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Life-history traits in closely related secondary parasitoids sharing the same primary parasitoid host: evolutionary opportunities and constraints

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Abstract

Thus far, few studies have compared life-history traits amongst secondary parasitoids attacking and developing in cocoons of their primary parasitoid hosts. This study examines development and reproduction in *Lysibia nana* Gravenhorst and *Acrolyta nens* Hartig (both Hymenoptera: Ichneumonidae), two related and morphologically similar secondary parasitoids that attack pupae of the gregarious endoparasitoid, *Cotesia glomerata* L. (Hymenoptera: Braconidae). On black mustard, *Brassica nigra* L. (Brassicaceae) plants in a field plot, adults of *L. nana* and *A. nens* frequently emerged from the same cocoon broods of *C. glomerata*. Based on similarities in their phylogeny and morphology, it was hypothesized that both species would exhibit considerable overlap in other life-history traits. In both *L. nana* and *A. nens*, adult wasp size increased with host cocoon mass at parasitism, although *L. nana* wasps were slightly larger than *A. nens* wasps, and completed their development earlier. Adult females of both species emerged with no eggs but matured eggs at similar rates over the following days. When provided with 20 host cocoons daily, fecundity in female *L. nana* was slightly more skewed towards early life than in *A. nens*, although lifetime fecundity did not differ between the two species. Longevity was significantly reduced in females of both species that were provided with hosts. Both parasitoids were found to exhibit strong similarities in life-history and development traits and in their ecological niche, thereby supporting our general hypothesis. Competition between *L. nana* and *A. nens* is presumably diffused because their preferred host (*C. glomerata*) is relatively abundant in open habitats.

Introduction

One of the major challenges faced by many organisms is in the allocation of resources towards competing fitness functions, such as reproduction and survival (van Noordwijk & de Jong, 1986; Stearns, 1992). Parasitoid wasps make excellent subjects for studying resource-related constraints on life-history and ontogenetic traits. Parasitoids develop on or in the bodies of other insects (the 'host'), whereas the adults are free-living (Godfray, 1994). Unlike insect predators, which may need to consume multiple prey in order to reach maturity, parasitoids depend on the finite resources contained in a single host, which is often not much larger than the adult parasitoid (solitary parasitoids) or which is much larger than the adult because it is shared among members of a brood (gregarious parasitoids). Moreover, in female parasitoids, resources carried over from larval feeding must be divided between maintenance and reproduction (Ellers, 1996; Ellers & van Alphen, 1997; Ellers et al., 2000). Consequently, parasitoids are under intense selection for the optimal exploitation and allocation of host-derived resources (Jervis et al., 2008).

Resource limitation in parasitoids has been overcome in either of two ways. First, adult parasitoids are able to allocate more resources to reproduction and less to maintenance by feeding on alternate sources of nutrients in the

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field, including floral nectar (Jervis et al., 1993). These sources, which are rich in sugars and carbohydrates, enable stored nutrients such as lipids to be diverted towards the production of eggs, rather than soma. Furthermore, parasitoids have evolved egg production strategies that generally closely reflect host availability. For instance, parasitoids attacking scarce host stages (such as pupae), or hosts that are highly dispersed often possess low fecundities but greatly extended life-spans (Jervis & Kidd, 1986; Pexton & Mayhew, 2002; Jervis et al., 2008). Moreover, these parasitoids are generally synovigenic, meaning that adult females emerge with only a small fraction of their potential complement of mature eggs (Ellers et al., 2000; Jervis et al., 2001, 2008). Some synovigenic parasitoids also invest more per capita resources into each egg (producing so-called 'anhydropic' eggs which are yolk-rich), and thus maximal egg loads are low in these species (Jervis & Kidd, 1986). By contrast, parasitoids attacking numerous host stages (such as eggs or young larvae) often possess a completely opposite set of reproductive traits (e.g., large numbers of small, hydropic yolk-poor eggs; see reviews by Jervis & Kidd, 1986; Jervis et al., 2008).

Although trade-offs in life-history characters, such as fecundity and longevity, are well-studied in individual parasitoid species (Kraaijeveld & Godfray, 1997) and among species that do not share the same host (Jervis et al., 2001, 2003; Jervis & Ferns, 2004), they are less well-studied in parasitoid complexes associated with the same host species and stage. Harvey (2008) found that two closely related secondary parasitoids, *Lysibia nana* Gravenhorst and *Gelis agilis* Fabricius (both Hymenoptera: Ichneumonidae), attacking cocoons of the same gregarious endoparasitoid species, exhibited striking differences in daily patterns of progeny production, lifetime reproductive success, and other morphological and behavioral traits. Importantly, significant differences in the degree of host specialization exhibited by the two parasitoids may have accounted for the observed variation in some traits. By contrast, some parasitoids with a common ancestry share a number of important morphological and physiological traits but differ in other respects. For example, Harvey & Strand (2002) compared host usage and development strategies in the solitary endoparasitoids *Venturia canescens* Gravenhorst and *Campoletis sonorensis* Cameron (both Hymenoptera: Ichneumonidae). Both parasitoids share a close phylogenetic affinity, occupying the same subfamily (Campopleginae) and tribe (Campoplegini). In spite of this, *V. canescens* exhibited a development strategy which favored increased size over reduced development time, whereas the reverse was true for *C. sonorensis*. The authors attributed this variation to strong differences in the biology and ecology of their hosts. A final scenario occurs where two or more parasitoid species demonstrate homology in various life-history traits because of a shared ancestry. This can be seen when closely related parasitoids attacking the same host species exhibit very little variation in their biology, morphology, and ecology (Price, 1970, 1972).

This study compares development and reproductive strategies in *L. nana* and a related species, *Acrolyta nens* Hartig (Hymenoptera: Ichneumonidae), that also attacks fully cocooned pupae of the same primary parasitoid host, *Cotesia glomerata* L. (Hymenoptera: Braconidae). Both *L. nana* and *A. nens* occupy the same subfamily (Cryptinae) as well as the same tribe (Hemitelini: Acrolytina). Their host, *C. glomerata*, is a gregarious primary endoparasitoid that parasitizes young larvae of cabbage white butterflies, including *Pieris brassicae* L. (Lepidoptera: Pieridae). Furthermore, it is a koinobiont whose hosts continue feeding and growing after parasitism, with host growth only arrested in the final instar. *Lysibia nana* and *A. nens* are obligate secondary parasitoids that only attack cocoons of closely related primary parasitoid hosts in the braconid subfamily Microgastrinae (Schwarz & Shaw, 2000).

The aims of the study are (a) to determine whether the two species of secondary parasitoids co-occur in the field, (b) to compare development in *L. nana* and *A. nens* and to determine whether both species exploit given amounts of host resources similarly, (c) to measure daily fecundity patterns and lifetime reproductive success of the two species when provided with excess numbers of hosts, and (d) to compare other reproductive traits, including the temporal dynamics of egg production in *L. nana* and *A. nens*. Finally, morphological and reproductive traits in these secondary parasitoids are compared and contrasted with those in *G. agilis*, which is related with *L. nana* and *A. nens* (Cryptinae, Hemitelini: Gelina). We argue that host range strongly influences the evolution and expression of various phenotypic traits amongst closely related secondary parasitoids.

Materials and methods

Insects

Hosts and parasitoids were maintained at 25 ± 2 °C under a L16:D8 h regime. Cultures of *C. glomerata* and *P. brassicae* were obtained from insects reared at Wageningen University (WUR), The Netherlands, and were originally collected from agricultural fields in the vicinity of the university (51°58′N, 5°38′E). All *P. brassicae* larvae used in these experiments had been maintained on *Brassica oleracea* L. var. Cyrus (Brussels sprouts; Brassicaceae) at WUR. In these experiments, *P. brassicae* larvae were maintained on *Brassica napus* L. plants that originated from seeds from plants growing along a road at Driel (51°57′N,

5°46′E), near the Institute of Ecology in Heteren, The Netherlands.

Cotesia glomerata were reared according to the protocol described in Harvey (2000). Adult female wasps oviposit 10–40 eggs into first (L1) to third (L3) instars of *P. brassicae*. During their development, parasitoid larvae feed primarily on host haemolymph and fat body, and emerge from the host caterpillar late during its final instar. After emergence, wasp larvae immediately spin cocoons on the host plant adjacent to the host, which perishes within a few days.

Lysibia nana, *A. nens*, and *G. agilis* (Figure 1A–C) were originally obtained from cocoons of *C. glomerata* recovered from leaves of *B. napus* growing in a garden plot adjacent to the Institute of Ecology. They were reared according to the protocol described in Harvey (2008). Like many ectoparasitic idiobionts, adult females of these secondary parasitoids perforate the host cocoon with their ovipositor and inject permanently paralyzing venom into the pre-pupa or pupa. Following envenomation, the wasps lay a single egg on the moribund host. After the parasitoid egg hatches, the larva perforates the host cuticle with its mandibles and imbibes haemolymph, but as it grows it begins attacking other tissues indiscriminately and eventually consumes the entire host, pupating within the cocoon of *C. glomerata*. Unlike female *L. nana* and *A. nens* wasps, *G. agilis* must first host-feed on *C. glomerata* pupae in order to mature eggs. Consequently, individual wasps of this species were provided with parasitoid cocoons over the course of a week in order to ensure that they could produce progeny. In culture, the secondary parasitoids were maintained exclusively on 1- to 2-day-old pupae of *C. glomerata*. After emergence, secondary parasitoids were kept in large (20 cm diameter) Petri dishes at 10 °C.

Rates of secondary parasitism of *Cotesia glomerata* cocoons in the field

In order to determine the abundance of *L. nana* and *A. nens* in the field, cocoons of *C. glomerata* were collected during the final week of June, 2005. A plot of *B. nigra* seedlings was sown in late April, 2005; the seedlings were planted 2 m apart in five rows of 20 (a total of 100 plants) near Wageningen, The Netherlands. Wild *P. brassicae* butterflies oviposited on these plants during the month of May, and many of their larvae were parasitized by *C. glomerata*. By mid-late June, broods of parasitoid cocoons appeared, and these were allowed to remain on the plant for 5 days before being collected and taken to the laboratory (n = 62 broods). The broods were kept individually in covered Petri dishes (12 cm diameter). Primary and secondary parasitoids that emerged from the cocoons were counted and identified to species.

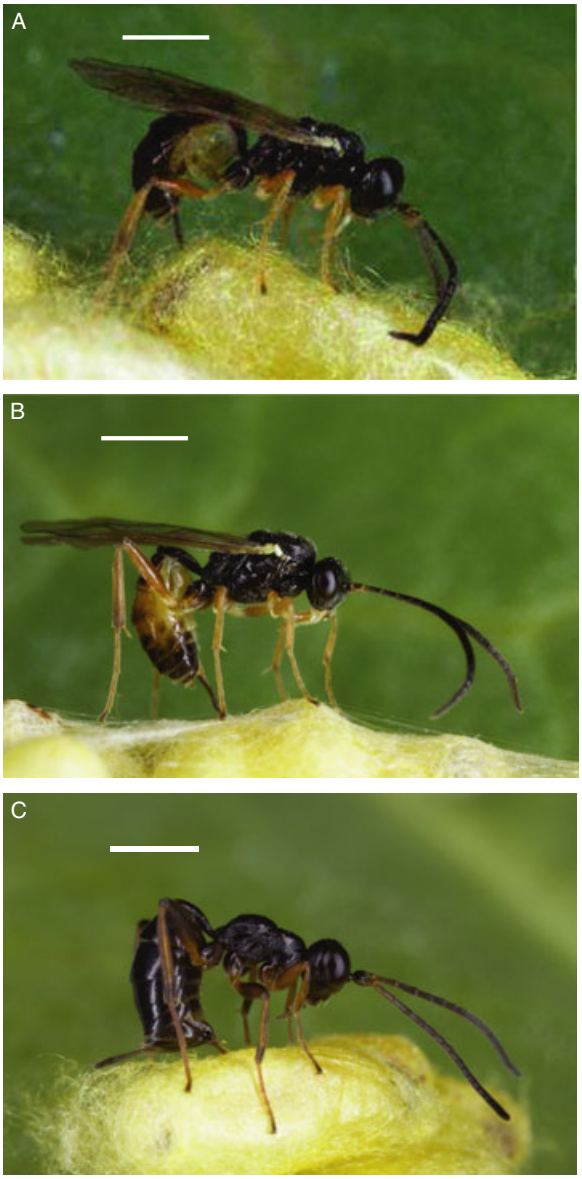

Figure 1 Adult female of (A) *Lysibia nana*, (B) *Acrolyta nens*, and (C) *Gelis agilis* ovipositing into cocoons of *Cotesia glomerata*. The white line bar represents 2 mm in length. Note the similarity in morphology of *L. nana* and *A. nens*, compared with *G. agilis*. The three species are closely related and are of approximately the same size, but also exhibit marked differences in reproductive characteristics. Photos courtesy of Tibor Bukovinszky and 'Bugs in the Picture'.

Relationship between host cocoon mass and adult secondary parasitoid mass

Larvae of *P. brassicae* were parasitized in the 1st instar (L1) by mated females of *C. glomerata* by presenting individual larvae to parasitoids at the end of a brush in plastic vials. Parasitism was verified by allowing wasps to sting hosts for

at least 10 s, which enables a full brood to be laid (Harvey, 2000). Parasitized caterpillars were immediately placed in large rearing cages (100 × 60 × 60 cm) containing four *B. napus* plants. These were refreshed every few days as required.

Between 12 and 24 h prior to parasitoid emergence, larvae of *P. brassicae* spin a silken mat on the surface of a leaf or on the inside of the rearing cage. These larvae were collected and placed individually into Petri dishes (10 cm diameter). Upon parasitoid egression, separate broods of *C. glomerata* cocoons were collected and separated carefully using a pair of forceps and a caecum. The cocoons of *C. glomerata* from the various broods were then mixed together and placed in a large Petri dish (20 cm diameter). Individual cocoons were taken from the Petri dish and weighed on a Mettler-Toledo MT5 Electrobalance (Mettler-Toledo, Greifensee, Switzerland) with an accuracy of ±1 μg. They were then presented to mated females of either *L. nana* or *A. nens* in small plastic vials (2 cm diameter × 5 cm long). Parasitism was visually observed and verified on the basis of insertion and removal of the ovipositor by the female secondary parasitoid. This process typically takes ca. 5 min in both *L. nana* and *A. nens*. Once a *C. glomerata* cocoon had been parasitized, the date and time (as well as the cocoon mass) was written onto an adhesive label that was applied to the vial. The cocoons were then monitored until either (1) primary parasitoid eclosion, (2) secondary parasitoid eclosion, or (3) neither species emerged, recorded as 'host death'. Upon eclosion, newly emerged adults of *L. nana* and *A. nens* were anesthetized using CO_2 and weighed on the Mettler microbalance. We used fresh mass to measure body size, because in both *L. nana* and *A. nens* there is a highly significant correlation between fresh and dry mass (Harvey et al., 2006). Offspring sex was also determined. Oviposition-to-adult development time was recorded in hours.

Measurement of reproductive and life-history parameters in *Lysibia nana* and *Acrolyta nens*

Newly emerged wasps were weighed on the microbalance, placed individually in Petri dishes (12 cm diameter) and provided ad libitum with honey. A small ball of cotton wool soaked in water was also added, to ensure that the honey remained partly in solution (and accessible to the wasps, which cannot imbibe dry honey with their mouthparts). For both control and treatment wasps, honey and water were refreshed on a daily basis, and, upon death, adult longevity (in days) was recorded. To measure temporal variation in egg production in both secondary parasitoids, different age-cohorts of *L. nana* and *A. nens* were maintained as described above. Beginning on the day of eclosion, 10 female wasps were dissected in a drop of water on a glass slide using two pairs of forceps and a caecum, and the mature (= ovulated) eggs in their ovaries were counted. This process was repeated with 10 wasps each on days 2, 4, 6, 8, and 10 post-eclosion.

Newly emerged adult secondary parasitoids were weighed and thereafter maintained in Petri dishes (as described above) with honey and water. Beginning 3 days after eclosion, cocoons of *C. glomerata* that were <24 h old (containing young pupae) were presented daily to individual female secondary parasitoids of both species. It was important to evaluate how many cocoons of *C. glomerata* would need to be provided to both *L. nana* and *A. nens* in order to ensure that cocoon number was not a limiting factor in daily oviposition and reproductive success. This was estimated on the basis of the ovary dissections (above) and the results of a previous study (Harvey, 2008). Based on these criteria, for the lifetime reproductive success experiments, *L. nana* and *A. nens* were provided with 20 fresh *C. glomerata* cocoons daily. At the end of each 24 h period, cocoons were removed and placed in labeled plastic vials. Data on the fate of all of the cocoons was determined (producing an adult secondary parasitoid, primary parasitoid, or precocious death). Cocoons that failed to produce wasps were dissected and those containing pharate (fully developed) adults of either species were identified (where possible). If the pharate adult was one of the secondary parasitoids, this was added to the fecundity data.

Longevity of *L. nana* and *A. nens* was also measured by recording the number of days between adult eclosion and death. The longevity of approximately 20 male and 20 female secondary parasitoids of both species that had no host access was also measured. These wasps were supplied ad libitum with water and honey.

Statistical analysis

The relationship between cocoon weight and adult male or female parasitoid body mass was determined using linear regression. Slopes were compared using a t-test. When slopes were not significantly different, Analysis of Covariance (ANCOVA) was used to test whether the relationship differed significantly between the two species. When slopes differed significantly, ANCOVA for separate slopes was used. Realized daily fecundity patterns of *L. nana* and *A. nens* were compared using repeated measures analysis. This analysis tests for differences between the two species independent of time, for time effects, and whether the fecundity patterns of the two species differ over time. To avoid missing values due to differences in date of death, data for the first 23 days were analyzed. Maximum egg loads and lifetime reproductive success in both secondary parasitoids were compared using t-tests. Daily egg loads were compared using repeated measures analysis. The

relationship between adult male and female mass and longevity were made using linear regression. Data for mean adult body mass and development time, and longevity in wasps with and without hosts were first tested for homogeneity of variance. Data from body mass were normally distributed and were analyzed using 2-way ANOVA with offspring sex and parasitoid species as factors. However, analyses for development time and longevity failed the homogeneity of variance tests and were therefore analyzed using non-parametric procedures and Kruskal–Wallis tests.

Results

Rates of parasitism of *Cotesia glomerata* cocoons in the field

Out of the 62 broods of *C. glomerata* that were recovered, only two (or 3.2%) were not parasitized and thus produced only adult *C. glomerata* wasps. The remaining 60 broods produced variable numbers of *L. nana*, *A. nens*, or both species. Out of a total of 2 741 collected cocoons, 671 produced adult *C. glomerata*, 515 produced adult *L. nana*, 960 produced adult *A. nens*, and the remainder (595) failed to produce any parasitoids. The corresponding percentages are shown in Figure 2A. Moreover, a higher proportion of broods of *C. glomerata* cocoons was parasitized by both species than by *A. nens* or *L. nana* alone (Figure 2B). No *G. agilis* emerged from these cocoon broods. However, in a separate study (JA Harvey, unpubl.), *G. agilis* and *A. nens* emerged from the same clusters of *C. glomerata* cocoons attached to leaves of *B. nigra* plants that had been placed in an open field near to the Institute of Ecology.

Development of *Lysibia nana* and *Acrolyta nens* in cocoons of *Cotesia glomerata*

The fate of *C. glomerata* cocoons which had been parasitized by either *L. nana* or *A. nens* did not differ significantly (χ^2 = 1.96, d.f. = 1, P = 0.16). In both species, more than 80% of cocoons in which a female wasp inserted and removed her ovipositor produced an adult parasitoid. There was a highly significant positive relationship between host cocoon mass and adult parastoid mass for males ($F_{1,307}$ = 905.4, P<0.0001) and females ($F_{1,72}$ = 385.4, P<0.0001), and the slope of the relationship for males was significantly higher than for females (t = 2.638, d.f. = 379, P = 0.0043). Regression slopes for *L. nana* and *A. nens* did not differ significantly in male wasps (t = 0.056, d.f. = 305, P = 0.96), but for females the slope for *L. nana* was significantly steeper than for *A. nens* (t = 2.47, d.f. = 71, P = 0.02). For a given host cocoon mass at parasitism, body mass in both *L. nana* males (ANCOVA: equal slopes, $F_{1,306}$ = 50.98, P<0.0001) and females (ANCOVA: separate slopes, $F_{1,70}$ = 4.16, P = 0.045) was significantly greater than *A. nens* body mass (Figure 3A, B). Mean

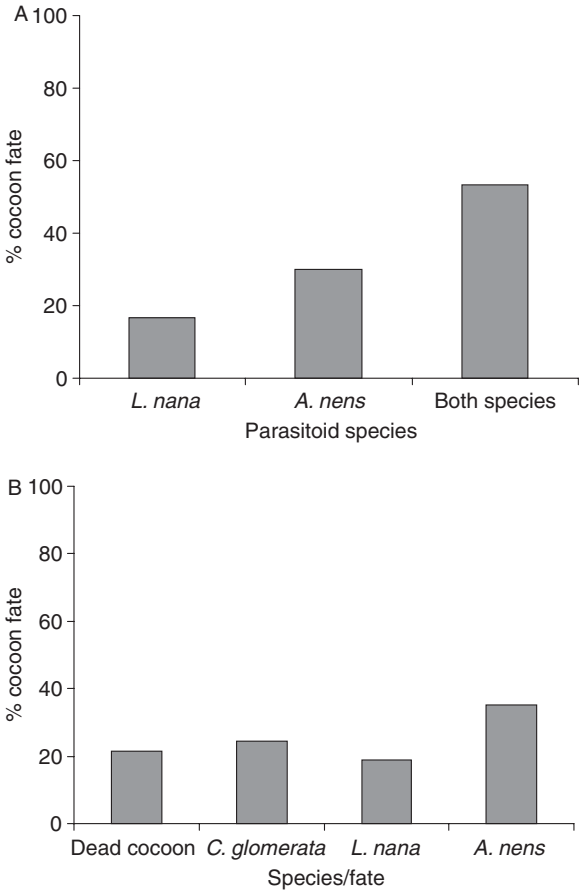

Figure 2 Interactions between cocoon clusters of *Cotesia glomerata*, *Lysibia nana*, and *Acrolyta nens* in a field plot of *Brassica nigra* plants. (A) Percentage of individual cocoon clusters of *C. glomerata* on *B. nigra* plants in a field plot that produced adult *L. nana* only, adult *A. nens* only, or in which both secondary parasitoid species emerged. Total number of cocoon clusters = 62. (B) Percentage fate of all individual cocoons of *C. glomerata* from all 62 broods collected from the field plot. Total number of cocoons = 2 741.

emerging adult body mass of *L. nana* and *A. nens* did not differ significantly between the two species ($F_{1,379}$ = 0.66, P = 0.42), but did so with offspring sex ($F_{1,379}$ = 4.11, P = 0.04), and the interaction between these parameters was also not significant ($F_{1,379}$ = 0.08, P = 0.78). Female wasps were larger than male wasps (Figure 3C). Egg-to-adult development time did not vary with species or sex (Kruskal–Wallis test: χ^2 = 7.13, d.f. = 3, P = 0.07). Both parasitoids typically took 380–400 h to complete their development (Figure 3D).

Measurement of reproductive and life-history parameters in *Lysibia nana* and *Acrolyta nens*

Maximum egg load of *L. nana* and *A. nens* was reached after 6 days and was on average 36. Maximum egg load

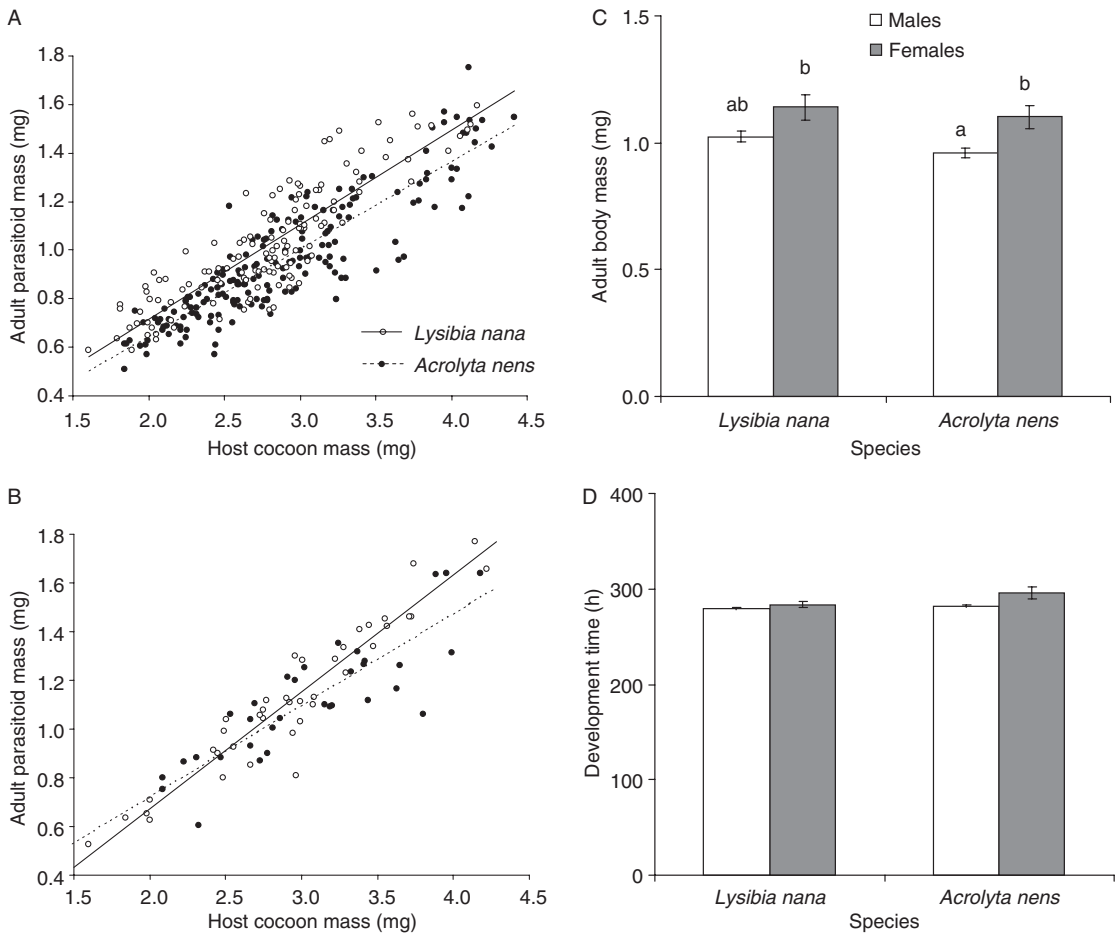

Figure 3 Developmental parameters in *Lysibia nana* and *Acrolyta nens*. Relationship between host cocoon mass of *Cotesia glomerata* and (A) adult male, and (B) female secondary parasitoid mass in *Lysibia nana* and *Acrolyta nens*. Regression equations: *L. nana* males: $y = 0.39x - 0.07$, $F_{1,126} = 398.09$, $P<0.0001$; *A. nens* males: $y = 0.36x - 0.08$, $F_{1,179} = 693.52$, $P<0.0001$; *L. nana* females: $y = 0.48x - 0.29$, $F_{1,38} = 44.79$, $P<0.0001$; *A. nens* females: $y = 0.38x - 0.03$, $F_{1,32} = 110.78$, $P<0.0001$. Overall mean (± SEM) emerging (C) adult body mass and (D) egg-to-adult development time in male and female *L. nana* and *A. nens*. Bars with the same letters are not significantly different (Tukey–Kramer tests: $P>0.05$). Sample size: *L. nana* males n = 128, females n = 39; *A. nens* males n = 182, females n = 36.

($t = 1.22$, d.f. = 18, $P = 0.23$) and egg maturation pattern (species: $F_{1,18} = 0.012$, $P = 0.91$; species*time: $F_{4,72} = 0.87$, $P = 0.49$) did not differ significantly between the two species (Figure 4A). Lifetime reproductive success also did not differ significantly between *L. nana* and *A. nens* females ($t = 1.73$, d.f. = 34, $P = 0.095$). During their lifetimes, female *L. nana* produced an average of 178.3 (± 6.3) progeny, whereas *A. nens* females produced an average of 158.6 (± 9.5) progeny. Fecundity of *L. nana* females declined faster over time than *A. nens* females resulting in a significant interaction between species and time ($F_{20,660} = 3.55$, $P<0.0001$; Figure 4B).

The number of progeny produced in both *L. nana* ($F_{1,16}<0.01$, $P = 0.97$) and *A. nens* ($F_{1,17} = 0.62$, $P = 0.44$) was not significantly correlated with female body mass. Moreover, longevity in female wasps of both species was not found to be significantly correlated with adult body mass, irrespective of whether the wasps had access to hosts (*L. nana*: $F_{1,16} = 0.33$, $P = 0.57$; *A. nens*: $F_{1,17} = 0.51$, $P = 0.49$) or not (*L. nana*: $F_{1,18} = 0.06$, $P = 0.80$; *A. nens*: $F_{1,16} = 0.16$, $P = 0.69$).

Longevity in female secondary parasitoids varied significantly with treatment and species (Kruskal–Wallis test: $\chi^2 = 53.02$, d.f. = 3, $P<0.0001$). The longevity of wasps with access to hosts was about 50% shorter than of wasps without hosts. Furthermore, longevity in *A. nens* wasps was generally higher than that of *L. nana* wasps (Figure 5).

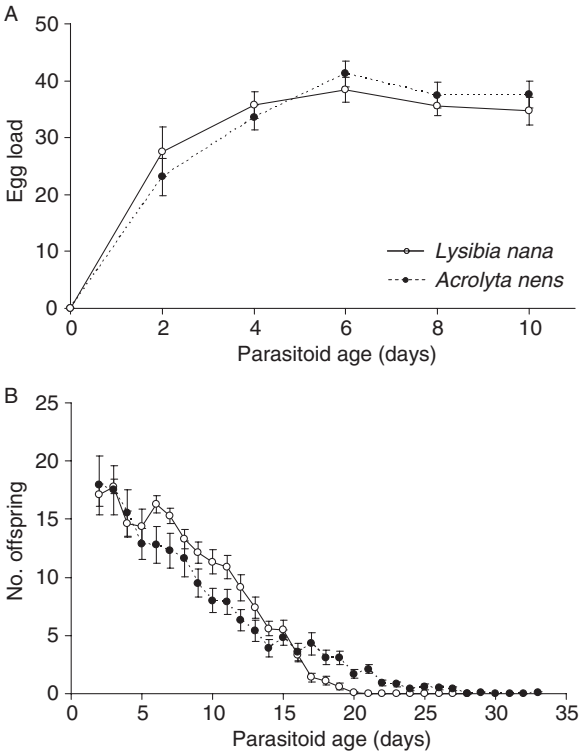

Figure 4 Reproductive parameters in *Lysibia nana* and *Acrolyta nens*. (A) Egg loads in female *L. nana* and *A. nens* wasps at different days post-eclosion. Sample size: n = 10 for each species and day. (B) Daily fecundity schedules in *L. nana* and *A. nens*. Line bars represent SEM. Sample size: n = 17 for *L. nana* and n = 19 for *A. nens*.

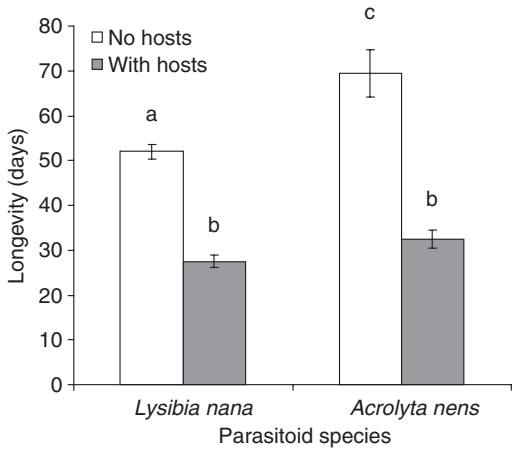

Figure 5 Mean (± SEM) longevity in *Lysibia nana* and *Acrolyta nens* females with hosts (gray bars) and deprived of hosts (open bars). Bars with the same letter are not significantly different (Tukey–Kramer tests: P>0.05). Sample sizes as in Figure 3B.

In *L. nana* males, there was a significant positive relationship between body mass and longevity ($F_{1,17}$ = 6.30, P = 0.02) and this was not the case in *A. nens* males ($F_{1,16}$ = 2.09, P = 0.10). Females of *L. nana* and *A. nens* that were deprived of hosts lived significantly longer than conspecific males (*L. nana*: t = 9.17, d.f. = 37, P<0.001; *A. nens*: t = 6.71, d.f. = 35, P<0.001). For both species females lived on average more than twice as long as males (data not shown).

Discussion

The two secondary parasitoid species studied here are closely related, occupying the same tribe and sub-tribe (Hemitelini, Acolytina), and exhibit a striking degree of similarity in body size and general appearance (Figure 1). However, *L. nana* is a slightly more 'robust' species, possessing shorter wings and a larger head capsule, relative to the overall body size, than the more 'gracile' *A. nens*. In both species, adult body mass was strongly positively correlated with host mass at parasitism. However, emerging *L. nana* adults were slightly larger than *A. nens* adults, revealing that larvae of the former species were able to exploit a given amount of host resources somewhat more effectively than larvae of *A. nens*. In general, pupal parasitoids, and particularly secondary parasitoids, are extremely efficient at utilizing and allocating host resources (Harvey et al., 2006, 2009).

Lifetime reproductive success in *L. nana* and *A. nens* was also quite similar, as were the dynamics of egg production in the ovaries over the first 10 days of life in wasps that were deprived of hosts. Moreover, fecundity curves in the two species were also similar in shape, but reproduction in *L. nana* was slightly more skewed towards early in life than in *A. nens*. Both wasps are completely synovigenic and thus adult females emerge with no eggs. However, egg production in *L. nana* and *A. nens* commenced soon after eclosion and female wasps attained maximal egg loads between 4 and 6 days later. In both species, oögenesis was arrested when egg loads had reached about 40, or approximately 20% of potential fecundity. Thereafter, it was assumed that metabolic resources were redirected for maintenance until hosts were located. The costs of egg storage are poorly understood in most insects, but the fact that many synovigenic parasitoids never carry more than a relatively small proportion of their potential egg complements suggests that it may be quite high (Jervis et al., 2001).

Strong synovigeny and low egg loads may also be linked with the amount of per capita resources invested per egg (Jervis et al., 2001, 2008). Like most other idiobionts, both *L. nana* and *A. nens* are ichneumonids that produce yolky 'anhydropic' eggs (Jervis & Kidd, 1986). By contrast, many

other ichneumonid clades are dominated by koinobionts that are also synovigenic but which produce large numbers of small, hydropic eggs (Jervis et al., 2001). For example, parasitoids in the ichneumonid subfamily Campopleginae attack larval lepidopteran hosts that may be extremely abundant, accounting for high egg production that has been found in some solitary parasitoids in this clade (Harvey et al., 2001; Harvey & Strand, 2002; Winkler et al., 2006). Ultimately, this reveals that there is immense within-family variation in the reproductive biology of parasitoids, with differences in the ecology and biology of their hosts probably playing a significant role in selecting for variation in these traits.

Lysibia nana and *A. nens* are both fairly long-lived species, and some females of *A. nens* fed on honey (but without host access) lived over 100 days. However, in both species the longevity of female wasps was reduced by more than 50% in females that had host access, compared with controls. This reveals that there are significant trade-offs between reproduction and survival in both species, presumably due to limits in their ability to acquire sufficient resources for vital metabolic functions (Reznick, 1985). The existence of trade-offs between survival and reproduction in parasitoids appears to be association-specific, with some studies reporting them (Orr & Boethel, 1990; Ellers, 1996; Bezemer et al., 2005) and others not (Bai & Smith, 1993; Harvey et al., 2001). The degree to which trade-offs between these parameters are expressed may critically depend on the amount of metabolic resources allocated for egg production vs. maintenance, and from the acquisition of exogenous resources that reduce the metabolic uptake of stored lipids (Ellers, 1996). This may, in turn, depend on such factors as adult female body size (Ellers & Jervis, 2003), or on the amount of resources invested per egg (Jervis et al., 2008). Because neither species host-feeds, the resources necessary for egg production are obtained entirely from the host during larval development. This places a significant strain on the premium use of internally stored resources, and probably accounts for the large trade-off between reproduction and survival in both species.

Maximum egg loads in both *L. nana* and *A. nens* closely reflect average single brood sizes of their host, *C. glomerata* in the field (Tagawa, 2000). Both species have short host-handling times (typically <1 min per host) and during an oviposition bout individual females often move from one host cocoon to another in rapid succession, eventually parasitizing most of the brood in 2 h or less. This suggests that *L. nana* and *A. nens* have evolved as specialist secondary parasitoids of gregarious hosts like *C. glomerata* with brood sizes typically around 30–40 eggs. Furthermore, these results contrast sharply with egg-maturation and oviposition patterns in a closely related species, *G. agilis*, which also attacks cocoons of *C. glomerata* in the field. Females of *G. agilis* produce much larger eggs than either *L. nana* or *A. nens*, have much longer host-handling times (usually >15 min per host), and never produce more than 3–4 progeny per day (Harvey, 2008; Table 1). Moreover, unlike the two secondary parasitoids studied here, *G. agilis* is 'anautogenous' and must host-feed to obtain proteins for oögenesis, with eggs taking up to several days to be matured after a host-feeding bout (Harvey, 2008; Table 1). Like most other species in the genus, *G. agilis* is wingless, meaning that its spatial area of search is likely to be much smaller than that of *L. nana* and *A. nens*, which are both efficient in flight (Figure 1). It is known that some *Gelis* species are effectively opportunist parasitoids, attacking a wide range of hosts in the field (Bezant, 1956; van Baarlen et al., 1996; Cobb & Cobb, 2004). Extreme generalism in dietary breadth may be an essential prerequisite for survival in wingless species that occupy such a small spatial area of their habitat during their lifetime.

Although rarely studied over its native range in Eurasia, cocoons of *C. glomerata* are attacked by five or more species of secondary parasitoids (that attack the primary parasitoid host after it has emerged from the herbivore host) and at least two species of primary hyperparasitoids (that attack the primary parasitoid while it is still developing within the herbivore host; Poelman, 2008). Under certain conditions, such as in structurally simple habitats, competition between secondary parasitoids for access to parasitoid cocoons as oviposition sites may therefore be quite intense. In *B. nigra* plants grown in rows with interstitial vegetation removed, 53% of cocoon broods of *C. glomerata*

Table 1 Comparison of reproductive and biological characteristics in *Lysibia nana*, *Acrolyta nens*, and *Gelis agilis*

	Species		
Trait	*Lysibia nana*	*Acrolyta nens*	*Gelis agilis*
Mature eggs at eclosion	0	0	0
Mean maximum egg load	38	41	4
Mean max. realized fecundity	178	158	40
Egg length (mm)	0.4–0.5	0.4–0.5	0.6–0.8
Resorb eggs?	No	No	Yes
Host-feed?	No	No	Yes
Oviposition duration (min)	3–5	3–5	>15
Mean max. longevity (days)	53	71	64
Host range	Small	Small	Large
Wings	Yes	Yes	No
Reproduction	Sexual	Sexual	Asexual

Data for *G. agilis* from Harvey (2008).

yielded adults of both *L. nana* and *A. nens*, although the latter species was more abundant. In pilot studies carried out in 2003–2005, *L. nana* and *A. nens* were the dominant secondary parasitoids of *C. glomerata* cocoons and both species emerged from host cocoons attached to leaves of *B. nigra* plants that had been placed in multiple sites. By contrast, *G. agilis* was only recovered from *C. glomerata* cocoons in a single field site. This reveals that *G. agilis* probably uses alternate hosts that are present within its habitat, and that they are opportunists, exploiting *C. glomerata* cocoons when they are available.

In summary, our study has reported that amongst two morphologically similar and closely related secondary parasitoid species sharing the same host species, differences in development and reproductive characteristics were only very subtle. Both species share a common phylogeny and have clearly evolved under a similar set of evolutionary constraints and opportunities. By contrast, reproductive and morphological traits in *L. nana* and *A. nens* differ sharply with those in the related species *G. agilis*, which also occasionally attacks *C. glomerata* cocoons in the field and readily attacks this host in the laboratory. It has also been recently shown that *G. agilis* is a 'tertiary parasitoid' that can successfully develop on pupae of *L. nana* and *A. nens* in *C. glomerata* cocoons, whereas the reverse does not happen (Harvey et al., 2009; F Pashalidou, pers. comm.). This reveals that *L. nana* is a highly specialized secondary parasitoid whereas *G. agilis* is not, and further, that vertical food chains involving plants and consumers may go to five trophic levels and perhaps even more (Harvey et al., 2009). Future studies conducted in the field will examine interactions amongst several secondary parasitoids of *C. glomerata* in order to better understand the importance of various factors (e.g., habitat patch size, characteristics of the surrounding vegetation, competition) that influence the structure of the primary, secondary, and tertiary parasitoid communities associated with this host.

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