of natal and adult experiences on their host preference for oviposition and host feeding. For each group, 20 parasitoid females were tested in the two-choice assays. Just prior to the beginning of experiments, wasp females were kept in the climatic chamber at 14 ± 1 °C and $50\pm 10\%$ relative humidity for 1 h; this made the females both easier to transfer into the arena and more reactive to stimuli, as assessed in preliminary trials. Female parasitoids were then introduced individually into the test arenas, consisting of glass petri dishes (diameter 15 cm) with a white paper filter disc on the bottom, and containing one leaflet of tomato infested by one *T. absoluta* larva (third-instar) and one leaf of upright pellitory infested by one *C. pulchrimella* larva (third- to fourth-instar). These leaves were placed in the middle of the arena side by side, separated by 30 mm and randomly located on the left or right, in order to avoid differences in exploitation of leaves by parasitoids due to the position. Test arenas were placed under the stereomicroscope in order to permit the observation of wasp behaviours. The two-choice assay lasted for 1 h, during which the parasitoid females in the arena were observed to detect the first host that they attacked by inserting the ovipositor into the mine. At the end, the parasitoid was removed and the leaves were carefully checked to assess host mortality, oviposition and host-feeding events. All of the two-choice assays were conducted at 24 ± 1 °C, $45\pm 10\%$ relative humidity and 150 ± 2 lx.

Statistical Analysis

In olfactometer bioassays, host choice of wasp females belonging to the same group was analysed using the chi-square test of goodness of fit. The null hypothesis was that parasitoid females had a 50:50 distribution across the two odour sources. Analysis of response proportion on the two host

species between the six groups was carried out using logistic regression via generalized linear models (GLMs), with a binomial distribution and logit link function. The same statistical analysis was carried out to analyse differences in the frequency of oviposition, host feeding, host killing and first attack on the two host species in two-choice assays. All statistical tests were performed using R (R Core Team, 2013).

RESULTS

Olfactometer Bioassays

The exotic host *T. absoluta*-tomato complex was in general the odour source preferred by the parasitoid (68.3%), but with noteworthy differences between experience groups (Fig. 1). There were significant effects of natal ($\chi^2_1=5.82$, $P=0.016$) and adult ($\chi^2_1=9.41$, $P=0.009$) experience on the choice of HPC by parasitoid females in olfactometer bioassays. No significant interaction between the two types of experience was found ($\chi^2_1=4.49$, $P=0.106$).

Natal experience showed a significant influence on host choice only in naïve parasitoid females. Naïve females reared on the exotic host *T. absoluta* more often chose *T. absoluta* (83.3%) than the native host *C. pulchrimella* (ATn: $\chi^2_1=13.3$, $P<0.001$), whereas naïve females reared on *C. pulchrimella* were unable to choose between volatiles coming from the two HPCs (ACn: $\chi^2_1=0$, $P=1$). On the other hand, there was an important effect of adult experience on host choice by parasitoid females that experienced the exotic host *T. absoluta*. Females with adult experience on *T. absoluta* (ATt and ACt) chose significantly more often the exotic host than the native host *C. pulchrimella* (ACt: $\chi^2_1=7$, $P=0.008$; ATt: $\chi^2_1=19.2$, $P<0.001$), whereas parasitoids exposed during adult life to *C. pulchrimella* (ACc and ATc) did not choose either of the two hosts (ACc: $\chi^2_1=0.533$, $P=0.465$; ATc: $\chi^2_1=0.533$, $P=0.465$).

The overall percentage of no response by parasitoid females was 19.4% with no significant differences between females with different natal and adult experience ($\chi^2_{5, 216}=8.11$, $P=0.15$; Fig. 1).

Two-choice Assays

When the parasitoid females were directly exposed to the exotic and native HPCs (*T. absoluta*-tomato and *C. pulchrimella*-upright pellitory) in a two-choice test, adult experience had a significant effect on the wasp's preference for oviposition, host killing and first-attacked host, with different outcomes for the two host species.

Oviposition preference of parasitoid females was significantly influenced by adult experience in its interaction with the host species, i.e. with different results for the two host species (Table 1).

Oviposition on the larvae of the exotic host *T. absoluta* occurred more frequently in females with both natal and adult experience on this host (ATt: 55%), and was significantly higher in comparison

with females with different adult experiences (ACn, ATn, ACc, ATc; Fig. 2). In contrast, oviposition on the larvae of the native host *C. pulchrimella* occurred more frequently than on the exotic host *T. absoluta* in parasitoid females with both natal and adult experience on this native host (ACc: 45%; $\chi^2_1=3.27$, $P=0.035$). Parasitoid females naïve or exposed to a different host species in their natal or adult life did not show any oviposition preference between host species (Fig. 2). Host-feeding activity was not influenced by any host experience previously had by parasitoid females (Table 1). The exotic host *T. absoluta* was preferred to the native host *C. pulchrimella*, regardless of the experience group. However, owing to the low number of host-feeding events, only wasp females reared on the native host and with adult experience on the exotic host (ACT) fed significantly more often on *T. absoluta* ($\chi^2_1=9$, $P=0.002$; Fig. 3).

Host-killing preference by parasitoid females (including both oviposition and host-feeding activities) varied significantly between the two host species, with the exotic host *T. absoluta* killed on average more often than the native host *C. pulchrimella* (61.2% and 39.2%, respectively; Table 1). Adult experience significantly affected the host-killing behaviour on *T. absoluta*. In fact, parasitoid females with adult experience on *T. absoluta* (ACT and ATt groups) on average killed *T. absoluta* larvae significantly more often (80%) than *C. pulchrimella* larvae (30%). Moreover, *T. absoluta* larvae were killed more frequently by parasitoid females with adult experience on the same host than by females with adult experience on the other host *C. pulchrimella* (47.5%; $\chi^2_1=4.6$, $P=0.032$) and naïve females (57.5%; $\chi^2_1=4.2$, $P=0.041$; Fig. 4).

Adult experience also had a significant influence on the host species first attacked by parasitoid females ($\chi^2_{2, 101}=6.16$, $P=0.045$; Table 1). Parasitoid females with adult experience on the exotic host *T. absoluta* (ACT and ATt) on average attacked this exotic host more often than females with adult experience on the native host *C. pulchrimella* (ACc and ATc). Also, wasp females with natal experience on *C. pulchrimella* and adult experience on *T. absoluta* (ACT) attacked first the exotic host significantly more often than the native host ($\chi^2_1=6.37$, $P=0.012$; Fig. 5). Neither the natal experience ($\chi^2_{1, 103}=0.14$, $P=0.701$) nor the interaction between natal and adult experience had influence on the first chosen host species (Table 1).

The results obtained in both olfactometer and two-choice assays are summarized in Table 2.

DISCUSSION

This study investigated the influence of natal and adult experience on the host preference of the parasitic wasp *N. tutae* on a native and an exotic host. To our knowledge, it is the first study that has specifically examined the effect of natal and adult experience on choices between native and exotic hosts of parasitoid females. The results suggest that host experience during both natal and adult life

may affect the HPC preference of this parasitoid species. The combination of different natal and adult experiences led to evident changes in odour attractiveness and foraging behaviour by parasitoid females. In particular, adult experience proved to influence host preference in both olfactometer and two-choice assays. By contrast, natal experience showed an effect on host preference only in olfactometer bioassays, where parasitoid females reared on *C. pulchrimella* with no adult experience (naïve) did not show any preference between the host species, as opposed to those with natal experience on *T. absoluta*, which preferred the exotic host in olfactometer assays (see Table 2). However, it was not always possible to clearly disentangle the effects of natal and adult experience on HPC selection, especially in olfactometer bioassays.

Effects of previous experience on host selection and exploitation by parasitic wasps have been investigated, both at the natal (Ghimire & Phillips, 2008; Morris & Fellowes, 2002; Rietdorf & Steidle, 2002; Rodriguez, Fuentes-Contreras, & Niemeyer, 2002; Villagra et al., 2007) and at the adult stage (Cortesero, Monge, & Huignard, 1995; Grasswitz, 1998; Guerrieri et al., 1997; Petitt et al., 1992; Takemoto et al., 2009). However, only a few studies have been performed on parasitoid species with similar ecological characteristics to *N. tutae*, an idiobiont ectoparasitoid of leafminer moth larvae (Cortesero et al., 1995; Gandolfi, Mattiacci, & Dorn, 2003b). Adult experience is often considered to have a higher impact on foraging behaviour than natal experience (Drost & Cardé, 1992; Petitt et al., 1992; Thiel & Hoffmeister, 2009). This assumption seems to be confirmed in our study, because only parasitoids with adult experience showed a host preference in foraging behaviours (i.e. oviposition, host feeding and host killing).

The influence of natal experience on host choice of naïve parasitoid females in olfactometer bioassays is particularly interesting, because it shows a greater response to the stimuli experienced during the natal period from the exotic host *T. absoluta* than from the native host *C. pulchrimella*. This is an unexpected result, since experience on the native host was expected to influence host preference to the same extent as experience on the exotic host. Surprisingly, the native host was never preferred by parasitoid females in olfactometer bioassays, regardless of the previous host experience. The native host was indeed preferred only for oviposition by parasitoid females exposed to *C. pulchrimella* during both the natal and adult stage. Different explanations are possible, such as: (1) *C. pulchrimella* may not be among the favourite primary hosts of *N. tutae*; (2) the exotic host *T. absoluta* lacks coevolved adaptive defences against the indigenous *N. tutae*, thus being more often detected and exploited than the native host *C. pulchrimella*; (3) *N. tutae* shows an innate preference for *T. absoluta*.

Actually, since the native host complex of the parasitoid *N. tutae* is not yet known (Gebiola et al., 2015), *C. pulchrimella* may not be the principal native host of this parasitoid. Moreover, its larvae

could have physiological, through the incorporation of allelochemicals or venom resistance, and behavioural defences (Gross, 1993), even if *N. tutae* is able to parasitize and develop successfully on this native species. Indeed, *C. pulchrimella* larvae seem to escape from the leaf mine more frequently than *T. absoluta* larvae, thus avoiding the foraging behaviours of the parasitoid (N. Bodino, C. Ferracini & L. Tavella, personal observation). Ultimately, the preference of the *N. tutae* for the exotic host *T. absoluta* (even if weakened by adult experience on the native host *C. pulchrimella*) may be due to the continuous mass rearing on this exotic host in commercial insectaries (Gandolfi et al., 2003a).

The effects of continuous rearing on the host *T. absoluta* could not be altogether eliminated, despite the switch to the rearing host made before starting the experiments. Even if we focused on natal experience influence, it is not possible to exclude altogether an influence of transgenerational adaptation occurring during the sequential rearing on two different hosts, since the parasitoids tested in the experiments came from groups reared for four to seven generations on two different HPCs. The transgenerational influence of the host species in mass rearing is still controversial; several studies have not found any influence of rearing host on subsequent host preference, even after many generations (Belda & Riudavets, 2012; Kölliker-Ott, Bigler, & Hoffmann, 2003; Van Bergeijk, Bigler, Kaashoek, & Pak, 1989), whereas others have shown behavioural differences after just one or two generations (Bourchier, Smith, Corrigan, & Laing, 1994; Jones et al. 2015). Therefore, the results seem to dramatically change in relation to the parasitoid species (Jones et al. 2015).

Interestingly, Gandolfi et al. (2003a) demonstrated that in another parasitoid species of the family Eulophidae, *Hyssopus pallidus*, learning of HPC stimuli takes place during natal development, and is not affected by a multigenerational adaptation due to mass rearing over 30 generations.

Considering the taxonomical and biological similarities (both species are idiobiont ectoparasitoids of moths), we think that the explanation of a multigenerational adaptation is unlikely to hold, and that, conversely, a significant effect of natal experience is more probable. However, further experiments are needed to clarify both of these issues. Nevertheless, our results show some clear effects of the host experience on host selection and exploitation preference on the two tested host species, suggesting NHPI and adult learning, if linked to direct experience on a host, as important components in switching between native and exotic hosts.

NHPI can increase not only the attractiveness of cues related to the natal environment (Gandolfi et al., 2003b; Giunti et al., 2015; Gutiérrez-Ibáñez, Villagra, & Niemeyer, 2007), but even the possibility of inheritance of environmental preferences via phenotypic plasticity (Corbet, 1985; Maturana-Romesin & Mpodozis, 2000; Rossiter, 1996; Van Allen & Bhavsar, 2014; Villagra et al., 2007). The inheritance of natal environment preferences may thus result in a parasitoid population

specializing on a host (Cornell & Hawkins, 1993; Gutiérrez-Ibáñez et al., 2007; Zepeda-Paulo, Ortiz-Martínez, Figueroa, & Lavandero, 2013). In contrast, learning of chemical cues during adult life seems to play a role in the host-switching process (Giunti et al., 2015; Li, Miller, & Sun, 2009; Papaj & Prokopy, 1989; Wei et al., 2013), and thus foster natural population splitting and even speciation (Pennacchio, Digilio, Tremblay, & Tranfaglia, 1994). Once the parasitoid female oviposits on a new host, NHPI can then reinforce the new host preference (Barron & Corbet, 1999; Douloumpaka & Van Emden, 2003). A similar scenario could be a valid explanation of the drivers that had contributed to the switch of the parasitoid *N. tutae* from the native host to the new exotic host *T. absoluta*.

In conclusion, our study suggests that host preference of the parasitoid *N. tutae* can be influenced by previous experience, especially if it occurred during adult life, resulting in a significant increase in exotic host selection by females exposed to the host *T. absoluta*. These results, applied in the context of biological invasions, can provide useful information for understanding the mechanisms underlying the host-switching behaviour that lead a native parasitoid to adapt to a new exotic host. Future research should be focused on host-parasitoid community dynamics, to determine the potential behavioural plasticity of native parasitoids and their response to a changing environment. The knowledge of these characteristics will be especially important to select the optimal candidate species among natural enemies for future biological control programmes against exotic insect pests.

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Table 1: GLM logistic regression of the effects of host-plant complex (HPC), natal host experience (N) and adult host experience (A) on frequency of foraging behaviours

Variable	df	Oviposition		Host feeding		Host killing		First attack	
		χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
HPC	1	1.87	0.176	21.51	<0.001	12.26	<0.001	---	---
N	1	0.02	0.880	0.11	0.736	0.16	0.691	0.15	0.702
A	2	2.98	0.225	0.17	0.918	1.09	0.579	6.16	0.046
HPC×N	1	2.77	0.096	0.04	0.841	0.86	0.356	---	---
HPC×A	2	13.50	0.001	3.47	0.176	12.22	0.002	---	---
N×A	2	1.82	0.402	0.82	0.663	0.31	0.856	0.60	0.740
HPC×N×A	2	1.06	0.589	2.92	0.232	1.02	0.599	---	---

Significant P values are shown in bold.

Table 2: Host-plant complex (HPC) preference of *Necremnus tuta* females in olfactometer and two-choice assays, based on their previous natal and adult experience

Assay	HPC preference	
	Exotic host <i>Tuta absoluta</i>	Native host <i>Cosmopterix pulchrimella</i>
Olfactometer	Females with adult experience on this exotic HPC and females naïve with natal experience on this exotic HPC	No preference
Two-choice		
Oviposition	Females with natal and adult experience on this exotic HPC	Females with natal and adult experience on this native HPC
Host feeding	High preference, especially females with natal experience on the native HPC and adult experience on this exotic HPC	No preference
Host killing	Females with adult experience on this exotic HPC	No preference

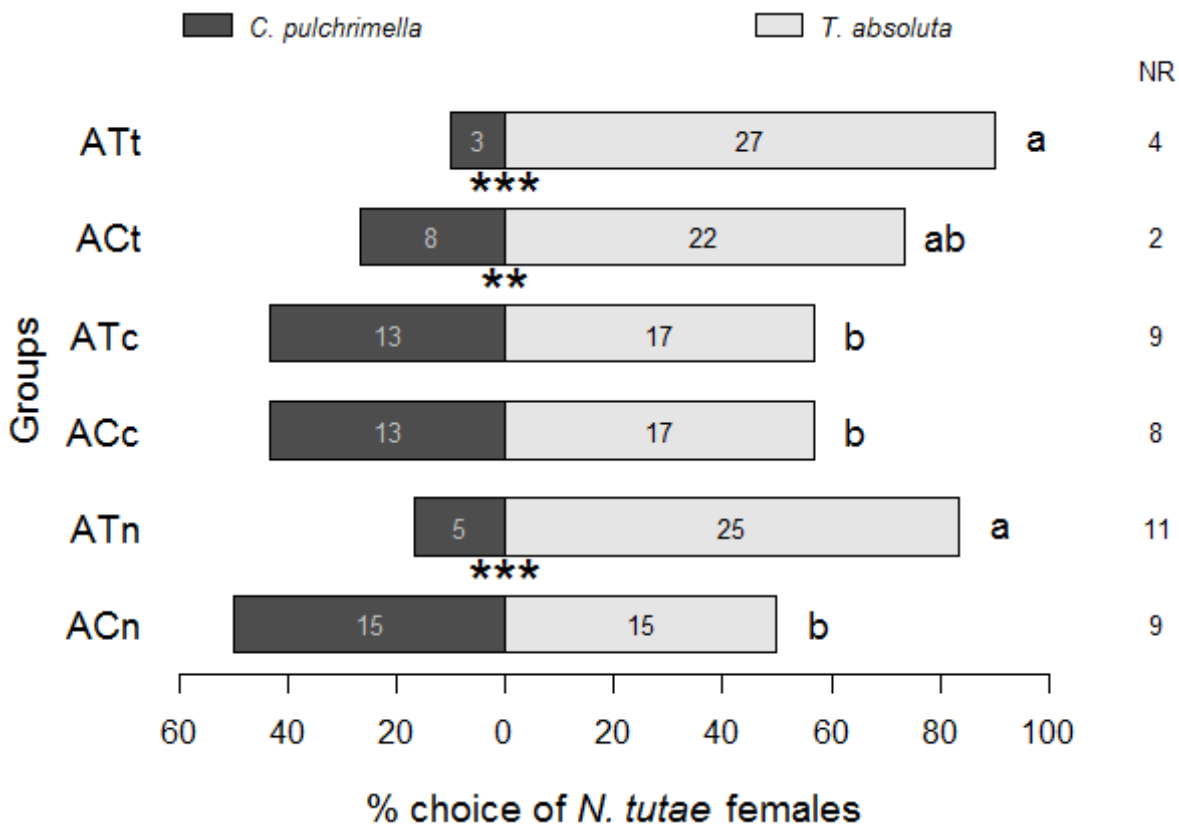


Fig. 1: Effect of natal and adult experience on the response of *Necremnus tuta* females to different host-plant complexes (HPCs) in a Y-tube olfactometer. The six groups of females are defined by two rearing conditions (AT = *Tuta absoluta*; AC = *Cosmopterix pulchrimella*) \times three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*). Asterisks indicate significant attraction for an odour source (χ^2 test: ** $P < 0.01$; *** $P < 0.001$). Same letters on bars indicate no significant differences (GLM: $P > 0.05$) between groups regarding the number of females choosing tomato leaves infested by *T. absoluta* larvae. The numbers in the bars represent females responding to the host plant odours, while the number of females not responding (NR) is indicated to the right of each bar.

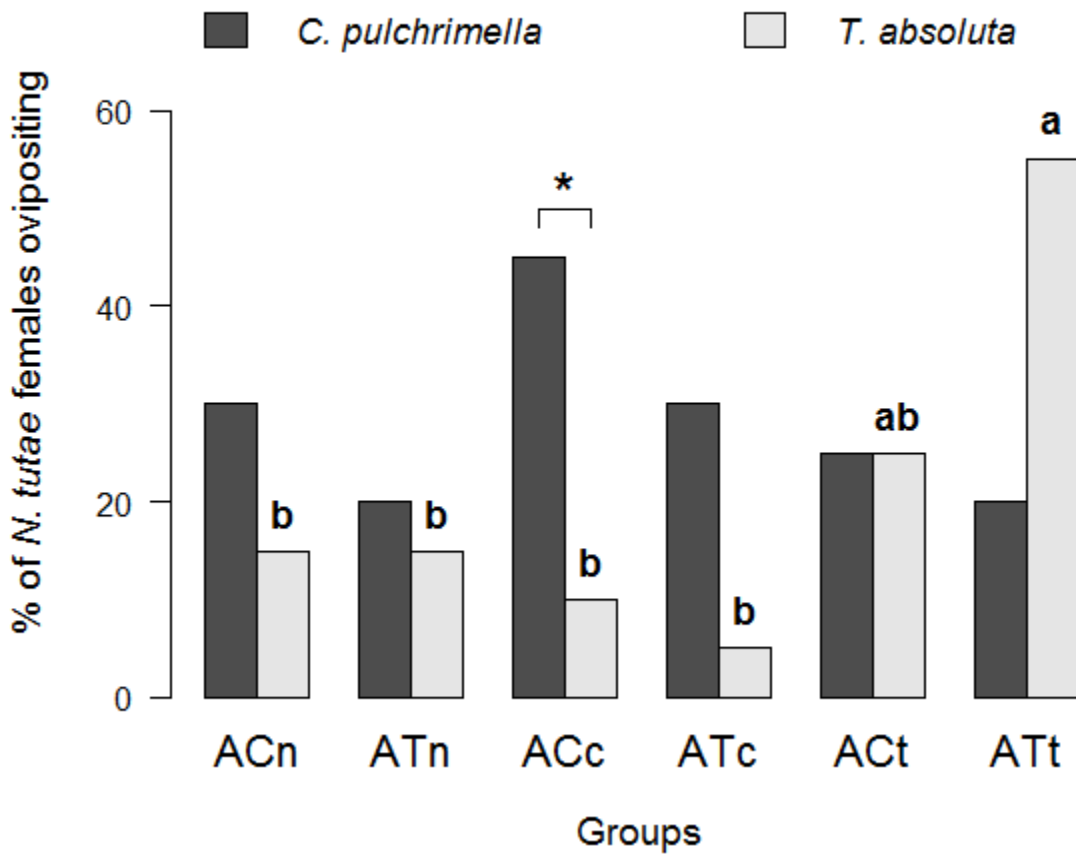


Fig. 2: Effect of natal and adult experience of *Necremnus tuta* females on oviposition preference for host species in the two-choice assays. The six groups of females are defined by two rearing conditions (AT = *Tuta absoluta*; AC = *Cosmopterix pulchrimella*) × three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*). Asterisk indicates significant difference in killing percentage between the two host species (χ^2 test: $*P < 0.05$). Same letters on bars indicate no significant differences between groups regarding the number of females ovipositing on *T. absoluta* host (GLM: $P > 0.05$).

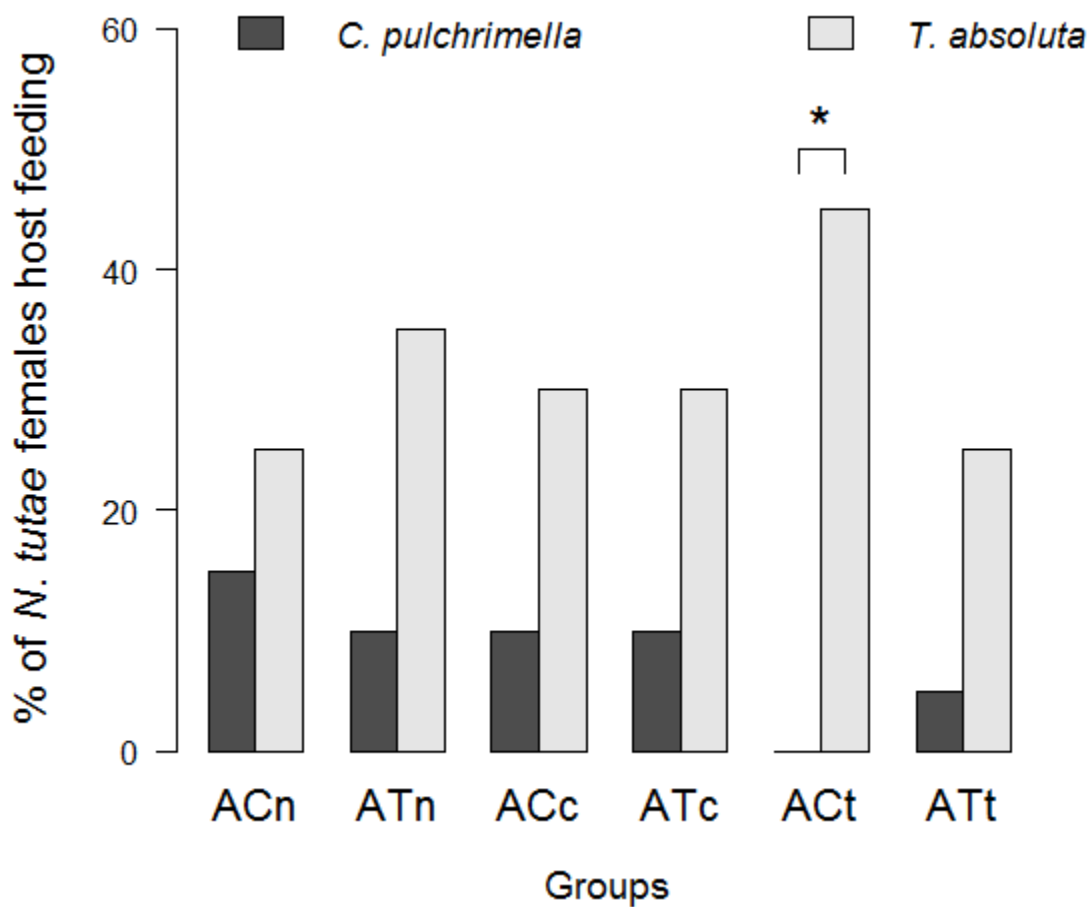


Fig. 3: Effect of natal and adult experience of *Necremnus tutae* females on feeding preference for host species in the two-choice assays. The six groups of females are defined by two rearing conditions (AT = *Tuta absoluta*; AC = *Cosmopterix pulchrimella*) × three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*). Asterisk indicates significant difference in killing percentage between the two host species (χ^2 test: * $P < 0.05$).

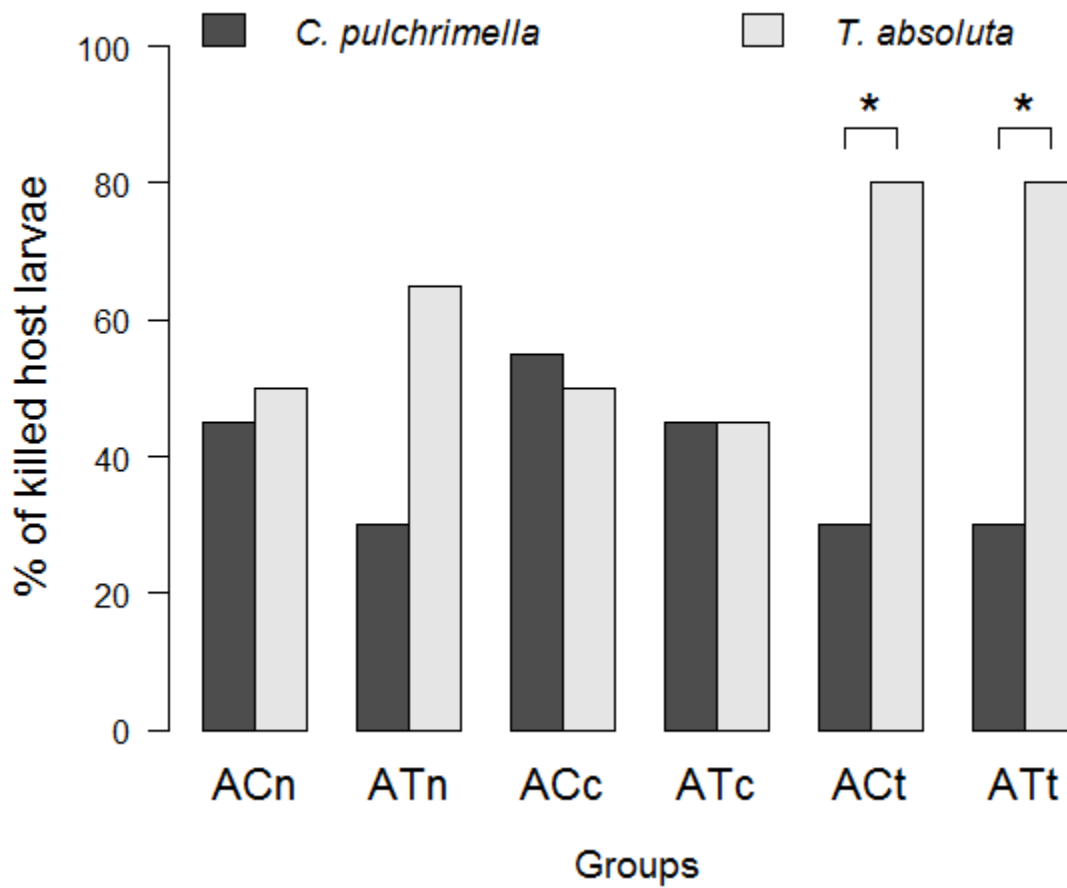


Fig. 4: Effect of natal and adult experience of *Necremnus tuta* females on killing preference for host species in the two-choice assays. The six groups of females are defined by two rearing conditions (AT = *Tuta absoluta*; AC = *Cosmopterix pulchrimella*) × three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*). Asterisks indicate significant differences in killing percentage between the two host species (χ^2 test: * $P < 0.05$).

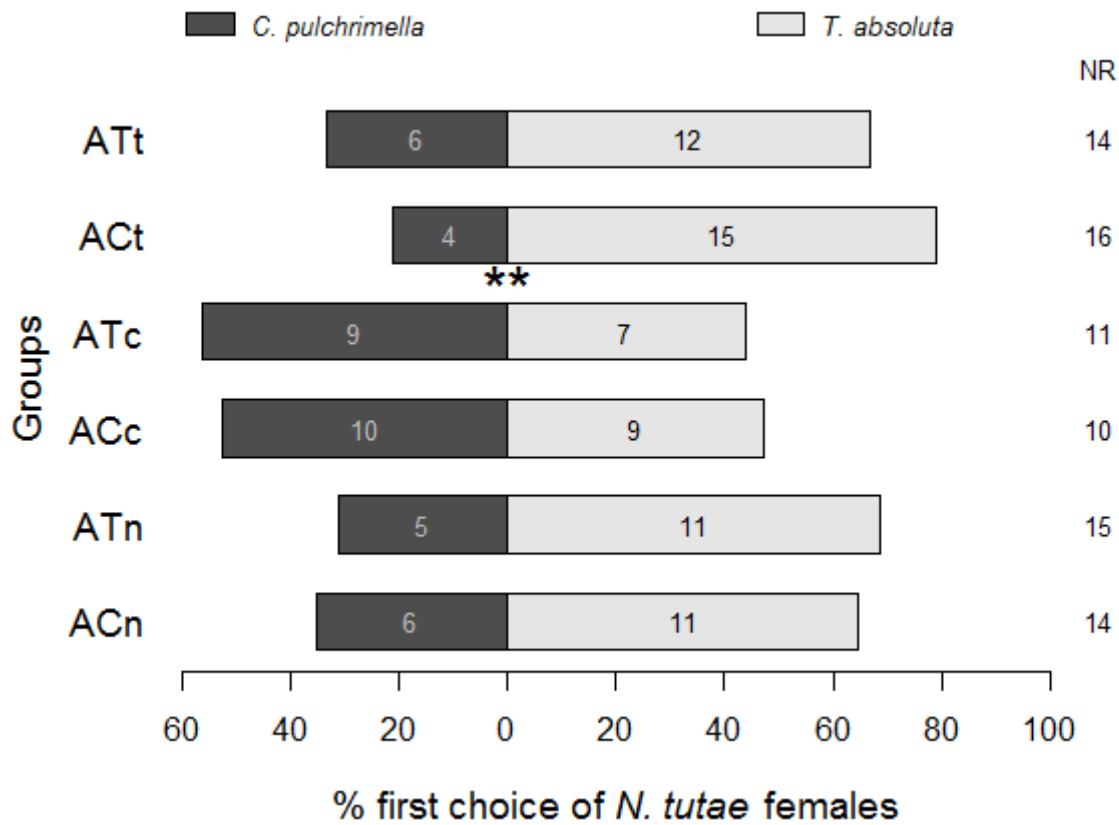


Fig. 5: Effect of natal and adult experience of *Necremnus tutae* females on the first host–plant complex (HPC) visited in the two-choice assays. The six groups of females are defined by two rearing conditions (AT = *Tuta absoluta*; AC = *Cosmopterix pulchrimella*) × three adult experiences (n = naïve; t = *T. absoluta*; c = *C. pulchrimella*). Asterisks indicate significant attraction for an odour source (χ^2 test: $**P < 0.01$). The numbers in the bars represent females visiting the HPC, while the number of females not responding (NR) is indicated to the right of each bar.