

Early nest emergence of females parasitised by Strepsiptera in protandrous bees (Hymenoptera Andrenidae)

J. STRAKA^{1,4}, K. REZKOVA¹, J. BATELKA² and L. KRATOCHVÍL³

¹Faculty of Science, Charles University in Prague, Department of Zoology, Viničná 7, 128 44 Praha 2, Czech Republic

²Nad Vodovodem 16, 100 00, Praha 10, Czech Republic

³Faculty of Science, Charles University in Prague, Department of Ecology, Viničná 7, 128 44 Praha 2, Czech Republic

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Styloposed (= parasitised by Strepsiptera Stylopidae) imagoes of *Andrena* (Hymenoptera Andrenidae) bees are known to exhibit intersexual morphology. Until now, their abnormal morphology has been thought to result from undernourishment of parasitised larvae during development. This hypothesis, however, does not fit to mass provisioning Hymenoptera. We hypothesised that induced changes in the suite of morphological characters might be a consequence of manipulation of sex-specific behavioural traits by a strepsipteran parasite. Thus, the masculinised morphology of styloposed females might be connected with shifts in their sexual behaviour. Here, we tested the effect of *Stylops* (Strepsiptera Stylopidae) infection on the timing of spring nest emergence in *Andrena* bees, where males generally emerged conspicuously earlier than conspecific females. We used two independent data samplings – pan trapping and direct observation – to avoid possible bias caused by one of the methods. In accordance with our hypothesis, we documented that the time of emergence/activity in styloposed females follows the temporal trend of uninfected, protandrous males. We ascribe this observation to host manipulation and briefly discuss the potential adaptive value of the altered host behaviour for the parasite. We discuss our results across three species: *Andrena strohmei*, *A. minutula* and *A. vaga*.

KEY WORDS: parasite, manipulation, protandry, sexual dimorphism, *Andrena*, *Stylops*.

INTRODUCTION

Parasites often possess conspicuous simplification of morphology and general body plan; however, their life cycles are frequently amazingly complex. Many parasites

⁴ Corresponding author: Jakub Straka, Faculty of Science, Charles University in Prague, Department of Zoology, Viničná 7, 128 44 Praha 2, Czech Republic (E-mail: straka.jakub.1@gmail.com).

provoke phenotypic changes in their hosts which substantially increase parasite fitness, mainly by enhancing its fecundity or chance of transmission to another host, but which at the same time harm the host severely (see ZIMMER 2000 for a popularised review). In some cases, the host manipulation can include host castration and changes in sex-specific morphology and behaviour. A well-known example of such a parasite is the morphologically highly simplified rhizocephalan barnacle (*Lernaeodiscus porcellanae* Müller 1862 (Cirripedia Rhizocephala)), a parasitic castrator. Once their host, the hermit crab (*Petrolisthes cabrilloi* Glassell 1945), is parasitised, the parasites fully exploit the host's parental care behaviour to their own reproduction. Male crabs develop several female characteristics, and females become even more feminised. Both crab sexes pursue the female-typical reproductive behaviour, i.e. they ventilate and clean the external parasite's reproductive sacs instead of their own eggs, a behaviour which helps to maintain and spread the parasites' propagules (RITCHIE & HØEG 1981). Due to the obviously favourable selective advantage garnered from exploitation of the host reproductive organs and its behaviour, castration is a common parasitic strategy (WHEELER 1910; BAUDOIN 1975). Interestingly, POTTS (1906) mentioned morphological changes induced by *Stylops* (Strepsiptera) in parasitised *Andrena* bees as an analogy to changes in sexual characters induced by rhizocephalan barnacles in crabs. Surprisingly, even more than a century later and in contrast to the now minutely examined crab–rhizocephalan barnacle interaction, the relationship of the strepsipteran parasite to its host remains largely unexplored.

Members of the order Strepsiptera are among the most specialised parasitic castrator insects. They are endoparasitic for almost their entire life cycle. Strepsipteran larvae, a free-living stage, attack eggs or larval stages of insect hosts and later induce sterility in host adults. With the exception of the subfamily Mengerillidae, the neotenic females never leave their hosts (KATHIRITHAMBY 1989). Winged males fly away from their host only after the completion of their development, and subsequently inseminate females through an opening in her cephalothorax extending from the host's body (BEANI et al. 2005). The free adult males are notorious for their extremely short life span (often less than 5 hr; HUGHES et al. 2004). Strepsipteran females are viviparous and may produce large number of larvae (triungulinids) (BATRA 1965; KATHIRITHAMBY 1989). In species parasitising bees, larvae are deposited on flowers, where they lurk for phoretic transport by host females to their nests (LINSLEY & MACSWAIN 1957; BATRA 1965), although a direct release of triungulinids on the host nest has been described in wasps (HUGHES et al. 2003; BEANI & MASSOLO 2007). Strepsipteran 'symbiosis' with hosts is thus closely linked to the parasites' reproductive success, and thus there is strong potential for host manipulation to be present.

HUGHES et al. (2004) recently ascribed dramatic behavioural changes in paper wasps (*Polistes*) parasitised by the strepsipteran genus *Xenos* to adaptive manipulation of host behaviour by the parasite. They observed that parasitised worker wasps do not work, desert the colony, and aggregate outside the nest (see also TATSUTA & MAKINO 2003 for similar observations in *Vespa*), which may increase the wasps' prospects of surviving during the winter, and consequently promote the completion of the parasite's life cycle.

It has been known for several decades that parasitisation by strepsipterans causes abnormal morphological development in some hosts, e.g. members of the orders Hymenoptera (families Andrenidae, Sphecidae and Vespidae) (SALT 1927, 1931) and Hemiptera (family Delphacidae) (LINDBERG 1939). In Hymenoptera, several secondary sexual characters are masculinised in females, but feminised in males, i.e. parasitised

individuals exhibit intersexual morphology (PÉREZ 1886; PIERCE 1909; SMITH & HAMM 1914; SALT 1927, 1931). In contrast, in Hemiptera, there is no acquisition of sex characters from the opposite sex, but rather a reduction or loss of sex characters, particularly in the stylopedised (= parasitised by the strepsipteran parasite) male, that superficially resemble a normal unstylopedised female (LINDBERG 1939). Females of hymenopteran hosts are usually more frequently parasitised and exhibit stronger modification by strepsipterans than males (SALT 1931; ASKEW 1971). In the solitary bees of the genus *Andrena*, the female-typical pollen-collecting apparatus is reduced, plumose setae of hind legs are shorter and sparser and thus look male-like, pygidium is flattened and narrower, and even the yellow facial coloration of males is attained in stylopedised females (PÉREZ 1886; BRANDENBURG 1953). SALT (1927) speculated that shifts to intersexual morphotypes result from undernourishment of infected host larvae during their development. He supported this interpretation with the observation that intersexuality occurs in mass provisioning hymenopterans, but it is not known in progressive provisioners, which provide additional food to their larvae *ad libitum* and where nutritional deficiency would thus be less likely to arise. In contradiction to this notion, however, intersexuality does not occur in bees of the family Halictidae, which are typical mass provisioners (BATRA 1965; STRAKA et al. 2006). It is possible that the cost of the strepsipteran parasite may be sustainable for the host (see HUGHES & KATHIRITHAMBY 2005). The occurrence of intersexuality in some but not all hymenopteran hosts also calls into question the possibility that intersexuality is simply induced by depletion of the host gonads through the obligatory castration by the parasites. These indications imply that morphological intersexuality could be a part of strepsipteran complex host manipulation. In our opinion, intersexuality induced by strepsipterans in certain hosts could be connected with changes in host sex-specific behaviour.

One of the conspicuous sex-specific behaviours in many solitary bees is the timing of nest emergence. This is the earlier nest emergence of males in comparison to conspecific females (protandry). For instance, active males can be typically found 4–14 days before the emergence of the first females in almost all central European species of the spring bee genus *Andrena* (WESTRICH 1989). Protandry in this genus seems to be adaptive, as it allows males to maximise their mating opportunities. As far as is known, females of these bees mate only once and thus to find and mate a virgin female is obviously highly advantageous (ALCOCK et al. 1978; EICKWORT & GINSBERG 1980). During mating, the male perfumes the female with an anti-aphrodisiac substance, causing a loss of her receptivity, resulting in rejection of all subsequent mates (KUKUK 1985).

At the extreme, we speculate that as strepsipteran parasites provoke morphological intersexuality in stylopedised *Andrena* imagoes, they could also alter normally sex-specific timing of the imagoes' nest emergence, as data in LINSLEY & MACSWAIN (1957) suggest. We tested this hypothesis by comparing the timing of spring nest emergence between uninfected bees and stylopedised individuals using pan-trapping. Although stylopedised *Andrena* bees also occur during the late spring or even summer, their findings at these times of year are scarce and spread over several months (KINZELBACH 1978; BATELKA & STRAKA 2005). Therefore, we focused our attention on early spring, when many species of *Andrena* occur 'en masse' and when high incidences of stylopedised bees are usually reported (first summarised by PIERCE 1909: 23–24, 30; LINSLEY & MACSWAIN 1957: 420–421). In addition, to test whether the difference in phenology between parasitised and uninfected *Andrena* females is an artefact of the pan-trapping method, we conducted direct observations of female nest behaviour in a bee species (*Andrena vaga*).

MATERIALS AND METHODS

Field data collection on spring emergence by pan-trapping method

The bees were sampled at two SSW exposed sites within an area of several hundred square meters situated near the Chýnice village, Czech Republic (southwestern periphery of Prague, 50°00'N, 14°15'E) in the standardised manner. The first site was an abandoned quarry of Devonian limestone possessing a thin layer of reddish clay soil. Vegetation was composed chiefly of sparse bushes (*Prunus* sp. and *Rosa* sp.), the dominant herbs being *Salvia* sp. and *Fragaria* sp. The second site, located about 500 m from the first one, was a short natural slope with similar geological and vegetative conditions. Specimens were trapped by 38 yellow and 28 blue pans (diameter 65 mm, depth 80 mm) containing a mixture of salt water and detergent from 24 March to 17 June 2006 (a total of 85 days). The sampling period represented the complete spring activity season of the *Andrena* bees. Spring 2006 arrived rather suddenly in the Czech Republic, after a long series of cold days unsuitable for nest emergence prior to 24 March. At the other end of the period, 17 June represented the end of spring activity as confirmed by the drop in numbers of collected *Andrena* bees. Traps were emptied once a week (for dates of sampling see Fig. 1). Failed traps (mostly damaged by rain, animals or falling stones) were replaced at each sampling visit.

The collected material was preserved in ethanol. The bees were identified to species, sexed, and checked for parasitisation by strepsipterans and morphological aberrations (i.e. presence of intersexuality). We examined morphology of hind legs and pygidial plate in females (according to SALT 1927 and BRANDENBURG 1953). Our qualitative evaluation was directed to length, density and shape of setae on the hind leg collecting apparatus and reduction of sculptures and altered shape of pygidial plate. There is a substantial individual variation in the form and range of altered morphology. For this reason, we checked the individuals for obvious presence of morphological modification and the individuals with only slight modification were identified as not aberrant. Males are less affected by the presence of parasites than females (SALT 1931; ASKEW 1971) and their morphology was thus not minutely examined. The sex of parasites in styloped bees was identified (males of *Stylops* form cephalothecae and pupate while females remain neotenic and do not pupate). All specimens were deposited in the personal collection of J. Straka.

Timing of nesting behaviour in parasitised and uninfected females in Andrena vaga

Daily observations of female bees were performed at a nesting site close to the Čelákovice village, Czech Republic (northeastern periphery of Prague, 50°10'N, 14°45'E) between 25 March and 25 April in 2007 and 28 March and 30 April in 2008. This nest aggregation is situated in bare alluvial sand with patches of grass on the margin of the secondary oak and pine forest. Systematic observation of males was not possible due to their higher flight activity and wide flight range. Females flying over the nesting site were collected by sweeping net, immediately checked for stylopedisation, marked and released. Morphology of marked individuals was not studied, as BRANDENBURG (1953) has described intersexuality in *Andrena vaga* in detail. Females searching for a new nesting place and females sitting inside the nest were not collected, because disturbance at that time affects their philopatry (K. REZKOVÁ pers. obs.). They were captured, examined, and marked on the earliest possible occasion. The marking was performed using oil-based colours on scutum and metasomal terga. Styloped females were not active after digging new nests and were thus difficult to observe later, but uninfected females were starting to collect pollen several days after establishing their nests.

Statistical analysis of phenology trends

Differences in phenology were tested by estimation and comparison of two parameters (κ ; λ) of the respective cumulative distribution functions for the Weibull distribution: $F(x) = 1 - e^{-(x/\lambda)^\kappa}$

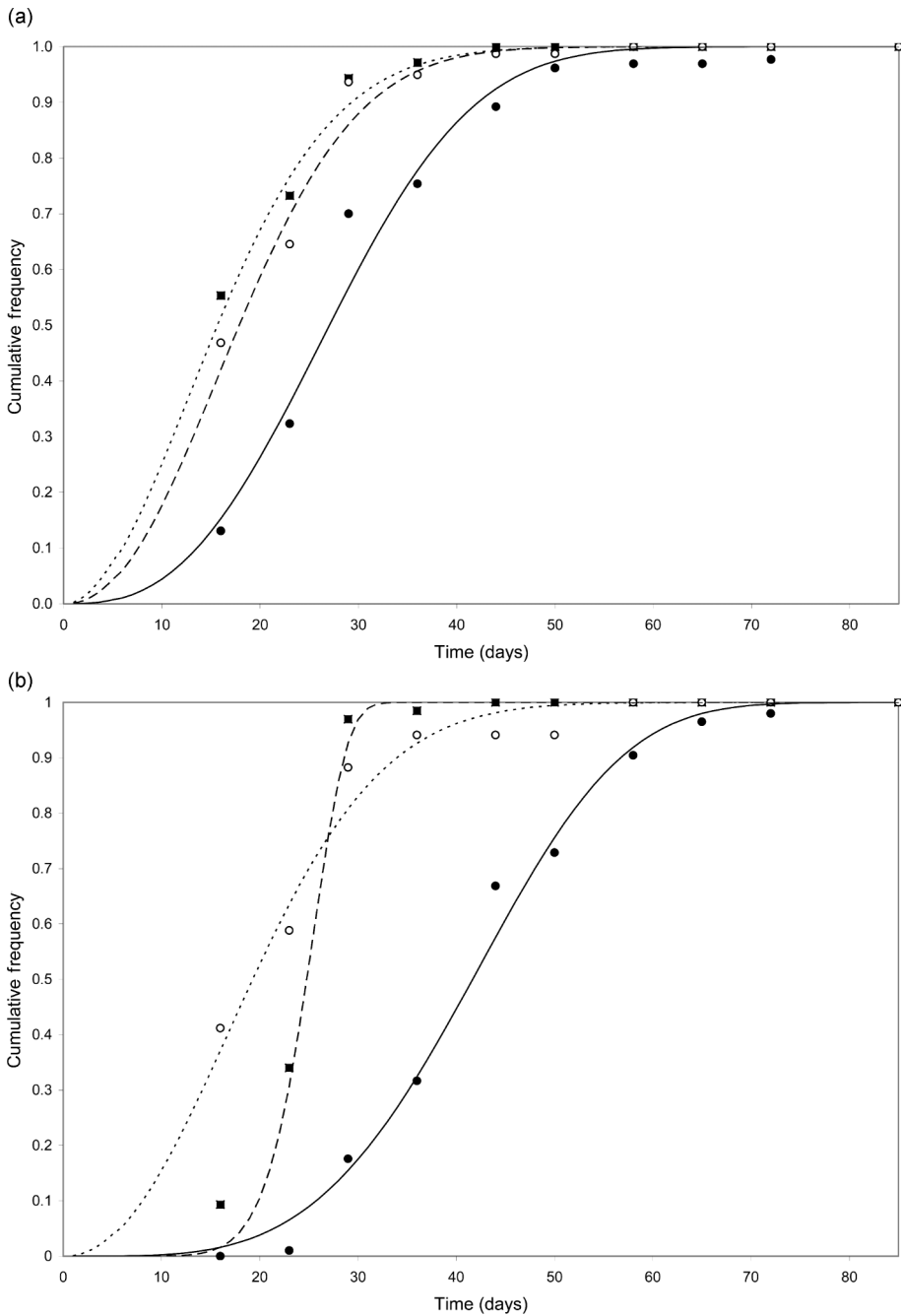


Fig. 1. — Temporal trends in numbers of uninfected males (squares, dotted line), uninfected females (closed circles, solid line), and styloped females (open circles, dashed line) in (a) *Andrena minutula* and (b) *Andrena strohmeella* sampled throughout the early spring in 2006. Note similarity between trends in uninfected bees and styloped females that largely differ from trends in uninfected females. Cumulative distribution functions for the Weibull distribution are fitted to each group.

(see e.g. PINDER et al. 1978). We compared temporal trends between sexes and between stylipised and uninfected individuals in pan-trapped *A. strohmella* and *A. minutula* bees during spring months in 2006. In the case of *A. vaga* nesting females, we compared cumulative distribution functions of marked individuals in stylipised and uninfected females in 2007 and 2008. Function fitting was performed in Statistica Vers. 6.0 (STATSOFT 2001) using the Levenberg–Marquardt algorithm.

RESULTS

Rates of parasitism by Stylops and effects on bee morphology

Species identity, the total number of bees trapped, sex ratios of uninfected *Andrena* bees, and the numbers of stylipised individuals collected to the pan traps near the Chýnčice village are summarised in Table 1. In total, we collected 2058 bees of 24 species of the genus *Andrena*. One hundred and forty-one (6.85%) individuals were parasitised by strepsipterans (Table 1). The abundance and the incidence of parasitisation varied greatly among species. The total sample consists of 36 male puparia (of which 31 were empty) and 120 females of *Stylops*. One bee contained two *Stylops* male puparia and 13 bees contained two females. Only one bee contained a male and a female together. It is important to note that stylipised host males formed the minority of the collected stylipised bees (34 male to 107 female hosts in total).

Concerning the external morphology of a high number of stylipised female bees, we observed a continuum from slightly to strongly modified traits: 40.2% of 107 stylipised females possess conspicuously reduced hairs on hind legs and altered morphology of pygidium, i.e. they demonstrated masculinisation. We found some effect of *Stylops* on host morphology in all host *Andrena* bee species collected.

Phenology trends in uninfected and parasitised bees revealed from pan trapping

The sex ratio of uninfected bees and the numbers of stylipised bees captured varied greatly over time. In the complete dataset (Table 1), 91% out of 1202 males and 94% out of 141 parasitised individuals (34 males and 107 females) were captured during the first three sampling periods, i.e. the first 30 days of collection, compared to only 43% of 715 uninfected females. The trend of early emergence of parasitised bees was also evident within the subgenus *Micrandrena*, where 73.4 % of all collected bees were parasitised at the beginning of the season (until 9 April 2006), while no stylipised individual was observed later in the season (after 7 May 2006). We restricted our analyses of emergence trends to the two host species with the highest incidence of parasitised bees: *Andrena minutula* and *A. strohmella* from the subgenus *Micrandrena* (Table 1).

In both of the tested species, the trends in emergence timing differ between uninfected males and uninfected females. The parameter λ , describing the respective cumulative distribution functions, is significantly different between these groups within each species ($P < 0.0001$ in both species; means \pm SE, *A. minutula*: males: $\lambda = 18.43 \pm 0.46$, $\kappa = 1.87 \pm 0.16$; females: $\lambda = 30.45 \pm 0.98$, $\kappa = 2.66 \pm 0.33$; *A. strohmella*: males: $\lambda = 25.38 \pm 0.30$, $\kappa = 8.42 \pm 0.89$; females: $\lambda = 45.28 \pm 0.77$, $\kappa = 3.85 \pm 0.33$; Fig. 1). These differences document that males were more active at the beginning of the spring activity season, but the numbers of collected males decreased much more steeply than those of the females. Our data thus confirmed protandry in these two species of the *Andrena* bees.

Table 1.

Summary of the *Andrena* bees collected by pan-traps. Styloped individuals are included in the numbers of males, females, and the total number of bees collected.

Species	No. of males	No. of females	Total	No. of styloped individuals
<i>Andrena (Andrena) fulva</i> (Müller 1766)	0	3	3	3
<i>Andrena (Andrena) helvola</i> (Linnaeus 1758)	0	1	1	–
<i>Andrena (Andrena) praecox</i> (Scopoli 1763)	12	0	12	1
<i>Andrena (Andrena) varians</i> (Kirby 1802)	4	1	5	3
<i>Andrena (Biareolina) haemorrhoea</i> (Fabricius 1781)	7	0	7	–
<i>Andrena (Chrysandrena) fulvago</i> (Christ 1791)	1	1	2	–
<i>Andrena (Euandrena) bicolor</i> Fabricius 1775	49	102	151	–
<i>Andrena (Hoplendrena) carantonica</i> Pérez 1902	1	3	4	1
<i>Andrena (Lepidandrena) florivaga</i> Eversmann 1852	22	26	48	–
<i>Andrena (Melandrena) cineraria</i> (Linnaeus 1758)	83	3	86	1
<i>Andrena (Melandrena) nigroaenea</i> (Kirby 1802)	1	7	8	–
<i>Andrena (Melandrena) nitida</i> (Müller 1776)	4	5	9	1
<i>Andrena (Micrandrena) falsifica</i> Perkins 1915	21	47	68	–
<i>Andrena (Micrandrena) floricola</i> Eversmann 1852	13	0	13	–
<i>Andrena (Micrandrena) minutula</i> (Kirby 1802)	338	209	547	99
<i>Andrena (Micrandrena) minutuloides</i> Perkins 1914	1	2	3	1
<i>Andrena (Micrandrena) strohmella</i> Stöckhert 1928	399	216	615	19
<i>Andrena (Micrandrena) subopaca</i> Nylander 1848	3	0	3	–
<i>Andrena (Poecilandrena) labiata</i> Fabricius 1781	2	9	11	–
<i>Andrena (Proxiandrena) proxima</i> (Kirby 1802)	2	0	2	–
<i>Andrena (Simandrena) dorsata</i> (Kirby 1802)	33	19	52	–
<i>Andrena (Simandrena) propinqua</i> Schenck 1853	2	2	4	–
<i>Andrena (Zonandrena) flavipes</i> Panzer 1799	222	139	361	12
<i>Andrena (Zonandrena) gravida</i> Imhoff 1832	16	27	43	–
Total	1236	822	2058	141

In *A. minutula*, the trend of emergence in the styloped females ($\lambda = 20.67 \pm 0.63$, $\kappa = 2.11 \pm 0.25$) significantly differs from the trend in uninfected females ($P < 0.0001$ for comparison of λ), but was not statistically distinguishable from the trend of uninfected males (NS differences in both parameters). In *A. strohmella* with a substantially

smaller sample size (cf. Table 1), the trend of stylopedised females ($\lambda = 22.41 \pm 0.74$, $\kappa = 2.09 \pm 0.25$) was statistically different from the trends in both uninfected females and uninfected males ($P < 0.05$); however, it was much more similar to the trend in uninfected males (cf. Fig. 1). We can thus conclude that, from dates of emergence, stylopedised females behave largely in a male-like fashion.

The trend of emergence in the stylopedised males of *Andrena minutula* ($n = 20$) seems not to be different from the trend in uninfected males; however, the number of collected individuals is too small to present reliable results.

Timing of nesting behaviour in parasitised and uninfected females in Andrena vaga

Observational data on female nest behaviour in *Andrena (Melandrena) vaga* Panzer 1799 strongly support our assumption that parasitisation affects the timing of female bee activities. For example, 98.3% of marked bee females were parasitised early in the season (28 March 2008), while only 8% were stylopedised in the late season (after 7 May 2008) (Fig. 2). In both observation years, parasitised females emerged on the nesting site significantly earlier than uninfected individuals ($P < 0.0001$ in both years; 2007: uninfected bees: $\lambda = 16.63 \pm 0.08$, $\kappa = 2.76 \pm 0.046$; parasitised bees: $\lambda = 4.88 \pm 0.14$, $\kappa = 1.12 \pm 0.05$; 2008: uninfected bees: $\lambda = 16.35 \pm 0.39$, $\kappa = 2.01 \pm 0.16$; parasitised bees: $\lambda = 3.32 \pm 0.23$, $\kappa = 0.55 \pm 0.03$; Fig. 2).

DISCUSSION

In *A. minutula*, *A. strohmella* and *A. vaga*, stylopedised females emerge and activate earlier in comparison to uninfected females (cf. Figs 1–2). This observation is also supported by the raw data published by LINSLEY & MACSWAIN (1957) in *Andrena suavis* Timberlake 1938 and *A. caerulea* Smith 1879. These data suggest that in *A. minutula* and *A. strohmella*, where we were able to compare the timing of their emergence to the trend in males, the phenology of stylopedised females follows rather the protandrous trend of uninfected males than the trend of uninfected females. The behaviour of stylopedised *Andrena* females conforms to their intersexual, i.e. masculinised, external morphology. Most conspicuously, legs of stylopedised females have a considerably reduced collecting apparatus closely resembling male legs (PÉREZ 1886; PIERCE 1909; BRANDENBURG 1953). This possible sex reversal in parasitised female emergence behaviour suggests that masculinisation is not confined to the external morphology, but also constitutes deep changes concerning an important behavioural characteristic. POTTS (1906) seems to have been right when he intuitively compared morphological and behavioural changes induced by rhizocephalan barnacles in crabs to changes induced by strepsipteran parasites in bees. If we are right in our explanation, it is an extreme case of host manipulation by parasite. However, we can hardly refute SALT's (1927) theory of undernourishment by our data, but his theory stands on less supported evidence than our result (see Introduction for details).

The adaptive value and physiological mechanism of the behavioural changes in bee females are unknown. The strepsipteran parasites may have exploited the pre-existing male behavioural strategy, protandry. Protandry in *Andrena* bees is probably adaptive, and it appears to have evolved due to strong competition among males to find a virgin, receptive female (ALCOCK et al. 1978; EICKWORT & GINSBERG 1980). By inducing sex reversal in the emergence time of female bees, the strepsipteran parasite shifts

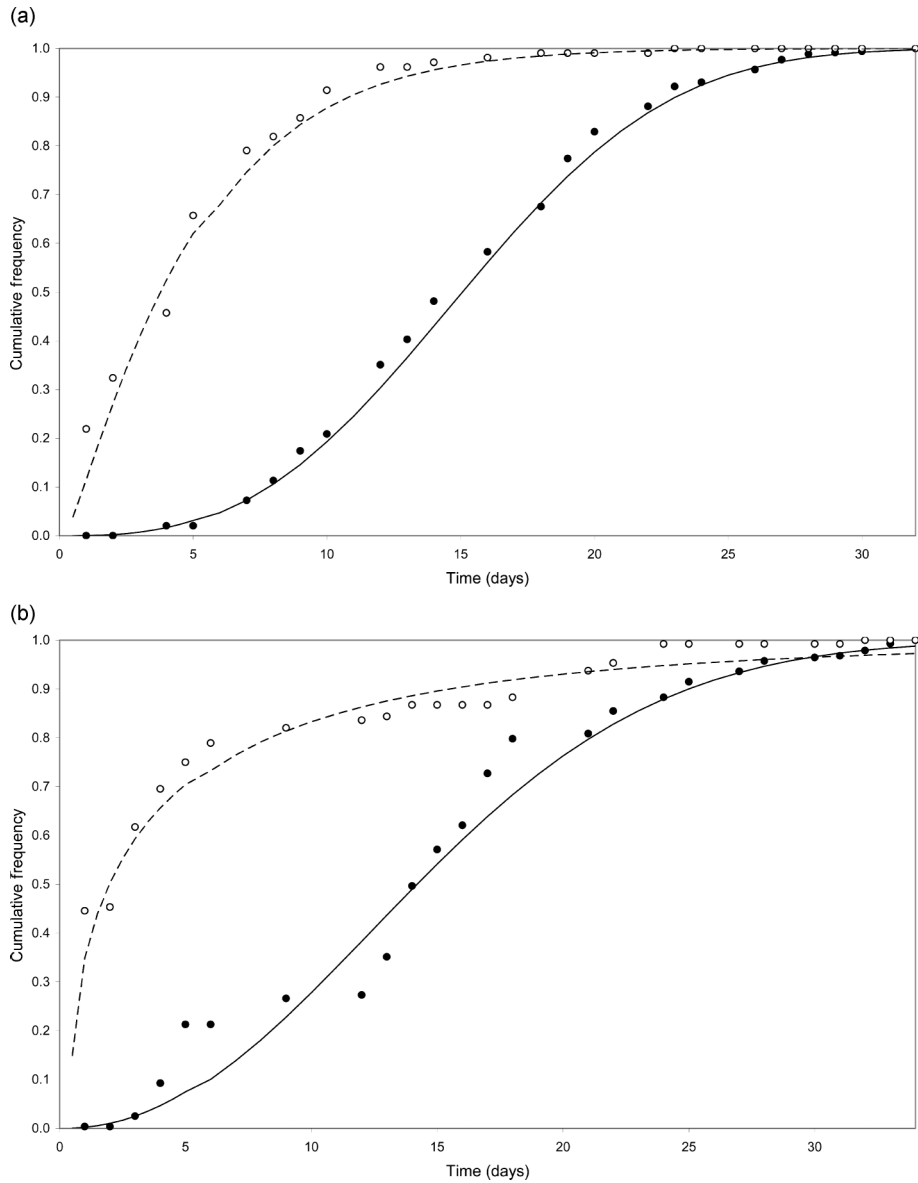


Fig. 2. — Temporal trends in numbers of marked uninfected females (closed circles, solid line) and styliposid females (open circles, dashed line) of *Andrena vaga* in (a) 2007 and (b) 2008 spring seasons. Styliposid females were active on the nesting site notably earlier than uninfected females in both years.

its own emergence time to an earlier period. By manipulating its host to emerge earlier, the parasite can obtain more time for development or dispersion of its larvae (see also KINZELBACH 1978: 18). According to LINSLEY & MACSWAIN (1957: 409), the time

between fertilisation and emergence of the first larvae is about 30–40 days in *Stylops*, which is surprisingly long in comparison with the active lifetimes of the host bees. The majority of spring species of *Andrena* are active for about 2 months at maximum (see WESTRICH 1989); however, the times the adult host females occur in abundance at nesting sites spans about 1 month (LINSLEY & MACSWAIN 1957) (e.g. in *Andrena fulva*, an individual imago lives for approximately 2 weeks on average (PAXTON 1991)). If the styloped and uninfected bees emerged at the same time, it would be difficult to find host bees available for infection by a new generation of the parasite. An early emergence could also be connected to success in sexual selection or other reproductive functions of *Stylops*. For example, the strepsipteran *Xenos vesparum* manipulates its host (wasps of the genus *Polistes*) to aggregate early outside the nests in the fashion of males or young queens. Infected individuals aggregate on vegetation and landmarks which are traditional nuptial arenas and on buildings which represent pre-hibernation sites (HUGHES et al. 2004; BEANI 2006). It appears that this manipulation enables male *Xenos* to more reliably find conspecific females (BEANI et al. 2005). The synchronised emergence of parasitised hosts in early spring could ensure the meeting of sexual partners in *Stylops* as well, even in the case of a low population density of their hosts. Another known example of phenological difference is described by LINDBERG (1939) from uninfected and styloped individuals of the family Delphacidae (Hemiptera). The strepsipteran parasite *Elenchus tenuicornis* probably manipulates individuals of the first host generation, inducing prolongation of their lifespan to overlap with the second generation of the host (see also KATHIRITHAMBY 1989: 53). This may ensure a better chance for *Elenchus* to infect the larvae of the second generation of the host. Unfortunately, little is known about the mating systems and the reproductive biology of Strepsiptera to further evaluate these possibilities.

Morphological and behavioural intersexuality and/or sex reversal could be linked functionally or by a common proximate mechanism inducing changes at both organisational levels. It will be interesting to explore whether the early emergence pattern induced by strepsipteran parasites occurs in halictids or other strepsipteran hosts where the parasite does not cause morphological intersexuality (BATRA 1965; STRAKA et al. 2006). The preliminary results suggest that the timing of emergence is not affected by strepsipteran parasitisation in *Bembecinus tridens* (Hymenoptera Crabronidae), an apoid wasp that does not demonstrate apparent parasite-induced intersexuality (J. BATELKA & J. STRAKA pers. obs.). Masculinised morphology has never been observed in bees of the subfamily Halictinae or in wasps of the subfamily Polistinae (SALT 1927). Male imagoes from both of these lineages do not overwinter, and thus masculinisation of host females by a parasite could increase winter mortality and not bring any adaptive advantage. Nevertheless, a broader comparative study will be needed to test the connection between the morphological and behavioural effect of strepsipteran parasites on their hosts.

Our data obtained by pan trapping (Table 1) showed high variability in infestation by *Stylops* among collected *Andrena* species. The most widespread parasitised host was *A. minutula*, with records of parasitisation by *Stylops* in 12 countries. On the other hand, *A. strohmeella* has been previously observed as a host of *Stylops* just once in Germany (KINZELBACH 1978) and its infestation rate was similarly comparatively lower in our data. We found close resemblance in spring emergence trends between uninfected males and parasitised females in *A. minutula*, but the trends were not as similar in *A. strohmeella* (cf. Fig. 1a–b). The differences between these two bee species could potentially be ascribed to the low number of styloped individuals in *A. strohmeella*, but could also reflect a closer coevolution of the *Stylops* parasite with *A. minutula*.

Future studies should test whether *A. strohmella* is a secondary host of a species of *Stylops* primarily parasitising the closely related *A. minutula*. With regard to recent systematics, only a single strepsipteran species (*Stylops melittae* Kirby 1802) is currently recognised as a parasite of the bees of the genus *Andrena* in Europe (KINZELBACH 1978). However, we cannot exclude the possibility that the parasite affects particular host species differently or that *Stylops melittae* in fact forms a species complex.

Looking at only the uninfected bees, we collected approximately 20% more males than females during the course of the sampling period. This biased sex ratio could be ascribed to a temporal overlap of our sampling period with the main activity period of males (cf. Fig. 1), to higher spring flight activity of males (LARSSON 1991), or to selectivity of the sampling method: females choose flowers more carefully (NE'EMAN et al. 2006), which can reduce their tendency to enter pan traps. However, we cannot exclude an actual biased sex ratio in the studied populations. Interestingly, in contrast to the male bias in uninfected individuals, females represent an overwhelming majority of parasitised bees in our sample. A similar situation has already been observed in other species of *Andrena* (PIERCE 1909; LINSLEY & MACSWAIN 1957), as well as for *Halictus* and *Lasioglossum* (STRAKA et al. 2006), while males and females of *Lasioglossum zephyrum* are reported to show equal rates of parasitisation (BATRA 1965). Female-biased sex ratios of active stylopid hosts could reflect higher mortality of overwintering parasitised males, which might be expected due to their smaller size and thus potentially higher sensitivity to parasitisation. Alternatively, strepsipteran parasites could predominantly specialise in female hosts, which could be advantageous due to the host's sexual differences in body size or life cycles (e.g. the main target of infection may be the first generation of females, i.e. the workers, in polistine wasps; BEANI 2006; and only females overwinter in wasps and most halictids; WESTRICH 1989; BEANI 2006). These alternatives can be tested only after identifying the initial proportion of the infected sexes (until now not even the total primary parasitised numbers were known) or by a study of host choice in the parasite. Here, the sex ratio and number of collected specimens could be biased by the collecting method (pan trapping) used in the 2006 experiment. However, for *Andrena vaga* we demonstrated shifts in emergence trends in uninfected and stylopid females generated by marking and observation of nesting individuals as well (Fig. 2), thus confirming the trends seen using pan trapping.

In conclusion, our study may be the first comprehensive documentation of sex reversal in emergence dates in females of protandrous bee species induced by strepsipteran parasites. Although the selective advantage of such induction remains unclear, we suggest that it could be attributed to adaptive (from the parasites' view) manipulation of hosts (HUGHES 2005), a way to increase the *Stylops* life span, and thus its reproduction. Future studies should evaluate this possibility and uncover the proximate mechanism of the parasites' extended phenotype, observable as morphological and behavioural changes in its bee hosts.

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