

Mixed life-history strategies in a local population of the ectoparasitic fly *Carnus hemapterus*

M. AMAT-VALERO^{1*}, R. VÁCLAV², T. MARTÍNEZ³ and F. VALERA¹

¹ *Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (EEZA-CSIC), Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain*

² *Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, 84506 Bratislava, Slovakia*

³ *C/Catavieja 31. 1ºB, 04007 Almería, Spain*

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SUMMARY

A major issue for the proper understanding of the evolution of life-cycle histories is the regulation of voltinism and its variation. Diapause characteristics are known to regulate voltinism, but the underlying mechanisms are poorly understood. This paper studies diapause duration and voltinism variation in a haematophagous diptera parasitizing 2 sympatric hosts with very different breeding phenologies. We hypothesize that bivoltinism will be more frequent in carnid flies parasitizing an early breeding, multi-brooded species than in flies parasitizing a late breeder, single-brooded species. We obtained evidence of the co-occurrence of uni- and bivoltinism in both clutches of the multi-brooded Spotless starling (*Sturnus unicolor*) as well as in clutches of the single-brooded European roller (*Coracias garrulus*). Unexpectedly, the proportion of bivoltine flies was similar in both host species. A remarkable degree of host-parasite synchronization at the population level was found for bivoltine flies. Our findings reveal the facultative nature of diapause in *Carnus*. We discuss the influence of abiotic conditions and host availability on polymorphism in life-history cycles and the consequences both for the parasite and the host.

Key words: *Carnus hemapterus*, voltinism, diapause, life-history strategies, phenotypic plasticity.

INTRODUCTION

One of the central issues in the evolution of life histories in insects is the mechanism behind variation in voltinism (Taylor and Spalding, 1988). Abiotic and biological factors, like food or natural enemies, are known to influence the number of generations of insects per year (voltinism) (Masaki, 1980; Tauber *et al.* 1986). These animals have evolved to use developmental mechanisms, particularly diapause, to synchronize their life cycles with seasonal changes in resource availability and climatic conditions suitable for their development, growth and reproduction. Diapause is a form of dormancy influenced both by genetic and environmental factors. It enables insects to survive unfavourable conditions, to synchronize their active stages with resource availability and to disperse and colonize new habitats efficiently (Tauber *et al.* 1986; Danks, 1987, 1992; Soula and Menu, 2003; Kostal, 2006). In insects, diapause is governed by reliable environmental signs preceding seasonal changes (Tauber *et al.* 1986). Duration of the seasons influences the diapause period, which may range between days and months, or even years. Therefore,

the number of generations per year varies. In habitats with long favourable periods and high resource availability, some species can shorten the cycle, develop fast with short diapauses, and give rise to a generation within days (Campbell and Mackauer, 1975; Tillman and Powell, 1991; Sabelis and Janssen, 1994). These are multivoltine species. In regions with short seasons and where the resources are not always available, multiple generations do not occur and many species are strictly univoltine (Danks and Footitt, 1989; Danks, 2002).

In many parasitic insect species, life-cycle duration varies within the population (Tauber and Tauber, 1981; Danks, 1987, 1992; Menu *et al.* 2000). In animals with facultative diapause, dynamic temporal risk spreading and adaptive developmental plasticity (Stearns, 1976) appear to have evolved to regulate diapause frequency and duration in response to uncertain environments (Bradford and Roff, 1993; Soula and Menu, 2003). Thus, a single genotype may produce multiple phenotypes, some of which enter diapause whereas others continue to develop and produce offspring (Hopper, 1999; He *et al.* 2010). Whereas the variation of voltinism over a geographical range as a result of local adaptation has been widely described in insects (Tauber *et al.* 1986; Danks, 1987), the phenotypic plasticity of voltinism in a local population has been seldom reported and is poorly understood (He *et al.* 2010).

* Corresponding author: Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (EEZA-CSIC), Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain. E-mail: amat@eeza.csic.es

Carnus hemapterus Nitzsch (1818) is a generalist parasite whose entire cycle, including diapause, takes place in its host's nest. It parasitizes a wide range of bird species whose phenology varies considerably (Grimaldi, 1997). A degree of synchronicity has been recorded between the appearance of the host and its parasite's emergence (Liker *et al.* 2001; Valera *et al.* 2003). It has therefore been assumed that some environmental signs must exist to regulate such synchronicity. Still poorly-known, the plasticity of *Carnus* diapause seems to be remarkable: (i) 3 different types of diapause have been described, namely a short diapause (Guiguen *et al.* 1983), a winter diapause to resist the adverse environmental conditions and food shortage (Guiguen *et al.* 1983; Grimaldi, 1997), and a long diapause that may prolong itself for years (Valera *et al.* 2006); (ii) experiments have proved that temperature changes at the end of the diapause influence the emergence phenology of the parasite, which ultimately means some plasticity in diapause termination in *Carnus* (Calero-Torrallbo and Valera, 2008).

This paper examines variation in voltinism in a local population of *Carnus hemapterus* in southern Spain. Resident bird species at temperate latitudes like ours start breeding early in the season and usually produce several clutches. These species usually co-exist with migrant bird species, which start breeding later and, therefore, produce a single brood. The starting hypothesis is that the length of the breeding season of bird species (i.e. *Carnus*' hosts) influences diapause duration and that the co-occurrence of various host species with different breeding phenology foster the co-existence of various life-cycle strategies (multi- *vs* univoltinism). Flies which parasitize early breeding, multi-brooded bird species are expected to undergo short diapause and to be multivoltine, while flies which parasitize late breeding, single-brooded bird species, like trans-Saharan migrants, are expected to undergo long diapause and to be univoltine. To this end, we studied the emergence phenology of adult flies in nests of a bird species with an early reproductive cycle as a model of a multi-brooded species, the Spotless starling (*Sturnus unicolor*), and also in nests of a bird species with a late reproductive cycle as a model of a single-brooded species, the European roller (*Coracias garrulus*).

MATERIALS AND METHODS

Study area and species

The main study area (~ 50 km²) lies in the Desert of Tabernas (Almería, SE Spain, 37°05' N, 2°21' W). The climate in this area is semi-arid with high annual and seasonal rainfall variability (mean annual rainfall ca. 218 mm), and strong thermal oscillations with inter-annual differences. Summers are long and hot and winters are usually mild.

Carnus hemapterus (Nitzsch 1818, Phylum Arthropoda, Class Insecta, Order Diptera, Family Carnidae) (hereafter *Carnus*) is a 2 mm long, highly mobile ectoparasitic fly that colonizes nestling birds (Grimaldi, 1997). It has a wide geographical distribution and parasitizes a variety of host species, although it shows some preference for birds nesting in holes (troglodytic species) (Dawson and Bortolotti, 1997; Grimaldi, 1997). Adult flies have a winged and wingless phase. After their emergence, adults are initially winged, but lose their wings as soon as they locate a suitable host (Roulin, 1998). Carnid flies do not need a host for transmission because they actively colonize hosts' nests during the winged phase of their life cycle (Grimaldi, 1997). Once emerged, the imagines can survive for slightly less than 3 days without feeding. Carnid flies complete their cycle in their hosts' nests. Adults mate in the nest, lay eggs which hatch approximately 5 days later, and the larvae live on the organic matter in the nest (Capelle and Whitworth, 1973; Papp, 1998). The 3 larval stages last 21 days (Guiguen *et al.* 1983) and pupation takes place in the nest. During the pupal stage, *Carnus* undergoes a diapause that usually lasts for months. *Carnus* emergence is usually synchronized with the occurrence of the host (i.e. hatching) and persists continuously throughout the whole nestling period (Roulin, 1998; Liker *et al.* 2001; Valera *et al.* 2003; Calero-Torrallbo and Valera, 2008). Guiguen *et al.* (1983) described a short, 8-day diapause in spring in laboratory conditions, and Valera *et al.* (2006) recorded a prolonged diapause that may last for years.

In southern Spain, the Spotless starling *Sturnus unicolor* and the European roller *Coracias garrulus* are common avian breeders. These species readily use nest boxes and are commonly parasitized by *Carnus* (Liker *et al.* 2001; Václav *et al.* 2010). The Spotless starling is a resident species in the Iberian Peninsula. The breeding period begins in early March and some pairs in our study area have a second clutch at the end of May. Incubation lasts for approximately 10 or 15 days, from the last laid egg until the first hatched egg (Peris, 1984). European rollers are migratory birds that arrive at breeding grounds when resident and secondary cavity-nesting birds are already settled. Rollers rear a single brood per year (Cramp, 1998). In our population incubation lasts for approximately 21 days and nestling rollers fledge about 20–22 days after hatching (R. Václav, *unpublished data*). European rollers are the latest breeders in our study area, with the tardiest nestlings remaining in the nest until as late as the third week of July.

Experimental approach

The number of generations per year and the existence of a short diapause in *C. hemapterus* can only be established based on the emergence of flies in newly

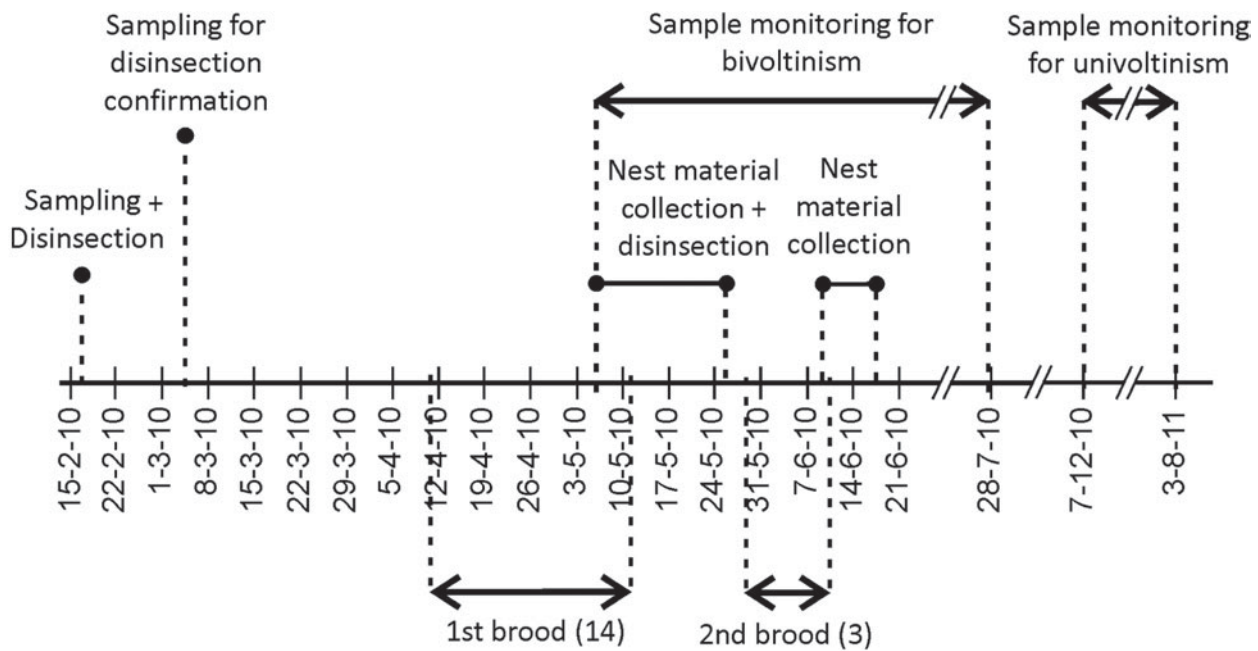


Fig. 1. Experimental approach for determination of *Carnus hemapterus* life history in the multi-brooded Spotless starling. Treatment, sampling and monitoring are shown above the line and bird breeding phenology (nestling period) is depicted below the line (number of infected nests in parentheses).

occupied nests. The emergence of flies after year-long diapauses can thus be discarded. Alternatively, the emergence of *Carnus* can be studied in used nests after due disinsecting. Our approach is based on the study of the emergence of carnid flies from diapausing pupae collected from first and second Starling broods and single Roller broods performed in previously disinsected nest boxes.

Twenty-four out of 55 nest boxes deployed in our study area as a result of several projects were selected in 2010. Some of them had been used by European rollers. The nest boxes were disinsected with a 10 ml/L solution of Arpon® (cipermetrine) on 17 and 18 February 2010. The solution was sprayed on the nest's inner surface to cover the material and walls and to soak the sand at the bottom of the nest. To test the efficacy of the insecticide, a sample of nest material (sand, feces and vegetal matter) was collected before spraying, from the 12 nest boxes that had been occupied the previous breeding season. The same nest boxes were sampled on 3 and 4 March, i.e. after disinfection (Fig. 1). The samples collected before and after disinfection were kept in plastic bags in the dark in a well-aerated room at the *Estación Experimental de Zonas Áridas* (EEZA-CSIC, Almería). The samples were checked every 3–4 days prior to collection and until fly emergence ceased (13 April 2010).

The disinsected nest boxes were then made available for nesting. Fourteen out of 24 nest boxes were used by Spotless starlings and were monitored to record the occurrence of *Carnus* based on the appearance of fecal spots on the eggs (López-Rull *et al.* 2007), or on insects on the chicks and in the nest.

The occurrence of *Carnus* in these nests means that flies emerged in other nests colonized the experiment's nests. The controls showed that 10 out of these 14 nests were parasitized by *Carnus*. The remaining 4 nests were infected purposely with 7–20 newly emerged adult *Carnus* obtained from pupae from European roller nests of the previous year. These *Carnus* adults were added when the nestlings were still featherless, i.e. 4–7 days after the first egg hatched.

Once breeding had finished, the material of the nest boxes was collected in plastic bags i.e. between 5 and 25 May, and kept at the EEZA in replicated natural conditions (i.e. ambient temperature moderated by partial enclosure and semi-darkness). Nest boxes were disinsected again and their floors covered with fresh sand (Fig. 1). Only 3 out of the 14 nest boxes used originally were occupied again, this time in late May. Second broods in these nests were monitored and *Carnus* was recorded in 2 nest boxes. The nestlings of the third brood showed *Carnus* wounds. Again, the material of the 3 nest boxes was collected after breeding, i.e. between 9 and 17 June (Fig. 1), and then kept as described above.

All the samples were monitored every 3–4 days from collection until 28 July 2010 for any possible cases of bivoltinism. A subsample of approximately one third of the nest material was selected and controls conducted from 7 December 2010 until 3 August 2011 for fly emergence (univoltinism) (Fig. 1). The emerged insects were preserved in 70% alcohol and were later identified.

Twelve European roller clutches in previously emptied, cleaned and disinsected nest boxes (from 25 March 2011 until 6 April 2011) were monitored

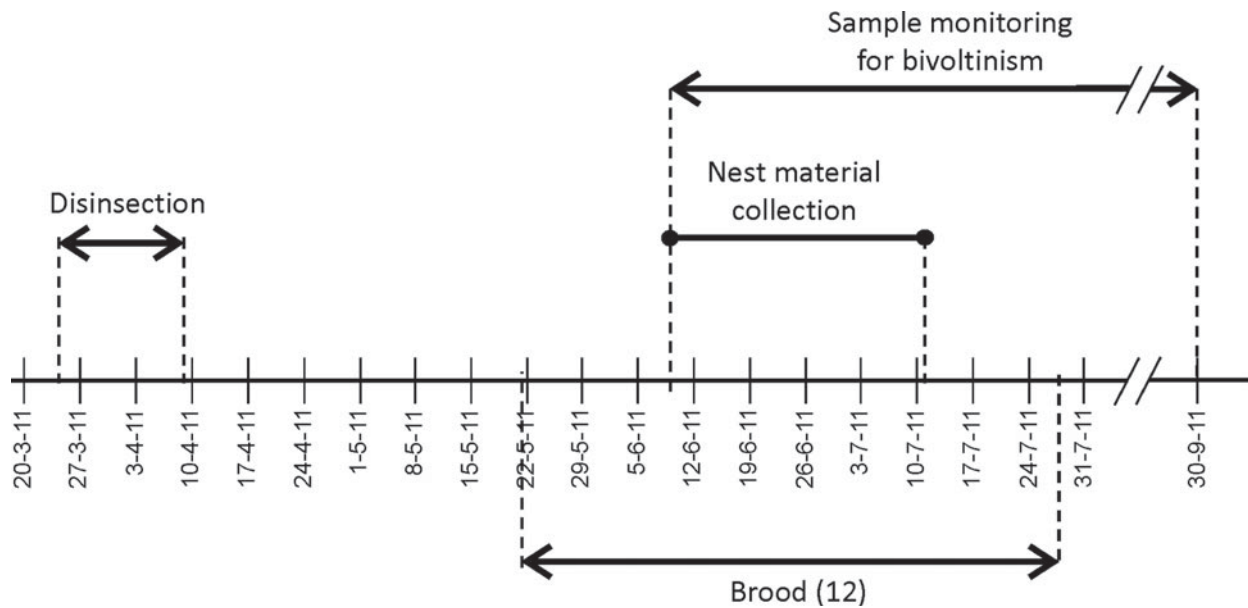


Fig. 2. Experimental approach for determination of *Carnus hemapterus* life history in the single-brooded European roller. Treatment, sampling and monitoring are shown above the line and bird breeding phenology (nestling period) is depicted below the line (number of infected nests in parentheses).

for bivoltinism in a late brood species during the 2011 breeding season. Sixteen to 19 days after the first egg hatched, i.e. between 9 June and 11 July, the nest material, containing both *Carnus* pupae and larvae in different stages of development, was sampled (Fig. 2). The material collected was kept in plastic bags as described above and monitored every 3–4 days since then until 30 September 2011. Data about the breeding phenology of rollers were obtained from 31 clutches laid in nest boxes in 2011.

The samples of Spotless starling nests were sieved in November 2010 and the samples of European roller nests were sieved in October 2011 for any viable *Carnus* pupae. The material was sieved through 8 mm, 4 mm, 1 mm and 0.4 mm sieves, and the remains of all the sieves were rejected except those retained by the 0.4 mm sieve, where the *Carnus* pupae remained. Two 1 g subsamples were selected randomly from every sample, and the *Carnus* pupae contained in the subsamples were counted for 2 min. A distinction was made between closed and apparently intact, i.e. viable pupae, and open, i.e. emerged pupae, or broken, i.e. unviable pupae.

Statistics

Prevalence (percentage of samples where emergence is recorded with respect to the total number of samples) and abundance (the number of emerged flies per sample) of *Carnus* was calculated. An exact unconditional test for the comparison of 2 prevalences was used following Reiczigel *et al.* (2008). Statistical tests were done with the program Quantitative Parasitology 3.0 (Reiczigel and Rozsa, 2005). Means and standard errors are shown and tests are 2-tailed unless otherwise stated.

RESULTS

Confirmation of treatment

Disinsection resulted in 0% prevalence of *Carnus* in the treated subsamples compared to 33% in the untreated subsamples (4 out of 12 subsamples) (exact unconditional test, $P=0.038$). The untreated subsamples also contained other insects (moths, hymenoptera, coleoptera) that were not found in the treated subsamples.

The *Carnus hemapterus* life cycle in a multi-brooded species: the Spotless starling

Carnus hemapterus emergence was recorded in 5 (35.7%) out of the 14 previously fumigated nest boxes where carnid flies were recorded in the first brood (Table 1). Two of these 5 samples come from the nests which had been infected purposely. The total number of flies was 49, averaging 9.8 ± 2.7 (S.E.) flies per nest (Table 1). In these nests, emergence occurred between 10 May and 5 June, partially overlapping with hatching of the second clutches (Fig. 3). The subsequent analysis of nest material revealed open pupae in 3 more nests, some with partially emerged, dead flies. Fly emergence in these nests may therefore have occurred before sampling. In that case, the percentage of nests with bivoltine *Carnus* emergence would rise from 35.7% to 57.1% (8 out of 14).

Three of the abovementioned 14 nests were occupied with a second clutch. In 2 nests (66.7%) carnid flies emerged in the following days (Table 1) and open pupae were also found in the third nest during sieving. The emergence period occurred between 25 June and 2 July (Fig. 3).

Table 1. Emergence of *Carnus* in first and second clutches of Spotless starlings during 2010 and 2011 and in European roller clutches during 2011

(Prevalence (on the basis of emerged flies) and abundance of emerging flies are shown. Ranges (in parentheses), sample sizes (in square brackets), mean values and standard errors are given.)

Study species and sample collection year		Emergence in 2010		Emergence in 2011	
		Prevalence	No. of flies ± s.e. (range) [n]	Prevalence	No. of flies ± s.e. (range) [n]
Spotless starling 2010	First brood	5/14 35.7%	9.8 ± 2.7 (1–16) [5]	4/5 80.0%	7.0 ± 2.3 (2–13) [4]
	Second brood	2/3 66.7%	4.5 ± 1.5 (3–6) [2]	1/3 33.0%	1.0
European roller 2011	Single brood	—	—	5/12 41.6%	22.6 ± 12.4 (7–72) [5]

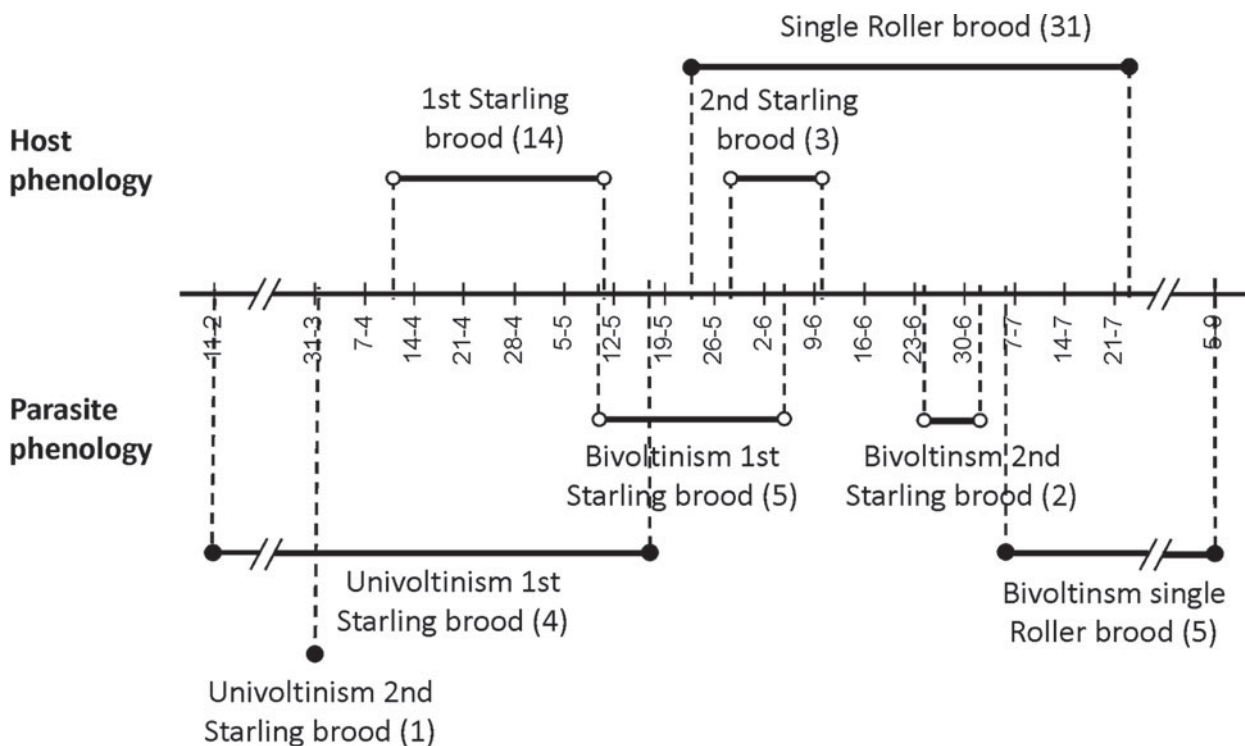


Fig. 3. Host availability (range of nestling period) in first and second starling broods and in roller broods and emergence period (range) of bivoltine and univoltine flies from starling and roller nests. White dots refer to data obtained in 2010 and black dots to data obtained in 2011. Sample size (nests) in parentheses.

Material from nests sieved in the autumn of 2010 revealed closed, apparently viable pupae in 8 out of 14 samples of the first broods (including 4 of the 5 nests where emergence was recorded in spring 2010) and in the 3 samples of the second broods. Further monitoring of the samples in spring 2011 detected *Carnus* emergence in 5 out of the 17 samples. Specifically, 4 (80%) out of the 5 nests where emergence was recorded after the first brood yielded flies in spring 2011 (Table 1). In these nests, emergence occurred between 11 February and 17 May 2011 (the earliest starling hatch of 2010 occurring on 11 April) (Fig. 3). Fly emergence was recorded only once in the samples of the second brood, specifically

on 1 April 2011. These results confirm that one and the same nest may contain both uni- and bivoltine pupae and this holds both for flies parasitizing first and second starling clutches.

The Carnus hemapterus life cycle in a single brooded species: the European roller

Carnus hemapterus emergence was recorded in 5 (41.6%) out of the 12 studied nests, averaging 22.6 ± 12.4 flies per nest (s.e.) (Table 1). Emergence occurred between 6 July and 5 September, partially overlapping with active roller nests (9 of 31 nests - 29% - had suitable nestlings for *Carnus* at this period)

(Fig. 3). Further examination of the sieved material for pupae revealed open pupae in 6 out of the 12 sampled nests, including the 5 nests where bivoltinism was recorded. Thus, the percentage of nests with bivoltine *Carnus* emergence would rise to 50%. Closed, apparently viable pupae were also found in these 6 nests. Thus, rollers' nests can contain bivoltine and non-bivoltine pupae.

Interspecific comparisons

The percentage of nests with bivoltine flies was similar for first starling broods and roller broods, both if we consider only those nests where emergence was recorded (exact unconditional test, $P=1.0$) and the nests where open pupae were found ($P=1.0$). Overall, the prevalence of bivoltine flies in starlings (first and second clutches) was similar to that registered in rollers (only nests with emerged flies: $P=1.0$).

DISCUSSION

Highlighting the variation in voltinism in insects and the underlying mechanisms is basic for a better understanding of the evolution of their life histories (Taylor and Spalding, 1988). This paper studies voltinism in a haematophagous diptera parasitizing 2 sympatric hosts with very different breeding phenologies: an early, resident, multi-brooded species, and a migrant single-brooded species. Our results show the co-occurrence of uni- and bivoltinism in a local population of the ectoparasitic fly *Carnus hemapterus*. We found evidence of bivoltinism in first and second clutches of starlings. The pupae in these nests underwent a short cycle and emerged within few days. Opportunistic observations of clutches of Hoopoes (*Upupa epops*) and of starling in new nest boxes also provided evidence of bivoltinism in 1 out of 3 hoopoe samples and in a Spotless starling sample (unpublished data). Evidence of bivoltinism in flies parasitizing a late breeder was also obtained; specifically bivoltine flies were found in at least 40% of European roller clutches.

Most of the specialized literature (Papp, 1998; Grimaldi, 1997; Roulin, 1998) describes a winter diapause in *Carnus* that may prolong itself over months. However, Guiguen *et al.* (1983) recorded a short cycle (34 days) with an 8-day diapause in laboratory conditions (22 °C and 95% relative humidity throughout the insect's cycle). Our experiment was not intended as a detailed study of the duration of short diapause in *Carnus*. Nevertheless, our data show that the life cycle may be even shorter than that described by Guiguen *et al.* (1983). The emergence of bivoltine adult flies in 6 out of the 7 starling samples collected in 2010 occurred between 20 and 27 days after carnid flies were first detected in the parasitized nests (mean \pm S.E.; 25.9 ± 3.5 , range

20–46, $n=7$ nests). Only in 1 nest did emergence occur after as long as 46 days. The contrast with the data of Guiguen *et al.* (1983) may be due to the different environmental conditions that the larvae and the pupae were subject to in each case.

To our knowledge, before this study, voltinism variation in a local population has only been reported for parasitic wasps (Parrish and Davis, 1978; Gag and Haynes, 1975; He *et al.* 2010). We could unambiguously show that some starling nests held univoltine and bivoltine flies, both in the first and the second clutches. Moreover, the occurrence of apparent viable pupae that did not emerge after 1 year suggests that some of them could also experience prolonged diapause, which has also been reported for this species (Valera *et al.* 2006). Thus, pupae with short diapause, with 1-year diapause and with prolonged diapause may co-exist in one and the same nest.

Co-occurrence of various life-cycle strategies in a local population may be a response to selective pressures associated with variation in abiotic and biotic factors and their interactions (Price *et al.* 2003; Winterhalter and Mousseau, 2007). In many insect species the number of produced generations increases with the length of the favourable season (Valimaki *et al.* 2008; Kivelä *et al.* 2009). Therefore, the occurrence of bivoltinism in an early, multi-brooded species like Spotless starling is not surprising. This strategy also results in remarkable host-parasite synchronization at the population level. Data from starling nests show that a fraction of the bivoltine flies from first clutches emerged at the time when nestlings had already hatched in second clutches, so flies could parasitize starlings again. Bivoltine flies from second clutches emerged too late to find starling nestlings, but they could still parasitize late breeders e.g. European rollers.

We expected a lower frequency of bivoltinism in rollers, because they are the last breeders in the study area and mistiming may have severe consequences for flies. Nonetheless, bivoltinism was common and a considerable fraction of the bivoltine flies (69.9%) emerged before 22 July when there were still roller nests with unfeathered nestlings. The remaining individuals emerged in August and September, i.e. when hosts were no longer available. Extemporaneous emergence of *Carnus* (i.e. out of the hosts' breeding season) has been both reported elsewhere (Matyukhin and Krivosheina, 2008) and observed by us (unpublished data), but this emergence is marginal.

Concerning univoltine flies, we also found a remarkable synchronization with the hosts' cycle. The emergence of univoltine starling flies in 2011 occurred within the hatching period observed for starlings in 2010.

Overall, which factors regulate the length of the *C. hemapterus* life cycle? The period during which environmental stimuli may induce diapause is known often to be limited to specific stages of the life cycle of

insects (Tauber and Tauber, 1970). Rapid larval development may allow some individuals to surpass the critical diapause induction age before the key abiotic conditions reach the levels that induce long diapause. For example, the determining factor for a long or a short diapause in the butterfly *Manduca sexta* is the photoperiod to which the larvae are subject (Denlinger and Bradfield, 1981). Temperature is a primary abiotic factor for the insect seasonal cycle (Hilbert *et al.* 1985; Roff, 1980, 1983). It is also known to influence termination of diapause in *Carnus* (Calero-Torrallbo and Valera, 2008). Thus, temperature is likely to participate in the regulation of diapause initiation in *Carnus*. Although this paper does not disclose any of the conditions that may induce short diapause, such conditions appear to occur between May and July in our study species.

Cases of partial bivoltine cycles in otherwise univoltine populations that cannot be related to climatic conditions have also been reported (Sota, 1988). Studies of such populations suggest that food limitation affects the population dynamics and that the seasonal change in food availability is a limiting factor for the bivoltine cycle (Sota, 1988 and references therein). The wide range of *Carnus* hosts, with varying breeding phenologies, entails a long period of resource availability for the parasite, actually over 5 months in the study area described here. The multivoltine cycle of *Carnus* can therefore be made possible by prolonged host availability (e.g. Kurota and Shimada, 2001) so that *Carnus* would produce long diapausing and short diapausing individuals according to the food prospects. A similar process has been described by He *et al.* (2010) for the parasitic wasp *Platygaster demades*: it seemingly regulates its population by entering aestivation in a proportion of individuals concordant with the expected scale of food shortage to avoid massive mortality when the future food source is expected to be short in different scales. This is more like the case of carnid flies infecting rollers than starlings, even though the percentage of bivoltinism is similar in both species.

Polymorphism in seasonal cycles is a common adaptation that can reduce the probability of extinction in unpredictable habitats and that allows populations to exploit seasonally variable habitats (Tauber and Tauber, 1986). Schlichting and Pigliucci (1998) pointed out that there are 3 evolutionary mechanisms to produce phenotypic variation within populations in differing environments: phenotypic plasticity, genetic variation (polymorphism with regard to diapause characteristics), and risk-spreading strategy. The phenotypic plasticity of voltinism in local populations is still poorly understood and has been reported rarely (He *et al.* 2010). In contrast, some genetic polymorphism and/or a risk-spreading strategy are considered as a reason for over-wintering stage variation within populations in insects (Takafuji

and Morimoto, 1983; Tyshchenko and Kind, 1983; Gerber, 1984). These strategies are very common in inactive stages of insects and give them the possibility to survive, diversify and adapt to stochastic conditions (Menu and Debouzie, 1993; Menu, 1993; Danforth, 1999; Menu and Desouhant, 2002).

In this study we did not control the origin of flies and, therefore, we cannot highlight the mechanisms producing intrapopulation variation in life cycles (nor was that our aim). The evidence of multiple immigrants colonizing single nests suggests that parasite genotypes within nests are mixed, so that flies genetically predisposed to be univoltine could co-exist with multivoltine-coded flies. In any case, the polymorphic life cycle of *Carnus* may have major effects both on the parasite and its host. Bivoltinism would allow *Carnus* to exploit successfully the time frame of resource availability during the breeding season by granting access to early breeders (e.g. resident species like starlings or hoopoes), resident species with a somewhat later phenology (Common kestrels *Falco tinnunculus*, Little owls *Athene noctua*) and late breeding trans-Saharan migrants (European rollers, Bee-eaters *Merops apiaster*). As to the host, the emergence of several generations of *Carnus* means a higher risk of parasitization (e.g. multi-brood species may be parasitized by *Carnus* throughout the breeding season). In turn, this may influence the host's strategies to avoid this pressure, e.g. by nest site selection, nest cleaning behaviour or development of strategies to increase the asynchrony between the parasite's cycle and the host's cycle. Our results also have implications at a practical level: the co-occurrence of univoltinism, bivoltinism and delayed voltinism in this species will make it difficult to distinguish individual generations, so population studies may become difficult. Thus, the length of the life cycle and emergence cannot be predicted easily and population control is probably ineffective in the absence of detailed information on the insects' stage and/or favourable conditions for the development of a short or long cycle.

This paper does not identify the conditions that may favour the induction of short diapause. Prolonged host availability and a range of nests where the parasite may thrive offer a wide range of environmental conditions that may favour a plastic response (Via, 1992; Scheiner, 1993). Thus, experimental research based on precise knowledge of the natural conditions that *Carnus* larvae and pupae are subject to in spring, may disclose the factors governing diapause onset and termination in this species.

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REFERENCES

- Bradford, M. J. and Roff, D. A.** (1993). Bet hedging and the diapause strategies of the cricket *Allonemobius fasciatus*. *Ecology (Washington D C)* **74**, 1129–1135.
- Calero-Torralbo, M. A. and Valera, F.** (2008). Synchronization of host-parasite cycles by means of diapause: host influence and parasite response to involuntary host shifting. *Parasitology* **135**, 1343–1352.
- Campbell, A. and Mackauer, M.** (1975). Thermal constants for development of the pea aphid (homoptera: aphididae) and some of its parasites. *The Canadian Entomologist* **107**, 419–423.
- Capelle, K. J. and Whitworth, T. L.** (1973). The distribution and avian hosts of *Carnus hemapterus* (Diptera: Milichiidae) in North America. *Journal of Medical Entomology* **10**, 525–526.
- Cramp, S.** (1998). *Handbook of the Birds of Europe, Middle East and North Africa*. Vol. IV. Oxford University Press, Oxford, UK.
- Danforth, B. N.** (1999). Emergence dynamics and bet hedging in a desert bee, *Perdita portalis*. *Proceedings of the Royal Society of London, B* **266**, 1985–1994.
- Danks, H. V.** (1987). *Insect Dormancy: an ecological perspective*. *Biological Survey of Canada* No. 1, Ottawa, Ontario, Canada.
- Danks, H. V.** (1992). Long life-cycles in insects. *Canadian Entomologist* **124**, 167–187.
- Danks, H. V.** (2002). The range of insect dormancy responses. *European Journal of Entomology* **99**, 127–142.
- Danks, H. V. and Footitt, R. G.** (1989). Insects of the boreal zone of Canada. *Canadian Entomologist* **121**, 625–690.
- Dawson, R. D. and Bortolotti, G. R.** (1997). Ecology of parasitism of nesting American Kestrels by *Carnus hemapterus* (Diptera, Carnidae). *Canadian Journal of Zoology* **75**, 2021–2026.
- Denlinger, D. L. and Bradfield, J. Y., IV.** (1981). Duration of pupal diapause in the tobacco hornworm is determined by number of short days received by the larva. *Journal of Experimental Biology* **91**, 331–337.
- Gag, S. H. and Haynes, D. L.** (1975). Emergence under natural and manipulated conditions of *Tetrastichus julis*, an introduced larval parasite of the cereal leaf beetle, with reference to regional population management. *Environmental Entomology* **4**, 425–434.
- Gerber, G. H.** (1984). Influence of date of oviposition on egg hatching and embryo survival in the red turnip beetle, *Entomoscelis americana* (Coleoptera: Chrysomelidae). *Canadian Entomologist* **116**, 645–652.
- Grimaldi, D.** (1997). The birds flies, Genus *Carnus*: species revision, generic relationships and a fossil *Meoneura* in amber (Diptera: Carnidae). *American Museum Novitates* **3190**, 1–30.
- Guiguen, C., Launay, H. and Beaucournu, J. C.** (1983). Ectoparasites des oiseaux en Bretagne. I. Répartition et écologie d'un diptère hematophage nouveau pour la France: *Carnus hemapterus* Nitzsch. *Revue Francaise d'Entomologie* **5**, 54–62.
- He, X. Z., Wang, Q., Walker, J. T. S., Rogers, D. J. and Lo, P. L.** (2010). A sophisticated life history strategy in a parasitoid wasp: Producing univoltine and multivoltine phenotypes in a local population. *Biological Control* **54**, 276–284.
- Hilbert, D. W., Logan, J. A. and Swift, D. M.** (1985). A unifying hypothesis of temperature effects on egg development and diapause of the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Journal of Theoretical Biology* **112**, 827–838.
- Hopper, K. R.** (1999). Risk-spreading and bet-hedging in insect population biology. *Annual Review of Entomology* **44**, 535–560.
- Kivelä, S. M., Välimäki, P., Oksanen, J., Kaitala, A. and Kaitala, V.** (2009). Clines of evolutionary stable reproductive effort in insects. *The American Naturalist* **174**, 526–536.
- Kostal, V.** (2006). Eco-physiological phases of insect diapause. *Journal of Insect Physiology* **52**, 113–127.
- Kurota, H. and Shimada, M.** (2001). Photoperiod- and temperature-dependent induction of larval diapause in a multivoltine bruchid, *Bruchidius dorsalis*. *Entomologia Experimentalis et Applicata* **99**, 361–369.
- Liker, A., Markus, M., Vozár, A., Zemankovics, E. and Rózsa, L.** (2001). Distribution of *Carnus hemapterus* in a starling colony. *Canadian Journal of Zoology* **79**, 574–580.
- López-Rull, I., Gil, M. and Gil, D.** (2007). Spots in starling *Sturnus unicolor* eggs are good indicators of ectoparasite load by *Carnus hemapterus* (Diptera: Carnidae). *Ardeola* **54**, 131–134.
- Masaki, S.** (1980). Summer diapause. *Annual Review of Entomology* **25**, 1–25.
- Matyukhin, A. V. and Krivosheina, M. G.** (2008). To the knowledge of Diptera (Insecta)- Parasites of birds. *Zoologicheskyy Zhurnal* **87**, 124–125.
- Menu, F.** (1993). Strategies of emergence in the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae). *Oecologia, Berlin* **96**, 383–390.
- Menu, F. and Debouzie, D.** (1993). Coin-flipping plasticity and prolonged diapause in insects: example of the chestnut weevil *Curculio elephas* (Coleoptera: Curculionidae). *Oecologia, Berlin* **93**, 367–373.
- Menu, F. and Desouhant, E.** (2002). Bet-hedging for variability in life cycle duration: Bigger and later-emerging chestnut weevils have increased probability of a prolonged diapause. *Oecologia, Berlin* **132**, 167–174.
- Menu, F., Roebuck, J. P. and Viala, M.** (2000). Bet-hedging diapause strategies in stochastic environments. *American Naturalist* **155**, 724–734.
- Papp, L.** (1998). Family Carnidae. *Manual of Palaearctic Diptera* **3**, 211–217.
- Parrish, D. S. and Davis, D. W.** (1978). Inhibition of diapause in *Bathyplectes curculionis*, a parasite of the alfalfa weevil. *Annals of the Entomological Society of America* **71**, 103–107.
- Peris, A. S.** (1984). Descripción y desarrollo del pollo del estornino negro. *Ardeola* **31**, 3–16.
- Price, T. D., Qvarnstrom, A. and Irwin, D. E.** (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London, B* **270**, 1433–1440.
- Reiczigel, J., Abonyi-Tóth, Z. and Singer, J.** (2008). An exact confidence set for two binomial proportions and exact unconditional confidence intervals for the difference and ratio of proportions. *Computational Statistics and Data Analysis* **52**, 5046–5053.
- Reiczigel, J. and Rozsa, L.** (2005). *Quantitative Parasitology 3.0*. Budapest, Hungary. Distributed by the authors.
- Roff, D.** (1980). Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. *Oecologia, Berlin* **45**, 202–208.
- Roff, D.** (1983). Phenological adaptation in a seasonal environment: a theoretical perspective. *Series Entomologica, Dordrecht* **23**, 253–270.
- Roulin, A.** (1998). Cycle de reproduction et abondance du diptère parasite *Carnus hemapterus* dans le niches de chouettes effraies *Tyto alba*. *Alauda* **66**, 265–272.
- Sabelis, M. W. and Janssen, A.** (1994). Evolution of life-history patterns in the Phytoseiidae. In *Mites: Ecological and Evolutionary Analyses of Life-History Patterns* (ed. Houck, M. A.), pp. 70–98. Chapman and Hall; New York, USA and London, UK.
- Scheiner, S. M.** (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics* **24**, 35–68.
- Schlichting, C. D. and Pigliucci, M.** (1998). *Phenotypic Evolution: a Reaction Norm Perspective*. Sinauer Associates, Sinauer, MA, USA.
- Soula, B. and Menu, F.** (2003). Variability in diapause duration in the chestnut weevil: mixed ESS, genetic polymorphism or bet-hedging? *Oikos* **100**, 574–580.
- Sota, T.** (1988). Ecology of a gall-forming thrips, *Ponticulothrips diospyrosi*: colony development and gall-associated arthropod community (Thysanoptera: Phlaeothripidae). *Applied Entomology and Zoology* **23**, 345–352.
- Stearns, S. C.** (1976). Life history tactics: a review of the ideas. *Quarterly Review of Biology* **51**, 3–47.
- Takafuji, A. and Morimoto, N.** (1983). Diapause attributes and seasonal occurrences of two populations of the citrus red mite, *Panonychus citri* (McGregor) on pear (Acarina: Tetranychidae). *Applied Entomology and Zoology* **18**, 525–532.
- Tauber, M. J. and Tauber, C. A.** (1970). Adult diapause in *Chrysopa carnea*: Stages sensitive to photoperiodic induction. *Journal of Insect Physiology* **16**, 2075–2080.
- Tauber, C. A. and Tauber, M. J.** (1981). Insect seasonal cycles: genetics and evolution. *Annual Review of Ecology and Systematics* **12**, 281–308.
- Tauber, M. J., Tauber, C. A. and Masaki, S.** (1986). *Seasonal Adaptations of Insects*. Oxford University Press, Oxford, UK.
- Taylor, F. and Spalding, J. B.** (1988). Fitness functions for alternative developmental pathways in the timing of diapause induction. *The American Naturalist* **131**, 678–699.
- Tillman, P. G. and Powell, J. E.** (1991). Developmental time in relation to temperature for *Microplitis croceipes*, *M. demolitor*, *Cotesia kazak* (Hymenoptera: Braconidae), and *Hyposoter didymator*

(Hymenoptera: Ichneumonidae), endoparasites of the tobacco budworm (Lepidoptera: Noctuidae). *Environmental Entomology* **20**, 61–64.

Tyshchenko, V. P. and Kind, T. V. (1983). Neuroendocrine mechanisms regulating the seasonal cycles. *Trudy Vsesoyuznogo Entomologicheskogo Obshchestva* **64**, 82–117.

Václav, R., Valera, F. and Martínez, T. (2010). Social information in nest colonisation and occupancy in a long-lived, solitary breeding bird. *Oecologia* **165**, 617–627.

Valera, F., Casas-Crivillé, A. and Hoi, H. (2003). Interspecific parasite exchange in a mixed colony of birds. *Journal of Parasitology* **89**, 245–250.

Valera, F., Casas-Crivillé, A. and Calero-Torralbo, M. A. (2006). Prolonged diapause in the ectoparasite *Carnus hemapterus*

(Diptera: Cyclorhapha, Acalyptratae) – how frequent is it in parasites? *Parasitology* **133**, 179–186.

Valimaki, P., Kivela, S. M. and Jaaskelainen, L. (2008). Divergent timing of egg-laying may maintain life history polymorphism in potentially multivoltine insects in seasonal environments. *Journal of Evolutionary Biology* **21**, 1711–1723.

Via, S. (1992). Adaptive phenotypic plasticity: target or by-product of selection in a variable environment? *The American Naturalist* **142**, 352–365.

Winterhalter, W. E. and Mousseau, T. A. (2007). Patterns of phenotypic and genetic variation for the plasticity of diapause incidence. *Proceedings of the Royal Society of London, B* **274**, 1211–1217.