CrossMark

Preference and life history traits of *Aphelinus abdominalis* (Hymenoptera: Aphelinidae) when offered different development stages of the lettuce aphid *Nasonovia ribisnigri* (Hemiptera: Aphididae)

Govinda Shrestha · Henrik Skovgård · Tove Steenberg · Annie Enkegaard

Abstract The degree of parasitisation, host feeding, developmental time, adult emergence and female sex ratio of the parasitoid *Aphelinus abdominalis* were evaluated when different host stages of *N. ribisnigri* (1st, 2nd, 3rd, alatoid 4th instar or newly moulted apterous adult) were offered as hosts under no-choice conditions in the laboratory at 22 °C, 70 % RH and 16:8 L:D. In addition, the parasitoid preference between 2nd and alatoid 4th instar was examined. In the no-choice experiments, the highest degree of successful parasitisation (leading to mummy formation) (41–56 %) was observed when young and intermediate stages of the lettuce aphid were exposed for parasitism compared with older developmental stages. This pattern was supported in the choice experiment where significantly more 2nd instar lettuce aphids were parasitised than alatoid 4th instars, with Manly's preference index (mean ± SE) for the former stage being 0.79 ± 0.03. Incomplete parasitisation was recorded highest in 1st instar (mean ± SE: 29.95 % ± 4.71), with much lower values (1–8 %) attained in the 2nd, 3rd, alatoid 4th instar and apterous adult with no significant differences. Host feeding was low (2–4 %) and only recorded in 1st and 2nd instars. The mean development time of *A. abdominalis* from egg to adult emergence was longest for 1st instar (19.03 ± 0.12 days) and the shortest for apterous adults (16.59 ± 0.29 days). A high percentage of adult emergence (>80 %) from mummified aphids as well as a strongly female-biased sex ratio (>78 %) were found across all host stages of the lettuce aphid.

Keywords Biocontrol · Host instar · Hymenoptera · Parasitoid · Host selection

Introduction

The lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae), is an important oligophagous pest of lettuce. The aphid species is believed to have originated from the temperate regions of Europe and has now spread to most lettuce growing areas of the world (Blackman and Eastop 1984, 2000). *N. ribisnigri* feeds mainly inside lettuce hearts and heads (Mackenzie and Vernon 1988), and due to this cryptic behaviour, frequent application of insecticides has led to development of resistance against most insecticides available on the market today (Rufingier et al. 1997; Barber et al. 1999). Hence, without having reliable chemical methods for the control of *N. ribisnigri*, this pest may pose a serious threat to the future worldwide lettuce production.

G. Shrestha (✉) · H. Skovgård · T. Steenberg · A. Enkegaard
Department of Agroecology, Faculty of Science and Technology, Research Centre Flakkebjerg, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark
e-mail: Govinda.Shrestha@agro.au.dk

Among lettuce growers biological control as an alternative or supplement to chemical control of *N. ribisnigri* has attracted increased interest within the last ten years. Recent studies have demonstrated that several species of predators (Hopper et al. 2011; Shrestha and Enkegaard 2013), hymenopteran parasitoids (Nebreda et al. 2005; Shrestha et al. 2014) and fungal entomopathogens (Fournier and Brodeur 2000; Åsman 2007) have the potential either individually or as a part of an integrated solution to control this aphid species. Aphid parasitoids are especially regarded as effective and reliable biocontrol agents due to their generally high population growth rates and their ability to respond in a density dependent manner to increasing aphid densities (Hofsvang 1990; van Steenis 1995). Recently, five commercially available parasitoid species have been evaluated for their potential against the lettuce aphid, revealing *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae) as the most promising candidate (Shrestha et al. 2014).

A. abdominalis is a koinobiont endoparasitoid, i.e. it allows the host to continue development for a certain period of time after being parasitised. Thus, before depositing an egg inside a host, the female parasitoid has to consider the chance of successful development of her progeny against the risk of failure as host quality and host immune defence mechanisms may change in the course of parasitoid development (Sequeira and Mackauer 1992; Walker and Hoy 2003; Henry et al. 2005). Furthermore, host selection by a female parasitoid depends on host physiology (cuticular thickness) and host defensive behaviour (kicking, jerking, drop-off and walking away), which may differ from one host stage to another (Hagvar and Hofsvang 1991). Consequently, aphid parasitoids often show preference to certain host instars with the preference being reported to be both host and parasitoid species specific (e.g. Walker and Hoy 2003; Perdikis et al. 2004; Wyckhuys et al. 2008). Through their mechanism of host selection parasitoids can further influence the timing of their own life history events as for example developmental time (Mueller et al. 1992; Harvey 2005; Colinet et al. 2005), adult emergence (Walker and Hoy 2003) or sex ratio (Mueller et al. 1992; Honek et al. 1998) may be influenced by the host stage parasitised. It is therefore important to determine which aphid host stage is most effectively parasitised prior to undertaking large-scale releases of parasitoids under field or glasshouse conditions. No information is available on the influence of *N. ribisnigri* developmental stages on the preference and life history traits of *A. abdominalis*.

This study examined the level of parasitism by *A. abdominalis* on different developmental stages of *N. ribisnigri* under no-choice and choice conditions. In addition, the parasitoid fitness on *N. ribisnigri* instars was examined by measuring key life history traits such as development time, adult emergence rates and sex ratio.

Materials and methods

Plant and insects

Iceberg lettuce (*Lactuca sativa* L., cv. Mirette) plants were grown in 9 cm plastic pots filled with peat soil, perlite and vermiculite (mixed at 90:8:2) with a pH of 6–7. The pots were placed in insect-proof net-covered cages (68 × 75 × 82 cm) in a climate-controlled glasshouse compartment at 20 °C, 70 % RH and 16:8 L:D. Plants with three to four unfolded leaves were used for the experiments.

The lettuce aphid *N. ribisnigri* (clone-4580a), originally supplied by Dr. Gemma Hough (Warwick Crop Centre, University of Warwick, UK), was reared on Iceberg lettuce plants in similar cages and under similar conditions as described above. The parasitoid *A. abdominalis* was supplied as mummies by EWH BioProduction, Tappernøje, Denmark, and placed in climate cabinets at 22 °C, 70 % RH and 16:8 L:D in plastic Petri dishes (diameter: 15 cm). Mummies were checked daily for adult emergence. Four-day-old female parasitoids were used for experimentation as fecundity is low in the first few days after emergence (Jarošik et al. 1996). Females of standardised age were obtained by transferring (by aspirator) cohorts of adults emerging on the same day to new Petri dishes with a piece of cotton pad soaked with a solution of 10 % of honey water were left for two days at the same laboratory conditions as above. On the third day, adults were sexed under a stereomicroscope and females and males subsequently transferred pairwise by aspirator to a new Petri dish (diameter: 5 cm) and left for mating for 24 h prior to use in experiments on the following day.

Aphid cohorts

To obtain aphids of the same stage, unwinged and winged adults of lettuce aphids were transferred onto uninfested Iceberg lettuce plants using a fine camel hair brush and maintained in similar cages and at similar conditions as above. After 24 h, the 1st instar nymphs produced were either used directly for the experiments or transferred onto uninfested plants and maintained for another three, five and eight days for production of 2nd, 3rd and 4th instar nymphs with wing buds (alatoid 4th instar), respectively (Diaz and Fereres 2005). Eight days after birth, some a proportion of the 4th instar nymphs had moulted into apterous adults. These unwinged adults were used for experiments as well.

No-choice tests

The no-choice experiments were performed to determine the parasitisation rates and fitness of *A. abdominalis* on each of the developmental stages of *N. ribisnigri* by measuring total parasitism as well as a set of life history traits (development time, parasitoid emergence rate and sex ratio). The experiment was carried out in vented Petri dishes (diameter: 9 cm) lined with moist filter paper. A circular lettuce leaf disc (diameter: 5 cm) was placed at the bottom of each dish. Twenty aphids, either of 1st, 2nd, 3rd, alatoid 4th instar nymphs or newly moulted unwinged adults, were transferred to each lettuce dish using a fine camel hair brush. Aphids were allowed to settle on the leaf disc for 1 h before the introduction of a female parasitoid.

One mated female parasitoid (four days old) was released into each Petri dish arena containing 20 lettuce aphids and left for a 24 h parasitisation period in a climate cabinet at 22 °C, 70 % RH and 16:8 L:D. The female parasitoid was subsequently removed by aspirator and the number of dead and live aphids in each dish was counted under a stereomicroscope. The numbers of aphids dying due to host feeding by *A. abdominalis* were determined based on their shrunken appearance (Rohne 2002; Tatsumi and Takada 2005). Live aphids from the leaf discs were then divided equally between two uninfested leaves. The petiole of each leaf was wrapped with moist cotton and inserted into a 1.5 ml Eppendorf tube containing demineralised water to avoid leaf degradation. Each leaf was placed in a

new vented Petri dish (diameter: 15 cm) lined with moist filter paper and incubated in a climate cabinet at similar conditions as described above. After 4–5 days, the filter paper was replaced and a new fresh leaf was placed in the vicinity of the old leaf to allow the aphids to translocate themselves. Generally lettuce leaves remained fresh for at least six days using this setup.

Aphids were checked at 2–3 days intervals for nine days for recording of aphids dying without mummification. Subsequently the aphids were checked daily for additional five days for appearance of mummies. The aphid mummies that formed in each dish were gently collected using a fine camel hair brush and transferred individually into small transparent medicine cups (15 mm) with screened lids. Emergence of adult parasitoids was checked daily and emerged parasitoids sexed under a stereomicroscope. For each of the five treatments, 10–15 Petri dishes (replicates) were set up with a total of 60 female parasitoids tested in the experiment. Dishes in which any nymphs had been produced or in which the parasitoid had died during parasitisation were discarded. For the controls, the numbers of replicates were five for each host stage and the same procedure was followed without the addition of parasitoids. Petri dishes were placed in a completely randomised design in the climate cabinet.

Choice test

To examine whether *A. abdominalis* has a preference for either early or late instars of *N. ribisnigri*, 2nd and alatoid 4th instars were chosen for the study. The experimental procedures and experimental conditions were similar to the ones described above except that 2nd instar (n = 25) and alatoid 4th instar (n = 25) nymphs of *N. ribisnigri* were offered simultaneously on the same leaf disc. Second instar nymphs were introduced first and allowed to settle for 15 min in the Petri dish prior to the release of the alatoid 4th instar nymphs. Earlier instars of lettuce aphids were easy to handle and settled quickly compared to later aphid instars (G. Shrestha, pers. obs.). Alatoid 4th instar nymphs are easily distinguished under a stereomicroscope by their size and by having wing buds. The number of replicates for treatment was 15 and the controls (replicates = 5) were performed in absence of any parasitoids.

Statistical analysis

The aphid mortalities inflicted by parasitisation were grouped into three categories: successful parasitisation, incomplete parasitisation and total parasitisation. The unit of replication for the parasitisation data was the Petri dish. The percentage of successful parasitism of the different aphid stages was calculated as percent mummified aphids out of the total number of exposed aphids minus host fed aphids, incomplete parasitism as percent dead aphids (corrected for control mortality) without signs of mummification out of the total number of exposed aphids minus host fed aphids, total parasitism as successful parasitism + incomplete parasitism, female sex ratio as percent females and adult parasitoid emergence as percent of parasitoids emerging from mummified aphids. Dead aphids recorded in the incomplete parasitisation group (Colinet et al. 2005) were corrected for control mortality (Abbott 1925) prior to calculation and statistical analysis.

One-way analysis of variance (ANOVA) was performed to test for the effect of host stage on the percentage of successful parasitism, incomplete parasitism and total parasitism in the no-choice experiments and for any differences in successful parasitism or incomplete parasitism when two host stages were offered simultaneously in the choice experiment. A normal quantile–quantile plot was performed to confirm normality of data and equality of the variance. A transformation (angular) was required on the percentages to achieve normal distribution prior to statistical tests. Tukey contrast pairwise multiple comparisons were used to test for significant differences in means (Hothorn et al. 2008). To test for significant differences in development times from egg to mummy formation, from mummy to adult emergence and for total development, separate one-way analyses of variance were performed. No transformation of development data was required.

The sex ratio and adult emergence data were found to be non-normally distributed even after the angular transformation, and the non-parametric one-way ANOVA (Kruskal–Wallis test), was therefore used to test for differences. A Mann–Whitney U-test was used as a post-hoc test for multiple comparisons between the means followed by a Bonferroni correction to adjust the probability ($\alpha = 0.01$). The unit of replication for development, sex ratio and adult

emergence data was the Petri dish. The preference of *A. abdominalis* for a given host stage (2nd or alatoid 4th instar) was evaluated using Manly's preference index (Manly 1974):

$$\beta_1 = \frac{\log\left(\frac{e_1 + 0.001}{A_1}\right)}{\log\left(\frac{e_1 + 0.001}{A_1}\right) + \log\left(\frac{e_2 + 0.001}{A_2}\right)}$$

where β_1 is the preference index attaining values between 0 and 1. A β_1 value larger than 0.5 indicates a preference for 2nd instar aphids and below 0.5 indicates a preference for alatoid 4th instar aphids. A_1 and A_2 are the number of available 2nd instar and alatoid 4th instar aphids, respectively, and e_1 and e_2 denote the number of mummified 2nd and alatoid 4th instar aphids. The preference index was tested for significance (i.e. difference from a value of 0.5) with a one-sample t test. The data were analysed using the software statistical package R 2.15.1 (R Development Core Team 2012).

Results

No-choice tests

Parasitisation and host feeding

A. abdominalis was able to successfully parasitise on each of the five developmental stages of *N. ribisnigri* (Table 1), although the percentage of parasitised alatoid 4th instars and the newly moulted apterous adults was significantly lower than for 1st, 2nd and 3rd instars (df = 4, 54, $F = 7.63$, $P < 0.001$). There was no significant difference in the parasitisation of the three young to intermediate stages of the lettuce aphid (Table 1).

The percentage of incomplete parasitisation was highest for 1st instar lettuce aphids and significantly different from the percentages found for the four other aphid stages (df = 4, 54, $F = 10.09$, $P < 0.001$). Although not significantly different, the percentage of incomplete parasitism showed a tendency to decrease with increasing development stage with the lowest percentage recorded for newly moulted apterous adults (Table 1).

The highest percentage of total parasitisation was found when young and intermediate developmental

Table 1 Mean percentage (±SE) of successful parasitisation, incomplete parasitisation and total parasitism (sum of successful and incomplete parasitisation) of *Aphelinus abdominalis* in the different developmental stages of *Nasonovia ribisnigri* in no-choice test

Host stage	Successful parasitisation Mean ± SE	Incomplete parasitisation Mean ± SE	Total parasitisation Mean ± SE
1st instar (13)	40.61 ± 7.98a	29.95 ± 4.71a	70.56 ± 4.44a
2nd instar (15)	55.86 ± 6.82a	7.76 ± 2.58b	63.62 ± 5.80a
3rd instar (15)	56.00 ± 8.30a	4.91 ± 1.90b	60.91 ± 7.55a
4th instar (alatoid) (7)	5.00 ± 1.89b	3.64 ± 1.32b	8.64 ± 2.65b
Adult (apterae) (8)	8.33 ± 1.88b	1.26 ± 0.69b	9.59 ± 2.01b

Mean values within columns bearing the same letters are not significantly different (Tukey test, P > 0.05). The values in parentheses denote the numbers of replicates per treatment

stages of the lettuce aphid were exposed to *A. abdominalis* (61–71 %), while the lowest level of parasitism (9–10 %) was found in the older developmental stages (df = 4, 54, $F = 15.17$, $P < 0.001$). Aphid mortality in the controls was less than 8 % and non-significant across host instars (df = 4, 54, $F = 0.80$, $P > 0.05$). Host feeding percentage in the no-choice experiments was found to be negligible and only occurred in 1st (mean ± SE: 4.23 % ± 0.52) and 2nd instar (mean ± SE: 2.33 % ± 0.67) aphids.

Development time

The development time of *A. abdominalis* progeny was significantly influenced by the aphid host stage at the time of parasitisation: egg to mummy formation (df = 4, 54, $F = 32.64$, $P < 0.001$), mummy to adult emergence (df = 4, 54, $F = 5.43$, $P < 0.01$) and total development time from egg to adult emergence (df = 4, 54, $F = 34.93$, $P < 0.001$). The shortest total development time was found in the oldest aphid stages (alatoid 4th instars; newly moulted apterous adults), whereas medium development times were found in the intermediate stages (2nd and 3rd instar) and the longest in the youngest stage (Table 2).

Parasitoid emergence and sex ratio

Both parasitoid emergence and female sex ratio were significantly influenced by the aphid host stage parasitised (parasitoid emergence: $\chi^2 = 13.25$, df = 4, $P < 0.05$; female sex ratio: $\chi^2 = 18.27$, df = 4, $P < 0.01$). More than 80 % of mummified aphids emerged irrespective of aphid host stage, with 100 % emergence from mummies resulting from

parasitisation of alatoid 4th instars and adult aphids (Fig. 1). A female-biased sex ratio (>78 %) was found for all of the five developmental stages of the lettuce aphid (Fig. 1), with solely females emerging from mummified alatoid 4th instars and newly moulted apterae adults.

Choice test

The choice experiment showed that *A. abdominalis* successfully parasitised significantly more 2nd instar than alatoid 4th instar aphids (df = 1, 14, $F = 88.79$, $P < 0.0001$) (Fig. 2), resulting in a preference index β for 2nd instar aphids of 0.79 ± 0.03, significantly different from 0.5 (df = 14, $t = -8.12$, $P < 0.0001$). With respect to incomplete parasitisation, no significant difference was found between the two exposed host stages of *N. ribisnigri* (df = 1, 14, $F = 1.49$, $P > 0.05$) (Fig. 2).

Discussion

The understanding of female parasitoid preference for different host stages is an important aspect in the development of efficient biocontrol strategies against aphid pest species including the lettuce aphid *N. ribisnigri* (Hagvar and Hofsvang 1991). In this study, we have examined different host stage-specific parameters for *A. abdominalis* parasitising *N. ribisnigri*.

In the no-choice experiment, the highest parasitisation rate leading to formation of mummies (successful parasitisation) was observed when young and intermediate stages of the lettuce aphid were exposed for parasitism compared with older developmental

Table 2 Developmental time (days) (mean ± SE) of *Aphelinus abdominalis* in different developmental stages of *Nasonovia ribisnigri* in no-choice test

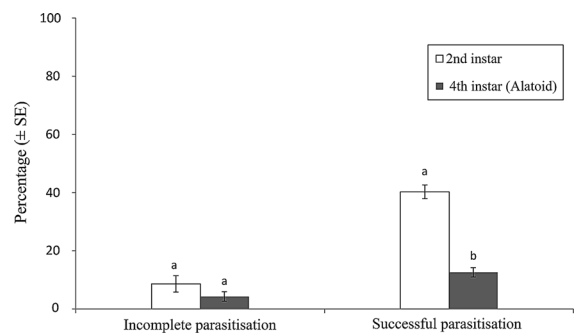| Host stage	Egg to mummy formation Mean ± SE	Mummy to adult emergence Mean ± SE	Egg to adult emergence Mean ± SE
1st instar (13)	7.90 ± 0.09 (101)c	11.13 ± 0.09 (89)b	19.03 ± 0.12 (89)c
2nd instar (15)	7.44 ± 0.10 (164)bc	10.83 ± 0.09 (151)b	18.27 ± 0.12 (151)b
3rd instar (15)	7.28 ± 0.05 (168)bc	10.96 ± 0.08 (147)b	18.24 ± 0.10 (147)b
4th instar (alatoid) (7)	6.25 ± 0.14 (7)a	10.75 ± 0.43 (7)ab	17.00 ± 0.35 (7)a
Adult (apterous) (8)	6.42 ± 0.24 (11)a	10.17 ± 0.34 (11)a	16.59 ± 0.29 (11)a

Mean values within columns bearing the same letters are not significantly different (Tukey test, P > 0.05). The values in parentheses in the host stage column denote the numbers of replicates per treatment. The values in parentheses in the development time columns denote the total numbers of individuals observed per treatment
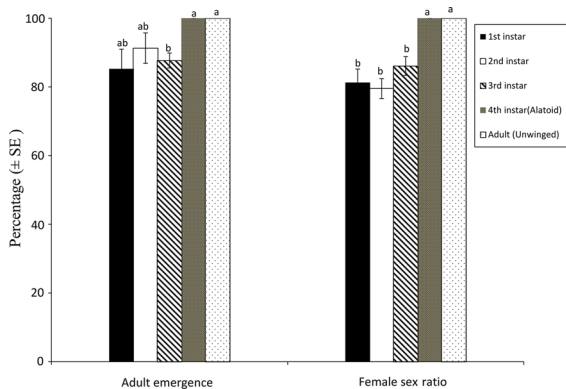
Fig. 1 Percentage (mean ± SE) of adult emergence and female sex ratio of *Aphelinus abdominalis* when different host stages of *Nasonovia ribisnigri* were offered as hosts under no-choice conditions. Different letters above bars indicate significant differences in adult emergence or female sex ratio [Mann–Whitney U-tests followed by Bonferroni correction ($\alpha = 0.01$)]

Fig. 2 Percentage (mean ± SE) of incomplete parasitisation and successful parasitisation of *Aphelinus abdominalis* when 2nd and alatoid 4th instar of *Nasonovia ribisnigri* were offered as hosts under choice conditions. Different letters above bars indicate significant differences in incomplete parasitisation or successful parasitisation (Tukey test, P < 0.05)

stages. This pattern was supported in the choice experiment where significantly more 2nd instars of the lettuce aphid were parasitised than alatoid 4th instars. The preference for young and intermediate host stages is in accordance with results reported for many other aphid parasitoids (Chau and Mackauer 2000; Perdikis et al. 2004; Wyckhuys et al. 2008), although in contrast to observations suggesting that *A. abdominalis* prefers 4th–1st instars of potato aphids, *Macrosiphum euphorbiae* (Thomas), and shows no significant differences in its parasitisation of intermediate and older stages of this aphid species (Honek et al. 1998).

Difference in successful parasitisation between host stages of aphids has been reported to be influenced by a number of factors such as developmental disruption of the parasitoid larvae (Beckage and Gelman 2004), aphid injury caused at the time of parasitisation (Rakhshani et al. 2004; Colinet et al. 2005), host physiology (Walker and Hoy 2003) and aphid defence behaviour (Gerling et al. 1990; Wyckhuys et al. 2008; He et al. 2011). The low parasitisation rate observed in the present study of alatoid 4th instars or newly moulted apterous adults seems best explained as a result of effective defence mechanisms, e.g. body shaking, kicking, walking away or clustering rather than internal defence mechanisms disrupting parasitoid larval development or the influence of host physiology. This suggestion is anchored in the fact that the number of older aphids dying without mummification was low and in contrast to the high degree of incomplete parasitisation when young aphid stages

(primarily 1st instar) were parasitised. This result is in line with previous findings reporting a decrease in incomplete parasitisation rate with increased aphid age (Rakhshani et al. 2004; Colinet et al. 2005). The higher mortality of 1st instar lettuce aphids due to incomplete parasitisation indicates that young nymphs are more susceptible to the injuries caused at oviposition, e.g. stinging or venom injection.

A. abdominalis was able to complete its development until adult emergence, irrespective of the aphid host stage parasitised. However, the lower adult emergence rate in the third instar together with the tendency ($P = 0.03$, $\alpha = 0.01$) to a lower emergence rate in the first instar of the lettuce aphids is consistent with the findings obtained by Rohne (2002), indicating that young stages of the lettuce aphids may provide suboptimal conditions for parasitoid progeny development.

The development time of *A. abdominalis* was affected by the aphid host stage parasitised as has been reported elsewhere in the literature (see review by Harvey 2005) where decrease (Mueller et al. 1992; Sengonca et al. 2008) as well as increase in parasitoid development time with aphid host age (Wyckhuys et al. 2008) have been observed. In some cases parasitoid development time has been recorded to be uninfluenced by host age (Cate et al. 1977) or been observed to attain the lowest value in intermediate aphid stages (Harvey et al. 2004; Colinet et al. 2005). No previous information seems to exist regarding the effect of host stage on development time of *A. abdominalis*. However, our results resemble the findings of Mueller et al. (1992) and Sengonca et al. (2008), who reported that the development time of *Aphelinus mali* (Hald) and *Aphelinus asychis* (Walker), respectively, decrease with the age of the hosts, the woolly apple aphid (*Eriosoma lanigerum* Hausmann) and the cotton aphid (*Aphis gossypii* Glover), respectively. Furthermore, developmental time of *A. abdominalis* on 2nd instar of the rose-grain aphid [*Metopolophium dirhodum* (Walker)], the bird cherry-oat aphid [*Rhopalosiphum padi* (Linnaeus)] and the grain aphid [*Sitobion avenae* (Fabricius)] is well comparable with our results (Haardt and Holler 1992).

The overall sex ratio of the progeny of *A. abdominalis* parasitising lettuce aphids was female-biased for all aphid host stages studied, although with some graduation as fewer females were produced when young and intermediate aphid stages were parasitised. This result is consistent with Honek et al. (1998), who reported a high

percentage (96–100 %) of female parasitoids resulting from parasitisation of 4th instar and adult aphids of *M. euphorbiae*, while parasitisation of young and intermediate aphid nymphs produced only 44 and 68 % females, respectively. In addition, female sex ratio seems to depend on the parasitoid isoline as Haardt and Holler (1992) demonstrated a sex ratio of 100 % *A. abdominalis* females resulting from parasitisation of 2nd instar of *M. dirhodum*, *R. padi* and *S. avenae* by one isoline of *A. abdominalis* but less female-biased sex ratios (33–80 %) after parasitisation by other isolines.

In conclusion, this preliminary research has provided information that may improve the utilisation of *A. abdominalis* for biocontrol of lettuce aphids. Our study suggests that young and intermediate host stages of the lettuce aphids would be the optimal target for *A. abdominalis* in field or greenhouse releases. The strongly female-biased sex ratio and the high adult emergence of *A. abdominalis*, irrespective of the mummified host stage, hold promises for a potential use in inoculative biocontrol programmes aimed at temporary establishment and reproduction in lettuce cropping systems. However, further investigation on the influence of plant architecture on the host-finding ability of *A. abdominalis* in whole lettuce plants is essential for evaluation of the possibilities for the parasitoid to access *N. ribisnigri* when feeding on lettuce heart leaves.

References

Abbott W (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Åsman K (2007) Aphid infestation in field grown lettuce and biological control with entomopathogenic fungi (Deuteromycotina: Hyphomycetes). Biol Agric Hortic 25:153–173

Barber MD, Moores GD, Tatchell GM, Vice WE, Denholm I (1999) Insecticide resistance in the currant-lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae) in the UK. Bull Entomol Res 89:17–23

Beckage NE, Gelman DB (2004) Wasp parasitoid disruption of host development: implications for new biologically based strategies for insect control. Ann Rev Entomol 49:299–330

Blackman RL, Eastop VF (1984) Aphids on the world's crops: an identification and information guide. Wiley, England

Blackman RL, Eastop VF (2000) Aphids on the world's crops: an identification and information guide. Wiley, England

Cate R, Archer T, Eikenbary R, Starks K, Morrison R (1977) Parasitization of the greenbug *Aphelinus asychis* and the effect of feeding by the parasitoid on aphid mortality. Environ Entomol 2:549–554

Chau A, Mackauer M (2000) Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): a preference for small pea aphids. Eur J Entomol 97:347–353

Colinet H, Salin C, Boivin G, Hance T (2005) Host age and fitness-related traits in a koinobiont aphid parasitoid. Ecol Entomol 30:473–479

Diaz BM, Fereres A (2005) Life table and population parameters of *Nasonovia ribisnigri* (Homoptera: Aphididae) at different constant temperatures. Environ Entomol 34:527–534

Fournier V, Brodeur J (2000) Dose-response susceptibility of pest aphids (Homoptera: Aphididae) and their control on hydroponically grown lettuce with the entomopathogenic fungus *Verticillium lecanii*, azadirachtin, and insecticidal soap. Environ Entomol 29:568–578

Gerling D, Roitberg B, Mackauer M (1990) Instar-specific defense of the pea aphid, *Acyrthosiphon pisum*: influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). J Insect Behav 3:501–514

Haardt H, Holler C (1992) Differences in life history traits between isofemale lines of the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). Bull Entomol Res 82:479–484

Hagvar E, Hofsvang T (1991) Aphid parasitoids (Hymenoptera, Aphidiidae): biology, host selection and use in biological control. Biocontrol News Inf 12:13–42

Harvey JA (2005) Factors affecting the evolution of development strategies in parasitoid wasps: the importance of functional constraints and incorporating complexity. Entomol Exp Appl 117:1–13

Harvey JA, Bezember TM, Elzinga JA, Strand MR (2004) Development of the solitary endoparasitoid Microplitis demolitor: host quality does not increase with host age and size. Ecol Entomol 29:35–43

He XZ, Wang Q, Teulon DA (2011) Host age preference behavior in *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). J Insect Behav 24:447–455

Henry LM, Gillespie DR, Roitberg BD (2005) Does mother really know best? Oviposition preference reduces reproductive performance in the generalist parasitoid *Aphidius ervi*. Entomol Exp Appl 116:167–174

Hofsvang T (1990) Advantages and disadvantages of parasitoids and predators of aphids when used in biological control. Acta Entomol Bohemoslov 87:401–413

Honek A, Jarosik V, Lapchin L, Rabasse J (1998) Host choice and offspring sex allocation in the aphid parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae). J Agric Entomol 15:209–221

Hopper JV, Nelson EH, Daane KM, Mills NJ (2011) Growth, development and consumption by four syrphid species associated with the lettuce aphid, *Nasonovia ribisnigri*, in California. Biol Control 58:271–276

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biom J 50:346–363

Jarošik V, Honek A, Rabasse J, Lapchin L (1996) Life-history characteristics of the aphid parasitoid *Aphelinus abdominalis* reared on *Macrosiphum euphorbiae*. Ochr Rostl 32:83–88

Mackenzie J, Vernon R (1988) Sampling for distribution of the lettuce aphid, *Nasonovia ribisnigri* (Homoptera: Aphididae), in fields and within heads. J Entomol Soc B C 85:10–14

Manly B (1974) A model for certain types of selection experiments. Biometrics 30:281–294

Mueller T, Blommers L, Mols P (1992) Woolly apple aphid (*Eriosoma lanigerum* Hausm., Hom., Aphidae) parasitism by *Aphelinus mali* Hal. (Hym., Aphelinidae) in relation to host stage and host colony size, shape and location. J Appl Entomol 114:143–154

Nebreda M, Michelena JM, Fereres A (2005) Seasonal abundance of aphid species on lettuce crops in Central Spain and identification of their main parasitoids. J Plant Dis Protect 112:405–415

Perdikis DC, Lykouressis DP, Garantonakis NG, Iatrou SA (2004) Instar preference and parasitization of *Aphis gossypii* and *Myzus persicae* (Hemiptera: Aphididae) by the parasitoid *Aphidius colemani* (Hymenoptera: Aphidiidae). Eur J Entomol 101:333–336

Rakhshani E, Talebi AA, Kavallieratos N, Fathipour Y (2004) Host stage preference, juvenile mortality and functional response of *Trioxys pallidus* (Hymenoptera: Braconidae, Aphidiinae). Biol Bratisl 59:197–204

R Development Core Team (2012). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available:http://www.R-project.org. Cited 14 July 2013

Rohne O (2002) Effect of temperature and host stage on performance of *Aphelinus varipes* Förster (Hym., Aphelinidae) parasitizing the cotton aphid, *Aphis gossypii* Glover (Hom., Aphididae). J Appl Entomol 126:572–576

Rufingier C, Schoen L, Martin C, Pasteur N (1997) Resistance of *Nasonovia ribisnigri* (Homoptera: Aphididae) to five insecticides. J Econ Entomol 90:1445–1449

Sengonca C, Schirmer S, Blaeser P (2008) Life table of the aphid parasitoid *Aphelinus asychis* (Walker) (Hymenoptera, Aphelinidae) parasitizing different age groups of *Aphis gossypii* Glover (Homoptera, Aphididae). J Plant Dis Protect 115:122–128

Sequeira R, Mackauer M (1992) Nutritional ecology of an insect host-parasitoid association: the pea aphid-*Aphidius ervi* system. Ecology 73:183–189

Shrestha G, Enkegaard A (2013) The green lacewing, *Chrysoperla carnea*: preference between lettuce aphids, *Nasonovia ribisnigri*, and western flower thrips, *Frankliniella occidentalis*. J Insect Sci 13(94):1–10

Shrestha G, Skovgård H, Enkegaard A (2014) Parasitization of commercially available parasitoid species against the lettuce aphid, *Nasonovia ribisnigri* (Hemiptera: Aphididae). Environ Entomol 43:1535–1541

Tatsumi E, Takada H (2005) Evaluation of *Aphelinus asychis* and *A. albipodus* (Hymenoptera: Aphelinidae) as biological control agents against three pest aphids. Appl Entomol Zool 40:379–385

van Steenis M (1995) Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse crops. Ph.D. Disertation, Wageningen Unversity, The Netherlands

Walker AM, Hoy MA (2003) Responses of *Lipolexis oregmae* (Hymenoptera: Aphidiidae) to different instars of *Toxoptera citricida* (Homoptera: Aphididae). J Econ Entomol 96:1685–1692

Wyckhuys K, Stone L, Desneux N, Hoelmer K, Hopper K, Heimpel G (2008) Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behaviour and parasitoid reproductive performance. Bull Entomol Res 98:361–370

Govinda Shrestha is a Ph.D. student at Aarhus University, Denmark. His research project investigates the possibilities for augmentative biological control of lettuce aphids.

Henrik Skovgård is a senior scientist at Department of Agroecology, Aarhus University. His main interest is biological control and integrated control of fly pests in livestock facilities.

Tove Steenberg is a senior scientist at Department of Agroecology, Aarhus University. Her primary research area is insect pathology and microbial pest control.

Annie Enkegaard is a senior scientist at Department of Agroecology, Aarhus University. Her main interest is biological and integrated control of crop pests.