

Serena Santolamazza-Carbone ·  
Adolfo Cordero Rivera

## Superparasitism and sex ratio adjustment in a wasp parasitoid: results at variance with Local Mate Competition?

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**Abstract** *Anaphes nitens* is a solitary parasitoid of the egg capsules of the *Eucalyptus* snout beetle, *Gonipterus scutellatus*. Some traits of its natural history suggest that Local Mate Competition (LMC) could account for sex ratio adjustment in this species. We tested whether males emerged early, a prerequisite for fully local mating, and investigated the occurrence and effect of superparasitism on adult size and pre-emergence mortality, factors that might influence sex ratio adjustment. We found in field-collected egg capsules that males emerged first. To investigate the effects of superparasitism on adult size, we compared the sizes of parasitoids that emerged early and late from egg capsules collected in the field, and from egg capsules parasitized and superparasitized in the laboratory. Superparasitism reduced parasitoid size, affecting females more strongly than males, and increased pre-emergence mortality. We estimated *A. nitens* sex ratio and parasitism rate in the field during 2 years in five localities and during 4 years in a sixth. Following LMC we expected an increase in sex ratio (proportion of males) with increasing parasitism rate (assumed to reflect parasitoid density). We found that sex ratio decreased from 0.38 when the parasitism rate was low (0–20%) to 0.21 when parasitism was high (80–100%). In contrast with field results, a laboratory experiment showed that: (1) at a low parasitism level sex ratio was clearly female biased ( $0.28 \pm 0.04$ ), (2) at a high parasitism level sex ratio increased ( $0.40 \pm 0.07$ ), (3) male larval survivorship was not lower than female survivorship, and (4) low-quality hosts (i.e. superparasitized) were allocated more males. We conclude that LMC cannot explain the sex ratio adjustment observed in the field, even at low parasitism rates, and alternative explanations for highly female-

biased sex ratios must be found. One such alternative is female-biased dispersal.

**Keywords** *Anaphes nitens* · Body size · Host density · Mymaridae · Pre-emergence mortality

### Introduction

Among hymenopteran parasitoids, optimal sex ratio adjustment can be influenced by the mother's life history (i.e. age, size, egg load, diet, time since insemination, genetic factors, experience), host species, host density and quality (i.e. size, sex, age, previous parasitism), adult population sex ratio, competition with other foragers, seasonal changes in temperature, photoperiod and humidity (King 1987; Godfray 1994). Furthermore, beyond the mother's decision, sex ratio can also be explained as the result of sperm reserve depletion or virginity (King 1987; Godfray 1990), larval developmental mortality (Wellings et al. 1986; Hardy et al. 1998), physical (Mackauer et al. 1992; Nénon et al. 1995; van Baaren et al. 1997) or physiological suppression due to superparasitism (Vinson 1980; Vinson and Hegazi 1998), *Wolbachia* infection (Stouthamer et al. 2002), ovicide (Antolin et al. 1995), hyperparasitism (Heinz 1996) or encapsulation (van Alphen and Visser 1990; Strand and Pech 1995). Therefore, we should distinguish between primary sex ratio (before any mortality factor occurs) and secondary sex ratio (after all mortality factors have acted) (Godfray 1994).

As the above list of factors clearly suggests, a single sex ratio theory is unlikely to predict sex ratio bias under all ecological circumstances. Nevertheless, the theory of Local Mate Competition [LMC (Hamilton 1967)] has been strongly supported by studies of solitary, gregarious and facultatively gregarious species (King 1987; King 1996; Godfray 1994). LMC predicts that in a haplodiploid structured population, interactions between relatives can lead to biased sex allocation. Solitary parasitoids that attack solitary hosts are likely to be more panmictic in

S. Santolamazza-Carbone (✉) · A. Cordero Rivera  
Grupo de Ecología Evolutiva,  
Departamento de Ecología e Biología Animal,  
Universidade de Vigo, Campus Universitario,  
E.U.E.T. Forestal, 36005 Pontevedra, Galicia, Spain  
e-mail: serena@uvigo.es  
Fax: +34-986-801907

mating than gregarious species due to host distribution (Waage 1986), but if they parasitize aggregated hosts, then sex ratio should be far from the Fisherian equilibrium (Fisher 1930), because competition among related males to mate with their sisters should take place. LMC provides an evolutionary explanation for the influence of superparasitism and parasitoid density on sex ratios (Godfray 1994). Superparasitism (self and conspecific) usually increases with the number of foragers (van Alphen and Visser 1990; Visser et al. 1990; van Dijken and van Alphen 1991), consequently the second female visiting the host should decide the offspring sex ratio in relation to the sex ratio and clutch size produced by the first female, and the general prediction is that the second female should produce fewer eggs and a more male-biased sex ratio (Werren 1980; Waage 1986; van Baaren et al. 1999). On the other hand, superparasitism can provoke important fitness penalties on size, longevity and fecundity, especially in species whose females have a positive relationship between size and egg supply (Godfray 1994; King 1987). Furthermore, superparasitism can also represent an important mortality factor, particularly in solitary parasitoids (Vinson 1980; Harvey et al. 1993; Potting et al. 1997; Hanks et al. 2000; King 1987).

In this study we tested LMC theory as a possible evolutionary explanation for sex ratio adjustment, using a system of three exotic species introduced to Spain: eucalypts, the eucalypt snout beetle *Gonipterus scutellatus* Gyllenhal (Coleoptera: Curculionidae) and its egg parasitoid *Anaphes nitens* Girault (Hymenoptera: Mymaridae).

*A. nitens* and its host have been studied since the beginning of the twentieth century by entomologists from many continents, because of important damage produced by the coleopteran to plantations of eucalypts and the use of the parasitoid for biological control (Tooke 1955; Arzone and Vidano 1978; Kidd and Jervis 1997; Cordero Rivera et al. 1999; Hanks et al. 2000). *G. scutellatus* was accidentally introduced to Galicia (NW Spain) in 1991 and in 1994 *A. nitens* was released as a pest control agent, producing good results very quickly (Mansilla and Pérez Otero 1996). *G. scutellatus* females lay clumped eggs on young eucalypt leaves, covered by a dark secretion mainly composed of faeces, which becomes very hard after some hours. These egg capsules contain a mean of eight eggs. Consequently, despite *A. nitens* being a strictly solitary parasitoid, it develops in a quasi-gregarious fashion.

Some evidence suggests that *A. nitens* is an appropriate species to test the generality of LMC theory in explaining sex ratio variation: (1) offspring emerge together from the same egg capsule (Tooke 1955), (2) they start immediately to mate, probably in part among siblings (Mossop 1929; Williams et al. 1951; Tooke 1955; Vidano et al. 1979), (3) in the laboratory and in the field *A. nitens* shows a female-biased sex ratio (Mossop 1929; Tooke 1955), and (4) females are able to distinguish between old and young hosts and between parasitized and unparasitized hosts (Santolamazza Carbone et al. 2003).

Here we tested whether males emerged early, because protandry could be a possible prerequisite for fully local mating (Hardy 1994). Moreover, we investigated the existence of superparasitism in the field and its negative effect on adult size and pre-emergence mortality, as factors that might influence sex ratio decisions. Finally we tested LMC through the study of the relationship between sex ratio and parasitism rate in *A. nitens*. If parasitism rate reflects parasitoid density (Cronin and Strong 1993), then it could be used as an indicator of competition inside the patch. Previous experimental evidence for sex ratio adjustment within the genus *Anaphes* has been obtained under laboratory conditions (van Baaren et al. 1999), but field studies are crucial to understand sex ratio variation in nature. With this aim, we sampled in five different eucalypt plots during 2 years and during 4 years in a sixth.

## Materials and methods

### Eclosion sequence

Some authors (Tooke 1955; Vidano 1979) in the context of *A. nitens* mass rearing for biological control have mentioned the early emergence of males, but until now no laboratory tests have been performed. On 18 April 1996, 144 egg capsules collected in a plot at Lourizán (NW Spain) were placed individually in plastic tubes (6×1 cm) and stored in the laboratory at room temperature (20°C, 70% relative humidity). Egg capsules were checked every day (until 24 May), and the sex of the eclosed parasitoids was recorded.

### Superparasitism effect on size and mortality

We hypothesized that adults from superparasitized hosts should be smaller, and should suffer higher pre-emergence mortality, because they have shared the host egg with several competitors.

### Field

During spring 1998 egg capsules were collected on six plots in NW Spain, when the egg production of *G. scutellatus* was at maximum (on 30 May and 13–14 June). All the egg capsules were individually placed in plastic tubes (6×1 cm), and checked daily over 1 month, recording the sex of the emerging parasitoids. Parasitism rate in this period reached 90–100%, so it is highly likely that superparasitism occurred.

Emergent adults were frozen ( $n=479$ ), and adult size was calculated by measuring right forewing length. The wing was mounted on a glass slide in a droplet of water and its image was digitized and measured using Global Lab 3.0 software. The response variate was wing length, and the effects of sex (fixed), date of eclosion (random), locality (random) and collection date (random) were tested with an ANOVA.

To measure pre-emergence mortality rate in the field we used 50 egg capsules, collected during spring 1997 in three localities with contrasting parasitism rates. Our expectation was to find an increase in mortality at higher parasitism rates (a measure of how likely was superparasitism). Egg capsules were maintained in the laboratory and the number of adult parasitoids and *G. scutellatus* larvae to emerge was recorded. After 1 month, the egg capsules were dissected under the microscope. This allowed us to estimate the total number of host eggs per capsule by adding to the emerged parasitoids and beetle larvae, the number of dead *G. scutellatus* larvae or undeveloped eggs and the number of dead adult

parasitoids. We considered that superparasitism should provoke parasitoid mortality at an early stage (Vinson 1980; Hanks et al. 2000), due to overcrowding inside the host, so the mortality rate was calculated as the number of undeveloped eggs divided by the total number of eggs per capsule. In this analysis, the dependent variable was the number of eggs that died during the first stages of development. We analysed the effect of the locality with a generalized linear model (GLM) with binomial errors and logit link, using the total number of eggs per capsule as the binomial denominator (Crawley 1993). To account for overdispersion, we used procedure extrabinomial in GenStat 6.1 software (GenStat 2000).

### Laboratory

To confirm if superparasitism plays a role in adult size and larval mortality, we measured the adults emerging from parasitized and superparasitized egg capsules obtained during the laboratory experiment on sex ratio (see below). The right forewing length was measured as described above. We compared the overall mean length of the two groups, the difference between sexes, as well as the relationship between forewing length and date of eclosion with an ANOVA.

All the egg capsules were dissected after parasitoid emergence. Mortality rate (see method described above) for each treatment (parasitized or superparasitized) was compared with a GLM with binomial errors as described above.

### Sex ratio and parasitism rate

If *A. nitens* females can assess host quality and competitor density in the patch, then they should adjust offspring sex ratio accordingly. We predict that if LMC takes place, sex ratio (proportion of males) must increase when parasitism rate (assumed to represent parasitoid population density) increases.

### Field

To study sex ratio in the field, six experimental plots (Lourizán, Tomba, Cotorredondo, Bora, Cotobade and Folgoso) were established in eucalypt plantations in Galicia (NW Spain). The first three plots were located on the coast, and the remaining ones far away from the sea, in order to have different climatic conditions. Distances between plots were in the range 10–20 km. At each plot we randomly selected ten small trees (2–3 m high) and marked them with a plastic tag. At intervals of 10–15 days we collected all the egg capsules found on the trees, transported them to the laboratory and placed them in plastic tubes. If the number of egg capsules was greater than nine per tree, a random sample of nine egg capsules per tree (90 per plot) was analysed. The tubes were examined every 2 days, and the number and the sex of the parasitoids that eclosed were recorded, as well as the number of host larvae that emerged. At Lourizán plot the study started in March 1996, and lasted until December 1999 (in this plot, we followed 25 trees and analysed all the egg capsules found during the first 2 years). The remaining plots were sampled from January 1998 to December 1999.

We obtained data on parasitoid sex ratio (number of males/total parasitoids), on parasitism rate and host density (number of egg capsules per tree). Given the large number of hosts analysed (5,460 egg capsules), we were unable to count egg mortality by host dissection. Therefore, we assumed that every host egg produced one parasitoid or one beetle larva, and estimated parasitism rate as parasitoids/(parasitoids+beetle larvae emerged). This is a conservative estimate due to parasitoids dying inside the egg capsule (see Results and also Cordero Rivera et al. 1999). We analysed the effect of parasitism rate and host density (mean number of egg capsules per tree), and the interaction between both, on the number of male parasitoids emerging daily, using a GLM with binomial

errors and logit link, with the total number of parasitoids emerging daily as the binomial denominator (Crawley 1993). To account for overdispersion, we used procedure extrabinomial in GenStat 6.1 software (GenStat 2000).

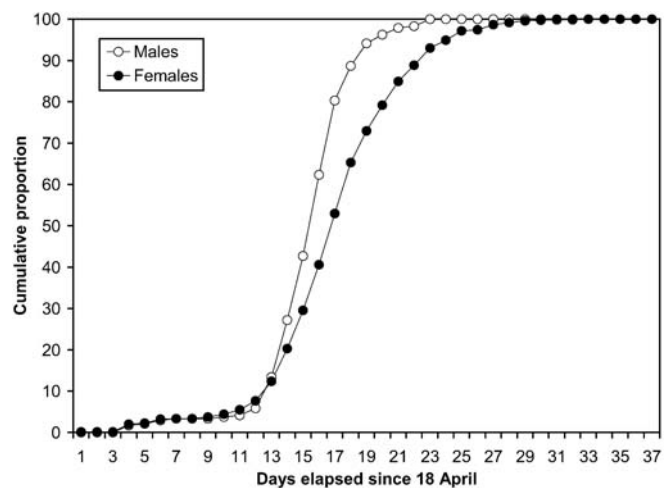
### Laboratory

We used 1-day-old adults of *A. nitens* that had emerged from egg capsules collected in the field, to obtain parasitized and superparasitized hosts. To obtain parasitized hosts, 40 females were mated and introduced individually into a Petri dish (10 cm in diameter, 2 cm in high) containing only one fresh egg capsule (1-day old), on a disk of wet filter paper. Egg capsules were obtained from adults of *G. scutellatus* captured in the field and maintained in the laboratory. Each female was continually observed under a binocular microscope and removed from the arena after parasitization, when she left the egg capsule for >2 min (typically after a visit of 40 min). To obtain superparasitized hosts ( $n=57$ ) we offered three egg capsules to groups of six mated females simultaneously. We observed females' behaviour under the microscope, to check that egg capsules were effectively superparasitized. After 1 h the egg capsules were taken from the Petri dish. Egg capsules were stored and checked daily as described above. The effect of treatment on the number of males emerging per egg capsule was analysed with a GLM as described above (the egg capsules that produced none or only one parasitoid were excluded from analysis).

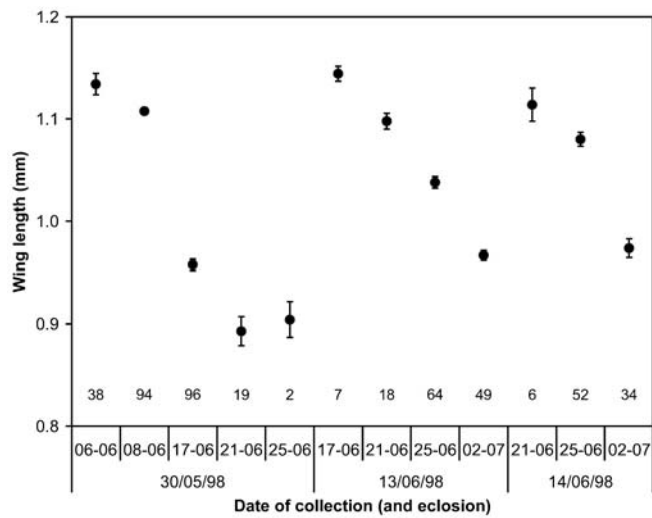
## Results

### Eclosion sequence

From the 144 field-collected egg capsules 239 males and 744 females emerged. The mean emergence date was earlier for males (Mann-Whitney  $U$ -test on the mean date of eclosion,  $U=2.83$ ,  $P=0.005$ ). The cumulative proportion of emergence indicates that 50% of the population emerged by day 15–16 (males) and 17 (females), and 98% had emerged by day 21 (males) and 27 (females) (see Fig. 1).



**Fig. 1** Cumulative emergence of male and female *Anaphes nitens* from 144 field-collected hosts (egg capsules were collected on 18 April 1996 and examined until 24 May). The mean emergence date was earlier for males (Mann-Whitney  $U$ -test on the mean date of eclosion,  $U = 2.83$ ,  $P = 0.005$ )



**Fig. 2** The effect of date of eclosion on size of *A. nitens*, from field-collected egg capsules ( $n=479$  adults) (mean $\pm$ SE). The last adult parasitoids to emerge were clearly smaller at all dates of eclosion ( $F_{5,465}=239.59$ ,  $P<0.0001$ ). Numbers indicate sample size

### Superparasitism effect on size and mortality

#### Field

The last adult parasitoids to emerge were clearly smaller at all dates of collection (Fig. 2). In all six plots males were larger than females (mean wing length, localities combined, males,  $1.01\pm 0.01$  mm,  $n=112$ ; females,  $0.98\pm 0.005$ ,  $n=367$ ). Although all factors were significant (sex,  $F_{1,465}=23.23$ ,  $P<0.0001$ ; date of eclosion,  $F_{5,465}=239.59$ ,  $P<0.0001$ ; locality,  $F_{5,465}=8.28$ ,  $P<0.0001$ ; date of collection,  $F_{2,465}=196.43$ ,  $P<0.0001$ ), date of eclosion was clearly the most important factor.

The average mortality rate (undeveloped eggs) of field-collected egg capsules was  $10.6\pm 3.9\%$  ( $n=14$ ) when parasitism was 28%,  $4.4\pm 1.9\%$  ( $n=24$ ) at 42% parasitism and  $24.9\pm 8.8\%$  ( $n=12$ ) at 83% parasitism. Results indicate a significant difference between localities (GLM with binomial errors corrected for overdispersion and logit link,  $P=0.012$ ). There was a positive relationship between mortality and parasitism rate (GLM with binomial errors corrected for overdispersion and logit link; localities combined;  $P=0.008$ ).

#### Laboratory

We compared right forewing length of males ( $0.94\pm 0.03$  mm,  $n=16$ ) and females ( $0.99\pm 0.05$ ,  $n=46$ ) emerged from parasitized egg capsules, and of males ( $0.91\pm 0.05$ ,  $n=16$ ) and females ( $0.89\pm 0.09$ ,  $n=16$ ) emerged from superparasitized egg capsules. An ANOVA with sex and treatment (parasitized vs. superparasitized) as factors indicates that sex does not have a significant effect on size ( $F_{1,90}=2.43$ ,  $P=0.122$ ), but individuals from superpara-

**Table 1** Spearman rank correlation coefficients ( $r$ ) between parasitism rate and sex ratio.  $n$  Number of days,  $P^*$  critical  $P$ -value after sequential Bonferroni correction per locality (Hochberg method)

Locality	Year	$N$	Spearman $r$	$P$	$P^*$
Lourizán	1996	8	0.54	0.170	0.050
	1997	19	-0.44	0.057	0.038
	1998	17	-0.76	<0.001	0.013
	1999	7	-0.89	0.007	0.025
Bora	1998	15	-0.51	0.054	0.025
	1999	14	-0.44	0.120	0.050
Cotobade	1998	22	-0.43	0.048	0.050
	1999	19	-0.58	0.009	0.025
Cotorredondo	1998	20	-0.51	0.022	0.025
	1999	14	-0.39	0.173	0.050
Folgosó	1998	14	-0.17	0.572	0.025
	1999	12	-0.58	0.046	0.025
Tomba	1998	17	-0.51	0.037	0.050
	1999	12	-0.63	0.027	0.025

sitized hosts were smaller ( $F_{1,90}=37.76$ ,  $P=0.0001$ ) and the interaction was significant ( $F_{1,90}=8.61$ ,  $P=0.004$ ). Female size was more affected by treatment than male size.

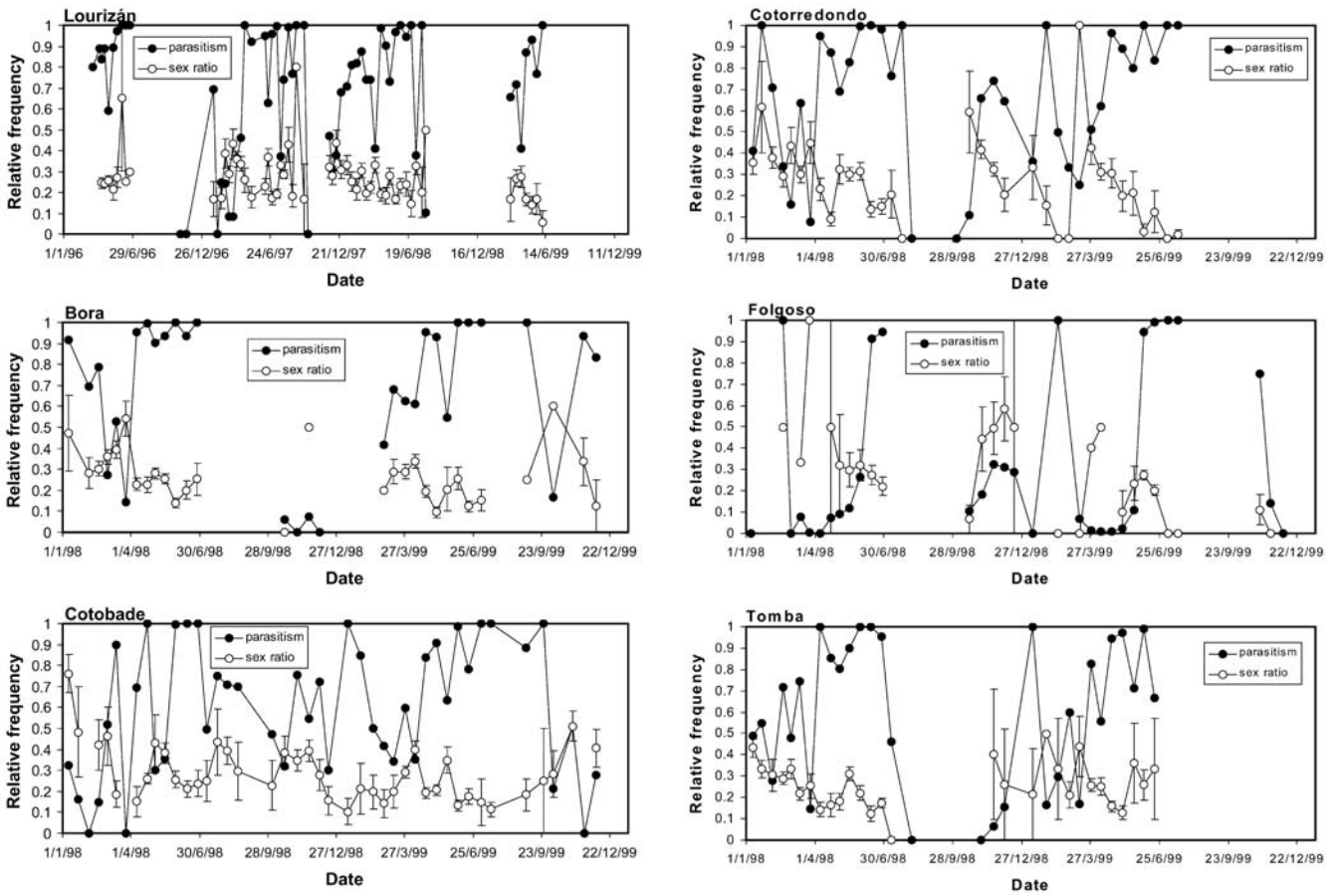
There was no significant effect of date of emergence on right forewing length of parasitoids ( $n=62$ ) which eclosed from parasitized hosts ( $F_{3,58}=0.84$ ,  $P=0.481$ ). This test could not be calculated for superparasitized eggs because adult emergence occurred on insufficient dates per egg capsule. We measured pre-emergence mortality in parasitized ( $0.05\pm 0.03$ ,  $n=40$  egg capsules) and superparasitized egg capsules ( $0.73\pm 0.04$ ,  $n=57$ ). Pre-emergence mortality rate was clearly higher in superparasitized eggs (GLM with binomial errors and logit link,  $P=0.001$ ).

### Sex ratio and parasitism rate

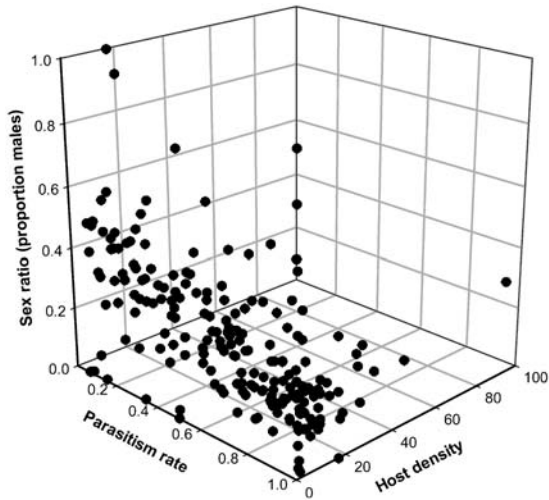
#### Field

The overall sex ratio in the field was  $0.24\pm 0.01$ , based on 5,460 egg capsules collected on 241 samples in all localities (27,943 parasitoids). At a low parasitism rate (0–20%) sex ratio was 0.38, but it decreased to 0.21 at a high parasitism rate (80–100%). Figure 3 summarizes the temporal changes in sex ratio and parasitism rate. In general, sex ratio was higher at the start of the year, and gradually decreased, while parasitism increased. Table 1 presents the relationship between parasitism rate (assumed to be an estimate of parasitoid density) and sex ratio. In all but one case ( $n=14$ ) this relationship was negative, and in six cases it was strong enough to be significant, after sequential Bonferroni correction by locality.

Given the above results, we merged all the data from the six plots to increase sample size and analysed the effect of parasitism and host density on sex ratio with a GLM with binomial errors (see Materials and methods). Results indicate that parasitism rate had a negative effect



**Fig. 3** The variation in parasitism rate of egg capsules of *Gonipterus scutellatus* by *A. nitens*, and the sex ratio (mean±SE) of the emerging parasitoids, in six localities of Galicia (NW Spain). Note that for Lourizán data span over 4 years



**Fig. 4** The effect of parasitism rate and host density (mean number of hosts per tree) on sex ratio in field plots of Galicia. Parasitism rate had a negative effect on sex ratio ( $P < 0.001$ ), but the effect of host density is not significant ( $P = 0.077$ )

on sex ratio (Fig. 4,  $P < 0.001$ ), but host density ( $P = 0.076$ ) and the interaction term were not significant ( $P = 0.052$ ), although both are close to significance.

*Laboratory*

The mean sex ratio obtained from the parasitized egg capsules was  $0.28 \pm 0.04$  ( $n = 36$  egg capsules and 235 adults examined), while from superparasitized egg capsules it was  $0.40 \pm 0.07$  ( $n = 32$  egg capsules and 108 adults; GLM,  $P = 0.017$ ).

**Discussion**

The prerequisite of inbreeding for LMC has been long debated, because it could only be a correlated effect of subdivided populations and not a selective agent for the observed sex ratio bias (Antolin 1993). Nevertheless, we consider that the early emergence of males, confirmed by the results of the first experiment, may favour inbreeding and should be considered as circumstantial evidence of local mating (Hardy 1994). If competition for mates takes place and females mate only once and are immediately

receptive after emergence, then selection might favour males that emerge early because this can increase the number of mates they can inseminate (Godfray and Cook 1997). If both sexes share the pupation site, as occurs in gregarious species or in solitary parasitoids that attack clumped hosts, then for the emerged male it is important to wait for the emerging females (Godfray and Cook 1997). *A. nitens* males have been occasionally observed in the field resting on the egg capsule, probably waiting for the emergence of new females (Tooke 1955; personal observation).

Our experiments indicate that superparasitism occurs in the field (see also Hanks et al. 2000) and that it has a clear negative effect on female size. Even if superparasitism could be adaptive under some circumstances (van Alphen and Visser 1990), it is reasonable to suppose that mothers should avoid it when it has low fitness returns. The laboratory tests indicate that: (1) in superparasitized egg capsules the reduction in size of adults was stronger for females (this is in agreement with the result from field-collected egg capsules and suggests that the reduction in size found in adults eclosed from field egg capsules is due to superparasitism, even if alternative explanations like climatic effects cannot be completely ruled out); (2) there is not a significant difference between wing length of first and last eclosed adults from parasitized hosts, which suggests that the lack of competition ensures large size.

In most parasitoid wasps there is sexual size dimorphism (Hurlbutt 1987). By measuring parasitoids emerging from field-collected egg capsules, we found that *A. nitens* males are larger than females (this difference was less clear from adults obtained in the laboratory, probably due to a smaller sample size). Nevertheless, in this species the most effective method to distinguish sexes is still by checking the number and the shape of antennal articles (Huber and Prinsloo 1990).

In female *A. nitens*, size is positively related to egg load at birth (Santolamazza Carbone and Cordero Rivera 2003). This means that reduced size could directly affect female reproductive fitness. Furthermore, being larger should be crucial for female dispersal ability, as has been shown for the solitary parasitoid *Asobara tabida* (Ellers et al. 1998). Whether small males of *A. nitens* suffer any disadvantage at mating, as has been shown in other parasitoids (van den Assem 1989), or in survivorship, is not clear at the moment, even if has been suggested that in *Anaphes flavipes* smaller males are less successful at mating (Anderson and Paschke 1969). Nevertheless, being smaller could be advantageous for males because they can experience a faster development and an earlier emergence, ahead of larger males, increasing in that way their mating opportunities (King 1987).

Pre-emergence mortality rate was significantly greater at higher parasitism rates in the laboratory and in the field. In a previous laboratory experiment (Santolamazza Carbone 2002) we found that multiple mated *G. scutellatus* females laid very few sterile eggs ( $4.2 \pm 0.01\%$ ,  $n=3,061$ ). Moreover, Hanks et al. (2000), describing a

biological control program to introduce *A. nitens* in California, noted that unviable eggs of *G. scutellatus* were almost absent before the biological control plan had started and that they increased with time as the parasitoid population increased. We agree with these authors that undeveloped eggs were mostly superparasitized eggs. The field result might be interpreted as the effect of differences in ecological factors between localities. Nevertheless, we think that the crucial factor is superparasitism, because there was a positive relationship between mortality and parasitism rate, and because laboratory experiments have supported the influence of superparasitism on parasitoid mortality.

Field data on sex ratio surprisingly indicate that in contrast with LMC predictions, when parasitism rate and presumably competition and superparasitism increased, sex ratio decreased (see Fig. 4). In a previous paper (Cordero Rivera et al. 1999) we pointed out the existence of fluctuations over the years in *A. nitens* and *G. scutellatus* populations, probably due to the high efficiency of *A. nitens* as a pest control agent, that provoke temporary local beetle extinction. In the present field experiment, the fluctuations in sex ratio confirmed that this variable was very sensitive to changes in ecological circumstances. Fluctuations and cycles are not unusual features in forest and agricultural entomology (Liebhold 2000; Hassel 2000), and they might be strictly related to host-parasitoid interactions (Godfray and Müller 1998). Sampling duration and total sample size are large enough to conclude that the observed sex ratio pattern is not an artefact. The trend shown in Figs. 3 and 4 is not consistent with the predictions of LMC. The sex ratio (0.21) found when parasitism rate is up to 100%, is lower than that expected from LMC. If we apply Hamilton's (1967) formula:  $r = (n-1)(2n-1)/[n(4n-1)]$ , where  $r$  is the expected offspring sex ratio and  $n$  the number of ovipositing females, then a sex ratio of 0.21 is the value expected when there are only two foundresses in the patch. On the other hand, the sex ratio at low parasitism rate (0.38) is still not completely in agreement with LMC predictions (0.38 is the offspring sex ratio predicted by Hamilton's formula when  $n=5$ ).

In the laboratory, we found that the difference between sex ratio from parasitized and superparasitized egg capsules was significant. In this case, the results were consistent with LMC predictions, because the sex ratio from superparasitized egg capsules ( $n=6$ ) was less female biased ( $0.40 \pm 0.07$ ) than the sex ratio from hosts parasitized by a single female ( $0.28 \pm 0.04$ ), but in contrast with the results from the field. This is an important finding, because it suggests that the situation of host and female density we created in the laboratory is different from the circumstances potentially present in the field, where such a high superparasitism level might not occur.

The intriguing *A. nitens* sex ratio adjustment found in the field seems to ask for a more careful analysis of the mechanism, evoking alternative sex ratio theories to LMC. As explained in the Introduction, larval competition inside the host is an important feature to explain

mechanistically secondary sex ratios. Other studies have shown that: (1) if mothers expect superparasitism occurrence, they could shift offspring sex ratio towards the better competing sex (van Dijken and Waage 1987), and (2) beyond the decision of the mother, a strongly sex-biased mortality could influence secondary sex ratio. This last case has been reported by van Baaren et al. (1999) with *Anaphes victus*, where the sex ratio from parasitized and superparasitized hosts did not differ significantly, both being female biased. The author indicated that in the superparasitized hosts the secondary sex ratio (ratio decided by the superparasitizing female), was effectively more male biased but that the greater male mortality led to a female-biased tertiary sex ratio (ratio of the emerged offspring).

*A. nitens* first instar larvae are sexually dimorphic (Tooke 1955). Females are known to be more aggressive and therefore the potential winners of contests with males (Tooke 1955), as has also been shown for *A. victus* (van Baaren et al. 1997, 1999). This situation suggests that daughters should have more chances to survive in a superparasitized host and that the strongly female-biased sex ratio observed in the field when parasitism rate is high should be due to greater male mortality. Nevertheless, we are reluctant to accept this explanation because the results from the laboratory test with superparasitized hosts have shown a consistent increase in sons, which means that even at a high superparasitism level male larvae survivorship could be high, and that low-quality hosts (i.e. superparasitized) are allocated more males. Despite Tooke's (Tooke 1955) observations, female *A. nitens* advantage during larval combat remains at the moment unverified.

Reduced female size as a consequence of superparasitism indicates that daughters developing in good quality hosts (i.e. unparasitized) gain more than sons, which is consistent with the major assumption of Host Quality Models (Charnov et al. 1981). Then, it is reasonable to argue that in the field superparasitizing mothers should invest more in sons than in daughters, as they behaved in the laboratory test. However, our results do not support this prediction. Consequently, at the moment we consider that Host Quality Models cannot satisfactorily explain the sex ratio calculated in the field.

Frequently, parasitoids shape clutch size and offspring sex ratio using contact with other females or with chemical traces, as a cue to assess competitor density (Godfray 1994). For a solitary parasitoid entering a patch when another has already left it (sequential patch visit), models predict that superparasitism should be observed more often when laying a second egg has a great pay-off and/or when the fraction of hosts parasitized by other females becomes larger (van Alphen and Visser 1990; Visser et al. 1992a). The optimal strategy when the patch is simultaneously depleted by a number of females is a theoretical problem, where it is expected that when the environment becomes sufficiently poor, the ESS switches from rejecting parasitized hosts to accepting them (Visser et al. 1992b; Visser 1995). Experimental evidence

indicates that superparasitism rate (and sex ratio) usually increases with the number of foraging females in the patch (King 1987; Visser et al. 1990; Visser and Driessen 1991). In an experiment on *A. nitens* adaptive superparasitism (Santolamazza Carbone and Cordero Rivera 2003), we showed that superparasitism rate (calculated as the number of multiple egg clutches per host) increased significantly with the number of females (one, two or four) exploiting the patch simultaneously. So, it is possible that if females in the field do not deplete the patch simultaneously, they could superparasitize less than expected.

In a previous study (Cordero Rivera et al. 1999) we found that within plots, parasitism rate (which should account for female density) does not always follow host density. Calculating the regression slope between egg capsule number and parasitism, we found that positive slopes were common only at low parasitism levels (range 0–40%, which means few females in the patch) and negative slopes only at high levels (range 50–100%, many females in the patch). This suggests that at a small spatial scale (i.e. the single tree) *A. nitens* shows a tendency for positive spatial density dependence when parasitism rate is low and negative spatial density dependence when parasitism is high. It is possible that females remain in a good quality patch only if parasitoid density is low. When female density increases locally, superparasitism risk and its penalties might be so serious that females gain more by dispersing.

Differential dispersal of males and females is predicted to result in biased sex allocation (Antolin 1993). The timing of dispersal relative to mating has an important effect on the equilibrium sex ratio, because if mating occurs after dispersal (partial local mating, see Hardy 1994 for a review), sex ratio is predicted to be biased in favour of the sex that disperses more (Ode et al. 1998; Hardy 1994). If some virgin females of *A. nitens* disperse to avoid overcrowding and mate off-patch, then this could account for the strong female-biased sex ratio. If females disperse intensively, competition among sisters for the resources decrease, but then inside the patch males will compete strongly for mates. To reduce competition among brothers, mothers should increase sex ratio bias towards females.

Details of *A. nitens* mating structure and its relationship with dispersal are unknown at the moment, but it has been mentioned as a parasitoid with a great dispersal capacity (Tooke 1955; Godfray 1994). Female-biased dispersal has been previously reported in *Anagrus delicatus* (Antolin and Strong 1987) and in *Bracon hebetor* (Ode et al. 1998). In the latter case, the existence of a certain percentage of constrained females (sensu Godfray (1990) and female dispersal have been proposed to explain the highly female-biased sex ratio in response to the presence of other mothers, an alternative to LMC.

In conclusion, this study suggests that LMC could not account for *A. nitens* sex ratio in the field. Daughters' dispersal before mating could increase mother investment in the sex that emigrates more from the patch, and is

therefore an interesting hypothesis that calls for further research on the *A. nitens* mating system and sex-biased dispersal.

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