Progeny Fitness of the Mealybug Parasitoid Anagyrus sp. nov. nr. Sinope (Hymenoptera: Encyrtidae) as Affected by Brood Size, Sex Ratio, and Host Quality

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PROGENY FITNESS OF THE MEALYBUG PARASITOID *ANAGYRUS* **SP. NOV.** NR. *SINOPE* (HYMENOPTERA: ENCYRTIDAE) AS AFFECTED BY BROOD SIZE, SEX RATIO, AND HOST QUALITY

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ABSTRACT

Anagyrus **sp. nov.** nr. *sinope* Noyes & Menezes (Hymenoptera: Encyrtidae) is a gregarious, koinobiont parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). We investigated effects of larval competition on development, survival, and progeny body size of the parasitoid over a range of brood sizes and sex ratios. We recorded the brood size, sex ratio, and fitness parameters of parasitoids developing from second instars and adult *P. madeirensis*. Adult mealybugs as hosts produce larger brood and progeny body sizes and more female progeny. Second instar *P. madeirensis* continued to develop after parasitism, and mummies achieved a more advanced developmental stage yielding parasitoid cohorts of larger brood and body sizes, female-biased sex ratio, and longer developmental time than mummies formed from younger host instars. Overall, the sexual composition within a brood had little or no effect on the development, survival and body size of the parasitoid progeny developing from second instar and adult mealybugs. Larval competition among brood-mates developing in adult mealybugs caused a reduction in the progeny tibial length with increasing brood sizes. In contrast, parasitoids developing from second instar mealybugs had a longer developmental time and little (although positive) or no response in the progeny tibial length to increasing brood size. We hypothesize that the continuous development of second instar mealybugs allows the hosts to accumulate additional resources, thus, reducing competition among parasitoid brood mates for limited resources. The continuous development of hosts of younger developmental stages after parasitism may have the potential to alter the direction and intensity of larval competition of the koinobiont gregarious parasitoid.

Key Words: growth potential, host stage preference, larval competition, *Phenacoccus madeirensis*, sexual asymmetry

RESUMEN

Anagyrus **sp. nov.** nr. *sinope* Noyes & Menezes (Hymenoptera: Encyrtidae) es un parasitoide koinobionte y gregario de la cochilla de Madeira, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae). Nosotros investigamos los efectos de la competencia de las larvas en el desarrollo, sobrevivencia y tamaño del cuerpo de la progenie del parasitoide sobre el rango en el numero de progenie y la razón sexual. Nosotros registramos los parámetros en el numero de la progenie de cada grupo, la razón sexual, y el estado físico de los parasitoides desarrollados sobre el segundo estadio inmaduro y adultos de *P. madeirensis*. Las cochinillas adultas como hospederas producen grupos de progenies mas numerosas, individuos mas grandes y con una mayor proporción de hembras. El segundo estadio inmaduro de *P. madeirensis* continuo su desarrollo después de estar parasitados y las momias lograron tener una etapa de desarrollo más avanzada, resultando en cohortes de parasitoides con grupos de progenie con mas individuos de cuerpos mas grandes, con una mayor proporción de hembras a machos y con el tiempo de desarrollo mas largo que las momias formadas de los estadios mas jóvenes de hospederos. Sobre todo, la composición sexual entre el grupo de progenie tuvo poco o no efecto sobre el desarrollo, la sobrevivencia, y el tamaño de cuerpo de la progenie del parasitoide que se desarrollaron de cochinillas de segundo estadio y adulto. La competición larval entre las parejas de grupo de progenie desarrolladas en cochinillas adultas causo una reducción en la longitud de la tibia de la progenie con un aumento en el numero de progenie por grupo. Por contraste, los parasitoides que se desarrollaron sobre el segundo estadio de la cochinilla tuvo un periodo de desarrollo mas largo y una respuesta ligera (aunque positiva) o ninguna respuesta en la longitud de la tibia de la progenie en relación al aumento en el numero de progenie por grupo. Presentamos una hipótesis de que el desarrollo continuo del segundo estadio de las cochinillas permite que el hospedero acumule recursos adicionales y reduzca la competición entre las parejas en el grupo de progenie del parasitoide para los recursos limitados. El desarrollo continuo de los hospederos en los estadios menos desarrollados despues de estar parasitadas puede tener el potencial para alterar la dirección y la intensidad de la competición larval de este parasitoide koinobionte y gregario.

A foraging gregarious parasitoid determines the number and the sex ratio of eggs deposited in a host, based on the host's age or quality, and the parasitoid's previous foraging experience and physiological conditions (Godfray 1994; van Alphen & Jervis 1996). The decisions on the brood size (i.e., the number of progeny emerged from each mummy) and the sex ratio (proportion of male progeny) within a single host have significant influence on the intensity of competition among brood mates, and subsequently, on the fitness of the progeny.

The influence of brood size on parasitoids' growth and development has often been explored on the basis of competition among brood mates for limited resources (Smith & Fretwell 1974; Godfray 1994). There are trade-offs between brood size and the fitness of parasitoids. Progeny which emerge from a larger brood suffer from a smaller body size (Bernal et al. 1999; Mayhew & van Alphen 1999; Fidgen et al. 2000), a shortened developmental time (Stapel et al. 1997; Harvey et al. 1998), and a reduced larval survival rate (Nakamura 1995; Allen & Hunt 2001; Milonas 2005). Adult body size is positively correlated to fitness of the parasitoids, which is measured by the number of progeny that can be produced by each female offspring and the number of females that can be sired by each male offspring (Ode et al. 1996; Sagarra et al. 2001). Larval competition reduces progeny body size, and thus, the fitness of the parents and offspring. An increase in larval competition has a greater impact on the fitness of female offspring, because smaller female parasitoids can produce fewer eggs than larger females, while small males can still produce many sperm for fertilization (Godfray 1994).

The sexual composition of a brood also had a significant influence on the fitness of gregarious parasitoids (Godfray 1986; Ode et al. 1996; West et al. 2001). Godfray (1986) suggested that the differential ability of female and male parasitoid larvae to compete for limited resources leads to sexual asymmetry in larval competition, which is one of the causes of variations in progeny sex ratio and brood size. Larvae of the less competitive sex will suffer to a greater extent in their fitness from the competition with their more competitive brood mates. When the female parasitoid larvae outnumbered the male larvae, the body size of male progeny was reduced with an increasing proportion of females (Ode et al. 1996; Fidgen et al. 2000).

In this study, we investigated the developmental response of *Anagyrus* **sp. nov.** nr. *sinope* Noyes & Menezes (Hymenoptera: Encyrtidae) to variations in brood size, sex ratio, and host quality. *Anagyrus* sp. nov. nr. *sinope* is a gregarious koinobiont parasitoid of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), a serious pest of greenhouse ornamental production. *Anagyrus* sp. nov. nr. *sinope* preferred third instar immature and prereproductive adult female *P. madeirensis* for oviposition and progeny development (Chong & Oetting 2006a). The parasitoids that developed in these preferred host stages had a shorter developmental time, a lower mortality rate, a higher proportion of females, and larger brood and body sizes. First and second instars were considered the least preferred and of the least suitable for parasitoid growth and development. However, younger nymphs that had continued development to become older nymphs before mummification produced a similar progeny number and sex ratio as those mealybugs that were parasitized as adults. Chong & Oetting (2006a) did not investigate the influence of brood size and sex allocation patterns on the fitness of *Anagyrus* sp. nov. nr. *sinope*. We conducted this study to detect the occurrence of larval competition and sexual asymmetry in larval competition among the brood mates of *Anagyrus* sp. nov. nr. *sinope*, and their consequences to the parasitoid's fitness.

MATERIALS AND METHODS

An *Anagyrus* **sp. nov.** nr. *sinope* colony was established in 2002 at the University of Georgia, Griffin Campus, Griffin, GA, USA, with individuals collected from a greenhouse colony of *Anagyrus loecki* Noyes & Menezes (Hymenoptera: Encyrtidae), which is another biological control candidate agent of *P. madeirensis*. The greenhouse colony of *A. loecki* at the Griffin Campus was established in 2000 with individuals collected from a colony maintained at the University of Florida, Mid-Florida Research and Education Center, Apopka, FL. *Anagyrus* sp. nov. nr. *sinope* was not detected in the original colony of *A. loecki* in Florida, leading us to believe that *Anagyrus* **sp. nov.** nr. *sinope* originated as a local contamination of the *A. loecki* colony established in Griffin, GA (Chong & Oetting 2006b). *Phenacoccus madeirensis* reared on sprouted russet potatoes (*Solanum tuberosum* L., Solanaceae) were provided as hosts in the laboratory colony. The mummies were collected from the laboratory colony, isolated individually in gelatin capsules, and held at a constant temperature of 25 ± 1°C in an environmental chamber until adult eclosion. After eclosion, each female parasitoid was paired with two males and isolated for 48 h in a glass vial supplied with a streak of honey solution as a source of moisture and carbohydrates. No mealybugs were provided in the glass vials; thus, the parasitoids had no oviposition experience at the beginning of the experiment.

Phenacoccus madeirensis was reared on sprouted russet potatoes in an insectary at the Griffin Campus. Second instars with a body length of 1.0-1.3 mm and pre-reproductive adult females of 2 to 3 mm were collected for the experiment. The experiment was conducted as a no-choice test where mealybugs of a single developmental stage were offered to the parasitoids at a time. Ten mealybugs of one of the two developmental stages were transferred onto an excised coleus leaf [*Solenostemon scutellarioides* (L.) Codd., Lamiaceae] and were allowed to settle over a 16-h period. The coleus leaf was kept fresh by inserting its petiole, through a hole drilled at the bottom of a petri dish, into a cup of water.

A single parasitoid was released from the vial and allowed to forage in a petri dish containing a cohort of 10 mealybugs for 24 h at 25 ± 1°C, 90 ± 2% relative humidity and 14:10 (L:D) h photoperiod. The parasitoid was removed after 24 h and the mealybug cohort was incubated in the environmental chamber. The mealybug cohort was examined every 5 d and the mummies were collected. The collected mummies were isolated in individual gelatin capsules and incubated in the environmental chamber at 25 ± 1°C until adult parasitoid eclosion. The developmental stage of the mealybugs at the time of mummification, the developmental duration from egg deposition to adult eclosion of the parasitoids, and the progeny number and sex ratio (percentages of males) were recorded for each mummy. All mummies were dissected at the end of the experiment to verify the survival of immature parasitoids. All adult parasitoids were collected and preserved in 70% ethanol within 6 h of eclosion, and their left hind tibial length was measured with a micrometer under dissecting microscopes. Hind tibial length was used as a surrogate for the body length and the fitness of the parasitoid (Sagarra et al. 2001; Chong & Oetting 2006a). Thirty seven and 46 replicates were prepared for the adult and second instars, respectively.

We first analyzed the distributions of brood sizes and sex ratios of *Anagyrus* sp. nov. nr. *sinope* reared from the 2 host stages by subjecting the frequency of mummies that yielded a specific brood size or percentage of males to the analysis of variance (ANOVA) after an arcsine-transformation (PROC GLM; SAS Institute 1999). The mean frequencies of brood size and sex ratio from each host stage were separated by the Tukey's honestly significant difference (HSD) test (PROC GLM; SAS Institute 1999). The influence of host stages at the time of mummification on the recorded fitness parameters also was analyzed with ANOVA and the means separated with Tukey's HSD test. The relationship between brood size and sex ratio, and the respective relationships of brood size or sex ratio with the developmental time (female and male combined), survival rate, and female and male tibial lengths of parasitoids developed from the 2 host stages were elucidated with linear regression analyses (PROC REG; SAS Institute 1999).

RESULTS

Parasitized second instar and pre-reproductive adult *P. madeirensis* continued to develop after parasitism. Fifty two percent of the parasitized pre-reproductive adult mealybugs produced eggs before mummification, while the rest remained as pre-reproductive females (Table 1). Adult progeny of *Anagyrus* sp. nov. nr. *sinope* emerged from these reproducing and non-reproducing adult mealybug mummies were of similar brood size (about 3 parasitoids per mummy), sex ratio (30-36% males), developmental time (about 16 days), survival rate (97-100%), and tibial length (averaged 0.37 mm for females and 0.30 mm for males).

The majority (46%) of the parasitized second instar mealybugs mummified at the second instar, while 21 and 32% of these mealybugs continued to develop into third instar and adult mealybugs, respectively, before mummification (Table 1). The hosts that were parasitized as second instars but achieved adulthood before mummification yielded significantly more female parasitoid progeny and larger brood and body sizes than the mealybugs mummified at second instar. With the continuous development of second instar hosts after parasitism, the parasitoid's developmental time from egg deposition to adult eclosion was significantly lengthened from 16 d, when the hosts were mummified at second instar, to 24 d, when the hosts were mummified as adults. Parasitoids which emerged from mealybug mummies that had achieved adulthood (whether the mummies were developing from second instars or pre-reproductive adults), had similar sex ratios, brood and body sizes, and developmental time.

Fifty eight percent of all mummies developing from the second instar mealybugs produced only one adult parasitoid from each mummy (Fig. 1A). This was by far the largest single value (ANOVA: $F_{7, 360} = 21.32$, $P < 0.0001$) and was observed predominantly in mummies of which the development was arrested in the second instar by the parasitoids' development and mummification. The mummies developing from the pre-reproductive adult mealybugs yielded a range of brood sizes from zero to 9 adult parasitoids per mummy, with the largest brood sizes collected at roughly equal proportion from mummies that achieved both pre-reproductive and ovipositing status. The most common brood sizes developed from adult mealybugs being composed of 2 (24.5%) or 3 (25.8%)

TABLE 1. MEANS (± SEM) OF SEX RATIO, BROOD SIZE, DEVELOPMENTAL TIME, SURVIVAL RATE, AND FEMALE AND MALE PROGENY TIBIAL LENGTH OF *ANAGRYUS* **sp. nov.** NR. *SINOPE* FROM DIFFERENT HOST STAGES AT THE TIME OF PARASITISM AND AT THE TIME OF MUMMIFICATION. THE DEVELOPMENTAL STAGES OF *PHENACOCCUS MADEIRENSIS* ARE SECOND INSTAR (N2), THIRD INSTAR IMMATURE FEMALE (N3), PRE-REPRODUCTIVE ADULT FEMALE (PRE-OVIP) AND OVIPOSITING ADULT FEMALE (OVIP).

Host stage at parasitism	Host stage at mummification	*n*	Sex ratio (% males)	Brood size	Developmental time (d)	Survival rate (%)	Female tibial length (mm)	Male tibial length (mm)
N2	N2	52	67.3 ± 6.6 a	1 b	16.1 ± 0.2 c	100	0.30 ± 0.01 b	0.26 ± 0.01 b
	N3	24	28.3 ± 4.9 b	1.3 ± 0.1 b	18.0 ± 0.3 b	100	0.36 ± 0.01 a	0.29 ± 0.01 a
	Pre-Ovip	36	23.6 ± 3.7 b	3.2 ± 0.2 a	23.9 ± 0.6 a	98.6 ± 1.4	0.35 ± 0.01 a	0.29 ± 0.01 a
ANOVA statistics								
	F value		33.37	85.75	107.30	1.06	22.82	13.04
	df		2, 109	2, 109	2, 109	2, 109	2, 72	2, 60
	P value		<0.0001	<0.0001	<0.0001	0.3512	<0.0001	<0.0001
Pre-Ovip	Pre-Ovip	47	30.3 ± 3.9	3.0 ± 0.2	15.7 ± 0.2	100	0.37 ± 0.01	0.30 ± 0.01
	Ovip	51	35.8 ± 3.7	2.9 ± 0.2	16.1 ± 0.3	97.1 ± 1.7	0.37 ± 0.01	0.31 ± 0.01
ANOVA statistics								
	F value		0.90	0.13	0.93	2.81	0.01	0.79
	df		1, 96	1, 96	1, 96	1, 96	1, 90	1, 74
	P value		0.3442	0.7210	0.3372	0.0971	0.9342	0.3770

adult parasitoids per mummy (Fig. 1A; ANOVA: $F_{7,396} = 3.88, P < 0.0001$). About 2% of all mummies from both the host stages failed to produce any parasitoids.

The mummies developing from the second instar mealybugs yielded predominantly single-sex broods (about 42% all-female and 35% all-male) (Fig. 1B; ANOVA: $F_{10,308} = 18.71, P < 0.0001$). Most of the all-male broods emerged from mummies that had achieved only second instar development, while the majority of all-female broods emerged from mummies that had achieved third instar and adult development. In contrast, the mummies developing from adult mealybugs mostly yielded broods containing 30% males (32.6%), followed by those that had sex ratios of 50% males (23.7%) and all-female (23%) (Fig. 1B; ANOVA: $F_{10,308} = 16.33, P < 0.0001$).

The proportion of male progeny in a single brood (i.e., the sex ratio) decreased with an increase in the brood size of parasitoids developing in the second instar mealybugs (% males = 0.53-0.07xbrood size; $F_{1,165} = 5.91, P = 0.0161$). However, there was only a weak relationship between the brood size and sex ratio of adult parasitoids developing from second instar mealybugs ($r^2 = 0.0346$). By contrast, no linear relationship was detected between the brood size and sex ratio of adult parasitoids developing from pre-reproductive adult mealybugs ($F_{1,133} = 0.10, P = 0.7555, r^2 = 0.0007$).

The developmental time of adult parasitoids from the second instar mealybugs increased strongly with the brood size (Fig. 2A; developmental time = 15.3 + 2.6xbrood size; $F_{1,165} = 125.76, P <$ $0.0001, r^2 = 0.4325$). On the other hand, the developmental time of parasitoids developing from adult mealybugs was not influenced by brood size (Fig. 2A; $F_{1,133} = 1.82, P = 0.1793, r^2 = 0.0135$). Survival rates of parasitoid larvae developing in both the second instar and adult mealybugs ranged from 83-100% and were not impacted by the parasitoid brood sizes (second instar: $F_{1,165} = 0.30, P = 0.5823, r^2 = 0.0018$; adult: $F_{1,133} = 1.51, P = 0.1190, r^2 = 0.0466$).

Although there was no significant relationship between brood size and female tibial length of parasitoids developing from the second instar mealybugs (Fig. 2B; $F_{1,165} = 0.01, P = 0.9170, r^2 = 0.0001$), the male tibial length increased slightly with an increase in brood size (Fig. 2C; male tibial length = 0.26 + 0.01xbrood size; $F_{1,165} = 15.12, P = 0.0002, r^2 = 0.1398$). An opposite pattern was observed in the relationship between brood size and progeny tibial length of parasitoids developing from adult mealybugs. The tibial length of the female and male parasitoids developing from adult mealybugs was significantly reduced with an increase in the number of parasitoids per brood (female tibial length = 0.41 – 0.02xbrood size; Fig. 2B; $F_{1,133} = 52.38, P < 0.0001, r^2 = 0.2970$; male tibial length = 0.35 – 0.01xbrood size; Fig. 2C; $F_{1,165} = 0.4841, P < 0.0001, r^2 = 0.3218$).

The developmental duration of parasitoids developing from second instar mealybugs was slightly shortened as the percentage of males was increased (Fig. 3A; developmental time = 20.7 – 2.7x% males; $F_{1,165} = 11.48, P = 0.0009, r^2 = 0.0651$). The development time was not influenced by the

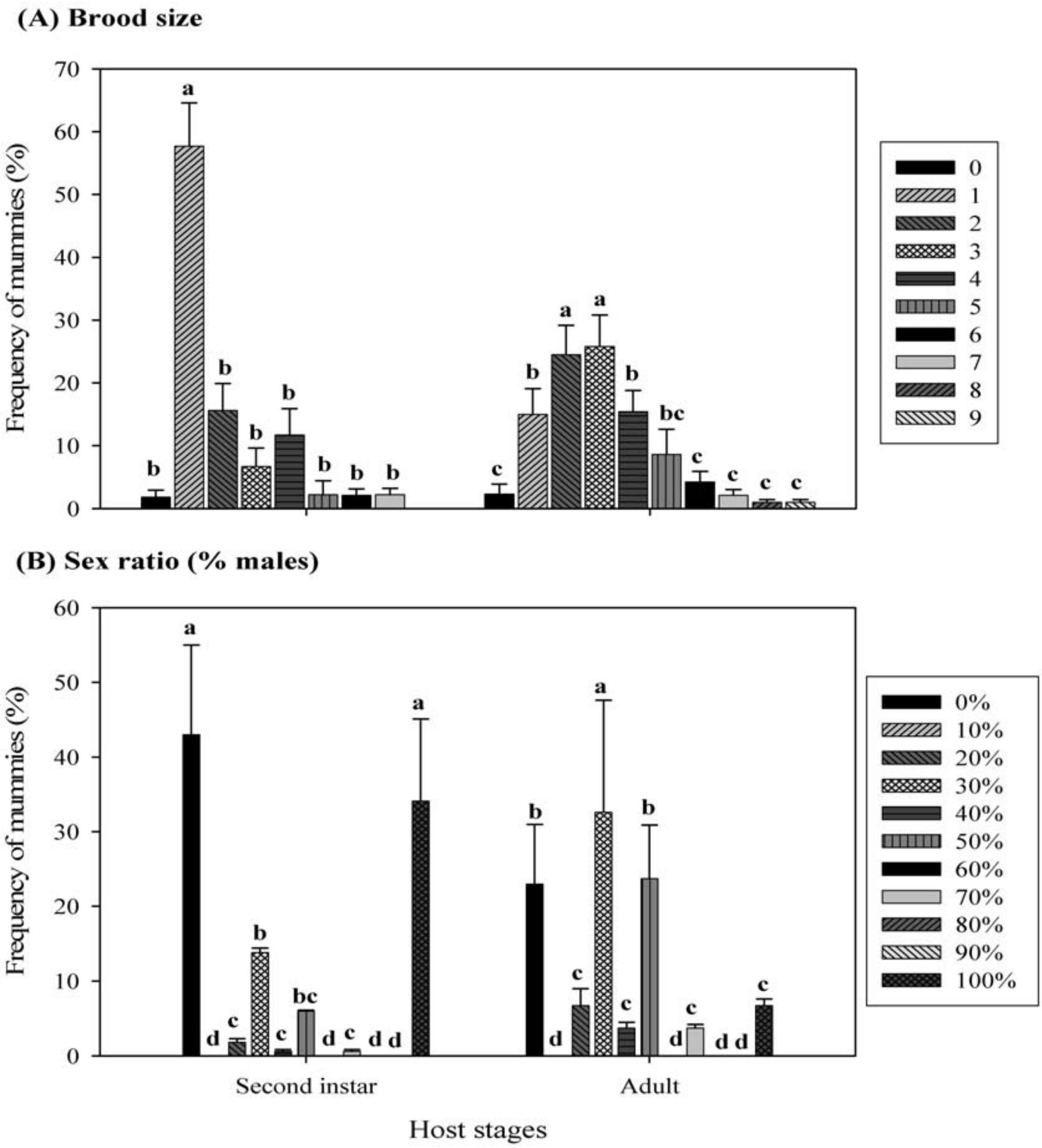

Fig. 1. Distribution of brood sizes (A) and sex ratios (B) of *Anagyrus* **sp. nov.** nr. *sinope* from the second instar and adult *Phenacoccus madeirensis*.

sex ratio of parasitoids which emerged from mummies developing from adult mealybugs (Fig. 3A; $F_{1,133} = 0.01$, $P = 0.9720$, $r^2 = 0.0001$). The proportion of male parasitoids in broods developing from the second instar and adult mealybugs did not influence the parasitoids' survival rate (second instar: $F_{1,165} = 1.73$, $P = 0.1905$, $r^2 = 0.0104$; adult: $F_{1,133} = 0.04$, $P = 0.8359$, $r^2 = 0.0003$) and female tibial length (Fig. 3B; second instar: $F_{1,165} = 0.01$, $P = 0.9620$, $r^2 = 0.0001$; adult: $F_{1,133} = 3.09$, $P = 0.0813$, $r^2 = 0.0243$). Male tibial length again showed different responses to an increase in the proportion of males in both host stages. The tibial length of male parasitoids developing from the second instar mealybugs was reduced as the percentage of males in a brood increased (Fig. 3C; male tibial length = 0.30 – 0.03x% males; $F_{1,165} = 18.15$, $P < 0.0001$, $r^2 = 0.1633$). By contrast, the male tibial length increased slightly with an increase in the sex ratio of broods developing from adult mealy-

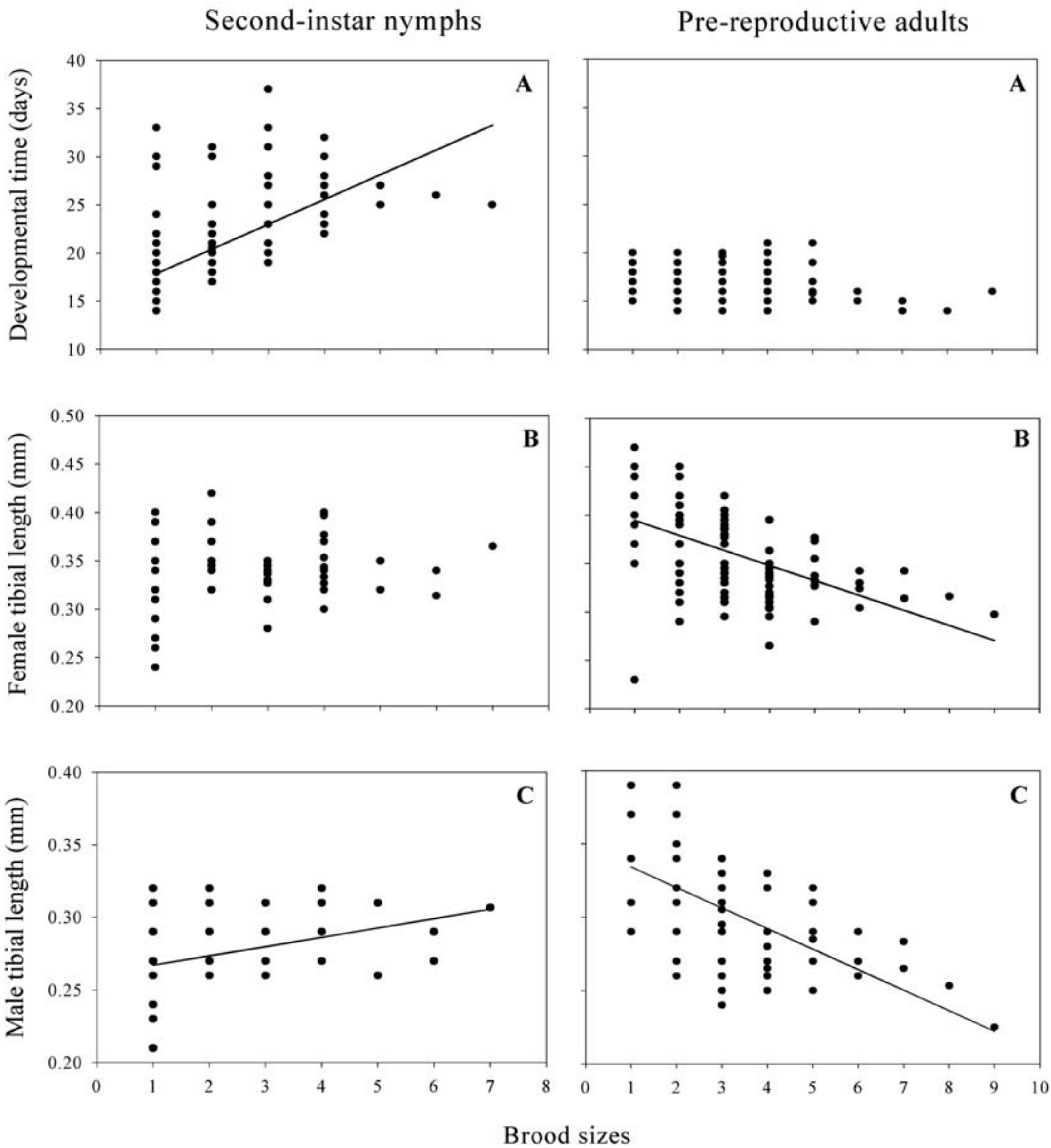

Fig. 2. Relationship of the brood size with the developmental time (A), female tibial length (B) and male tibial length (C) of *Anagyrus* **sp. nov.** nr. *sinope* developing in the second instar (left hand side) and adult (right hand side) of *Phenacoccus madeirensis*. Each point represents the mean measurements of the whole brood. The solid line is the best fitted linear regression model.

bugs (Fig. 3C; male tibial length = 0.27 + 0.08x% males; $F_{1,133}$ = 23.47, P < 0.0001, r^2 = 0.1871).

Discussion

Brood size (or clutch size) and sex ratio are major components of the life history strategy of many parasitoid species and have received much attention both experimentally and theoretically (Godfray 1994). Many optimality models of brood size and sex ratio have considered the effects of limited host resources or availability of time and eggs (e.g., Charnov & Skinner 1984; Waage & Godfray 1985; Godfray 1986). The primary brood size and sex ratio (i.e., the brood size and sex ratio at the time of parasitism) are determined by the female parasitoids based on their physiological status (e.g., energy reserve and egg load) and foraging

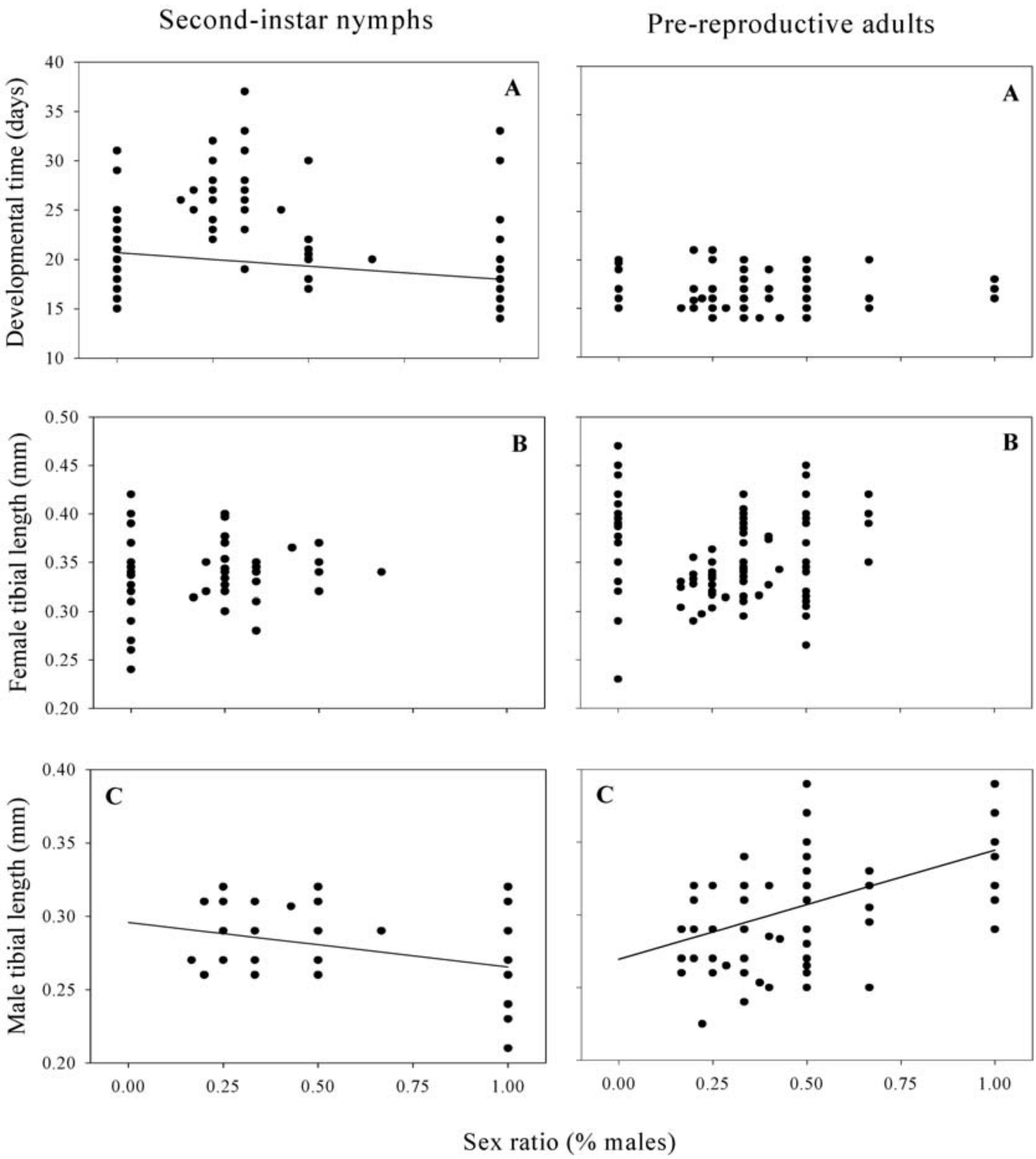

Fig. 3. Relationship of the sex ratio (% males) with the developmental time (A), female tibial length (B) and male tibial length (C) of *Anagyrus* **sp. nov.** nr. *sinope* developed in the second instar (left hand side) and adult (right hand side) of *Phenacoccus madeirensis*. Each point represents the mean measurements of the whole brood. The solid line is the best fitted linear regression model.

experience (e.g., host density and travel time), and the host's physiological status (e.g., host stage, size or age). The primary brood size and sex ratio provide the basis for larval competition among brood mates, which in turn determine the fitness of the progeny. We showed in this study that the fitness consequences of larval competition for the gregarious parasitoid *Anagyrus* **sp. nov.** nr. *sinope* were affected in part by the number of parasitoid larvae sharing limited resources within a brood, and weakly by the competitive pressure of one sex exerted on the other.

The model of sexual asymmetry in larval competition suggests that the fitness of the less competitive sex is reduced to a greater extent by an increase in the proportion of the more competitive sex (Godfray 1986). Overall, the sexual composition within a brood had little or no effect on the development, survival, and body size of *Anagyrus* **sp. nov.** nr. *sinope*. The body size (using the hind tibial length as the proxy) of female parasitoids developing from both second instar and adult *P. madeirensis* appeared to be constant over a range of sex ratios (% males). The conservation of body size is beneficial to the female parasitoids because their egg loads are reduced with even a small reduction in the body size (Godfray 1994). The sensitivity of female parasitoids to the reduction in body size often makes them more aggressive competitors (Ode et al. 1996; Fidgen et al. 2000) in an effort to secure more resources for their development to the optimal body size. An increase in the percentage of males within a brood reduced the intensity of competition from the female brood mates, which led to a slight increase in the body size of male *Anagyrus* **sp. nov.** nr. *sinope* developing from adult mealybugs. In contrast, the body size of male parasitoids developing from second instar mealybugs was only slightly reduced by an increase in the percentage of males. This is the reciprocal effect of the lower percentage of males in the mummies achieving adult development (more resources to produce larger parasitoids) and the higher male ratio (almost all males) in the mummies that remained as second instar nymphs (produced smaller parasitoids).

An increase in brood size reduces the amount of host resources available to individual parasitoid larvae, resulting in a reduction in the fitness of the emerging adult parasitoids (Godfray 1994; Ode et al. 1996). The negative effect of increased brood size on progeny fitness has been found in several studies (Nakamura 1995; Ode et al. 1996; Zaviezo & Mills 2000; Allen & Hunt 2001; Guinnee et al. 2005; Keasar et al. 2006). The body sizes of male and female *Anagyrus* **sp. nov.** nr. *sinope* developing from adult *P. madeirensis* were reduced with an increase in brood size, which agreed with the prediction of larval competition models. The developmental time of parasitoids developing from the second instar mealybugs was significantly lengthened because more time was required to obtain sufficient resources to complete development in larger broods. The increasing body size of male *Anagyrus* **sp. nov.** nr. *sinope* developing from the second instar *P. madeirensis* contradicted the expectations of larval competition. We believe the observed contradictions in the fitness responses of *Anagyrus* **sp. nov.** nr. *sinope* to larval competition when developing in second instar and adult mealybugs can best be explained by an alteration in the direction and intensity of larval competition in hosts of different quality and growth potential.

Larval competition among parasitoid brood mates occurs when there is a limitation in the amount of resources (i.e., host tissues) that can be provided by the hosts for the parasitoid's development. Many parasitoids, including *Anagyrus* **sp. nov.** nr. *sinope*, actively select hosts of a specific age or size that have sufficient resources to produce progeny of a higher fitness (Godfray 1994; Harvey et al. 1998; Zaviezo & Mills 2000; Milonas 2005; Chong & Oetting 2006a). Adult and second instar *P. madeirensis* represent hosts of two different qualities for *Anagyrus* **sp. nov.** nr. *sinope* (Chong & Oetting 2006a). The adult mealybugs did not continue to increase their body sizes through growth after parasitism; effectively providing the developing parasitoid larvae with a fixed amount of resources. Therefore, the developing parasitoid larvae in adult mealybugs were competing against each other for the limited amount of resources. The larval competition intensified with an increase in brood size, which reduced the amount of resources available to each parasitoid larva. As a result, the body sizes of female and male parasitoids were reduced as the brood size increased. On the other hand, the developing parasitoid larvae might have suppressed their development to allow the second instar mealybugs continued development after parasitism (Chong & Oetting 2006a). These growing mealybugs accumulated resources and provided the developing parasitoid larvae with an increased amount of resources. The increases in developmental time, brood size, proportion of females, and body size of *Anagyrus* **sp. nov.** nr. *sinope* in mummies that achieved more advanced developmental stage has already been demonstrated by Chong & Oetting (2006a). Hosts of a more advanced developmental stage provided sufficient resources to allow the development of a larger parasitoid brood without the expense on the body size of the developing parasitoids. This increase in available resources appeared to benefit the male progeny more than the female progeny (in terms of the gain in body size), perhaps because excess resources were available to the male progeny after satisfying the development requirements of the female progeny. The increase in the male parasitoid's body size to the increase in brood size and host quality was, however, weak even when the parasitoids stimulated the growth of the parasitized mealybugs (Vet et al. 1994).

This study showed that some aspects of the fitness of parasitoids are influenced by the number of parasitoid larvae and, to a lesser extent, the sexual composition within a brood. The growth potential of young hosts after parasitism may be able to alter the direction and intensity of competition among the larvae of *Anagyrus* **sp. nov.** nr. *sinope*, particularly among male progeny. The results of this study indicated the potential influence of larval competition on parasitoid fitness and the importance of considering the consequence of the quality or growth potential of a host stage on the evolution of brood size and sex ratio in gregarious koinobiont parasitoids.

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