Male egg-carrying in *Phyllomorpha laciniata* is favoured by natural not sexual selection

Gomendio M., García-González F¹, Reguera P. & Rivero A.

Reproductive Ecology and Biology Group, Department of Evolutionary Ecology, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain.

Correspondence to:

Montserrat Gomendio

Reproductive Ecology and Biology Group

Department of Evolutionary Ecology

Museo Nacional de Ciencias Naturales (CSIC)

José Gutiérrez Abascal 2, 28006 Madrid, Spain

Tel: +34-91-411 13 28 Fax: +34-91-564 50 78

E-Mail: montseg@mncn.csic.es

¹Present address: Centre for Evolutionary Biology, Zoology Building (M092), School of Animal Biology, The University of Western Australia, Nedlands, WA 6009, Australia.

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Abstract

There are two hypotheses to explain the evolution of paternal care: the sexual selection hypothesis proposes that caring males are more attractive to females and thus benefit from enhanced mating opportunities; the natural selection hypothesis proposes that males care when the benefits in terms of offspring survival exceed the costs. We use *Phyllomorpha laciniata* to test these hypotheses, a species where females can choose whether to lay eggs on plants or on conspecifics, and where the extent to which males carry eggs varies between populations. Our findings show that females do not choose to mate with egg-carrying males neither in natural populations, nor in experimental contexts. Thus, our data do not support the sexual selection hypothesis. We compare two populations which differ in the extent of male egg carrying and we show that in the population where male egg carrying is more prevalent, parasitism pressure is higher. Field experiments reveal that, in the population with high parasitism rate, egg mortality as a result of parasitoid attack is up to ten times higher on plants than on conspecifics. Egg carrying is thus an effective strategy that protects eggs against parasitoid attack. We conclude that the main benefit derived by males from egg carrying is a substantial increase in offspring survival and that males are sensitive to inter-population differences in egg mortality risks. Male care in this system has evolved despite intermediate levels of certainty of paternity because the impact on offspring survival is high, and the costs in terms of loss of mating opportunities low. Thus, our findings support the natural selection hypothesis.

Key words: Golden egg bug, natural selection, sexual selection, parasitism, parasitoids, parental care, paternal care, *Phyllomorpha laciniata*.

Introduction

Theory predicts that care for offspring will evolve through natural selection when the benefits in terms of offspring survival are greater than the costs to the parents residual reproductive value, such as reduced survival, fecundity and mating opportunities (Williams 1966; Trivers 1972). When offspring survival is substantially improved by parental care, it can be provided either by females, by males, or by both sexes. Which sex ends up caring for offspring depends partly on the balance of benefits and costs for each sex, and partly on historical constraints. Because of the costs involved, each sex would be better off if the other sex provides the care, so a conflict of interests between males and females is always present (Parker 1979; Westneat & Sargent 1996; Chapman *et al.* 2003). This implies that the behaviour of each sex will be influenced by the behaviour of the other, so the patterns that we observe in natural populations must be understood as the resolution of a game with three players: the male, the female and the offspring (reviewed in Parker *et al.* 2002).

Parental care in insects is rare, possibly because in most species parents can do little to protect or nurture the offspring (Zeh & Smith 1985; Tallamy & Wood 1986). Exceptions include species in which eggs or young face particularly harsh environments or high predation pressures, because in these species parents can improve offspring survival substantially by keeping environmental conditions at an optimal level for the development of the offspring (Smith 1976; Tyndale-Biscoe 1984; Wyatt 1986; Favila 1993; Halffter *et al.* 1996), protecting offspring against food competitors (Tyndale-Biscoe 1984; Wyatt 1986; Halffter *et al.* 1996), or protecting them against predators (Tallamy & Denno 1981; Nafus & Schreiner 1988; Kudô *et al.* 1989; Scott 1990; Kudô & Nakahira 1993; Kudô *et al.* 1995; Kudô & Ishibashi 1996) or parasitoids (Ralston 1977). The distribution of parental care among arthropods is such that in most species care is provided by the female alone, followed by biparental care and, finally, paternal care is the least frequent form of care (Zeh & Smith 1985; Clutton-Brock 1991). Exclusive care by males is uncommon in most taxa because male lifetime reproductive success is mainly determined by the number of females with whom they mate, and providing care frequently reduces to a great extent the opportunities for looking for females and mating with them (Zeh & Smith 1985; Clutton-Brock 1991; Queller 1997).

Recently, another hypothesis has been proposed to explain the evolution of care by males in insects, which suggests that sexual selection has played an important role (Tallamy 2000; Tallamy 2001). According to this hypothesis, caring males in these species benefit from female preferences to mate with males who demonstrate their willingness and ability to look after young. Thus, these males would not only avoid the costs of reduced mating opportunities, but they would in fact benefit from their enhanced attractiveness to females. This argument is similar to the ones developed to explain the evolution of paternal care in

fish, where territorial males look after the broods of several females simultaneously, and females prefer to lay eggs with guarding males (Reynolds *et al.* 2002, and references therein).

Most studies aimed at understanding the evolution of care by males and females have carried out comparative analyses to find out how the balance of costs and benefits for each sex varies between species (Clutton-Brock 1991; Thomas 1994; Reynolds *et al.* 2002). However, even closely related species often differ in several aspects of their ecology and life-history patterns making it difficult to control for confounding effects. Perhaps for this reason most hypotheses are still rather speculative (Balshine *et al.* 2002). To test which are the selective forces favouring the appearance of male care, ideally one should compare populations of the same species which differ in the extent to which care has spread in the population. Very few species show this degree of intraspecific variability, but the golden-egg bug, *Phyllomorpha laciniata* (Heteroptera, Coreidae), is an exception and therefore constitutes an ideal model organism to test these hypotheses.

In this paper, we examine which selective forces explain egg-carrying by males in the golden-egg bug (*Phyllomorpha laciniata*). Females of this species can lay eggs both on plants and on the backs of conspecifics (males and females). The main sources of egg mortality are predation (mainly by ants) and the parasitoid *Gryon bolivari* (Hym: Scelionidae). While eggs on plants show low survival rates (3%), being carried by an adult considerably improves egg survival rates (25%) (Reguera & Gomendio 2002). Given the benefits in terms of egg survival, females prefer to lay eggs on conspecifics if given the choice (Garcia-Gonzalez & Gomendio 2003a). Egg carrying, however, is costly for the individuals, who become more visible (eggs are bright yellow) and less able to escape from predators (Reguera & Gomendio 1999). Whether egg carrying by male *P. laciniata* constitutes a form of parental care or is merely the consequence of intraspecific parasitism remains a point of contention (see Kaitala *et al.* 2001; and Gomendio & Reguera 2001 for a reply). Kaitala and collaborators argue that natural selection acts on the ovipositing female, who lays eggs on both male and female conspecifics whenever the opportunity arises, such as when they encounter a pair engaged in copulation (Kaitala 1996). However, this hypothesis has not been supported by data from natural populations (Garcia-Gonzalez & Gomendio 2003b). In contrast, it has been argued that to understand patterns of care observed in natural populations it is important to consider not only the benefits to females of laying eggs on conspecifics, but also the selective forces operating on the sex who accepts to provide care (Gomendio & Reguera 2001). Thus, while it is clear that females would benefit from laying eggs on any conspecific (Garcia-Gonzalez & Gomendio 2003a), it remains to be explained why at the peak of the reproductive season all (95-100%) males carry eggs while only between 20-40% of females do so (Gomendio & Reguera 2001). Thus, while some of the

egg carrying by males and females may indeed be the result of female opportunistic behaviour, the high levels of egg carrying by males (over 77% of all eggs in the population are carried by males) must partly be explained by selection acting on the males to accept eggs (Gomendio & Reguera 2001; Garcia-Gonzalez & Gomendio 2003a; Garcia-Gonzalez & Gomendio 2003b; García-González *et al.* 2005), particularly when the evidence available shows that males can easily reject attempts by females to lay eggs on them simply by moving away (Gomendio & Reguera 2001). Females mate with several males, and several lines of evidence suggest that the certainty of paternity is intermediate (Tallamy 2001; García-González *et al.* 2003; Tay *et al.* 2003). Males attempt to increase their share of paternity by transferring more sperm and extending copulation when there are rivals in the vicinity (Garcia-Gonzalez & Gomendio 2004). Under such conditions, natural selection is expected to favour male egg acceptance only when the impact in terms of offspring survival is very high, and the costs in terms of reduced mating opportunities low (Garcia-Gonzalez *et al.* 2003; Garcia Gonzalez *et al.* 2005).

The aim of this paper is to disentangle the potential roles played by sexual and natural selection in favouring egg acceptance by males. For this purpose we compare populations in which egg-carrying by males has evolved to a different extent (Central and Southern Spain). A higher proportion of males carry eggs in Central Spain than in Southern Spain (81% and 50% respectively, Reguera 1999). The predictions from the sexual selection and the natural selection hypotheses are easily distinguishable:

- (1) The sexual selection hypothesis predicts that egg-carrying males should become more attractive to females than non-carrying ones, and that males in the two populations should differ in the extent to which they become attractive to females when they carry eggs.
- (2) The natural selection hypothesis predicts that males in the two populations should differ in the extent to which they benefit from offspring survival. Thus, parasitoid pressure, the main cause of egg mortality on plants (Reguera & Gomendio 2002), should be more prevalent in the population in which a greater proportion of males carry eggs, and male egg carrying should protect eggs from parasitoids.

Methods

Natural history

Phyllomorpha laciniata occurs in the Mediterranean area where it is typically found in the host plant *Paronychia argentea* and other *Paronychia* species (Mineo 1984; Reguera 1999 and references therein). In the Iberian Peninsula, individuals born on any given year overwinter as sexually immature adults, emerging from their refuges around March/April of

the following year, once temperatures start to rise. Soon after emergence, individuals start to copulate, which is followed by female oviposition either on plants or on other adults. Females mate with different males along the reproductive period and egg laying takes place throughout the whole active season (from March/April until August). Females lay eggs continuously throughout the breeding season, producing one egg at a time, and between 0-4 eggs daily on average. Eggs change in colour as they develop: eggs are white for some hours after being laid, then they turn to yellow and finally after about 10-12 days they hatch (Kaitala 1996; Reguera 1999). Hatched eggs remain solidly stuck to the backs of the individuals. Eggs parasitised by *Gryon bolivari* turn black. Nymphs start an independent life as soon as they hatch, and after five instars they become adults. The first adults born on any given year can be detected around June in Central Spain. These individuals are easily distinguishable from the sexually mature overwintering adults because instead of the dark, heavily sclerotised exoskeleton of the latter, newly emerging adults are pale and soft. These individuals do not copulate during the remaining of the breeding season, nor do they carry eggs. Individuals cease to be active during August when the host plant becomes dry, and emerge again after the winter (Reguera 1999; García-González 2002).

Tests of the sexual selection hypothesis

Field sampling

To test whether male egg carrying influences female choice field observations were conducted in five adjacent localities in Central Spain (Villaviciosa de Odón, Robledo de Chavela, Colmenar del Arroyo, Valdemorillo and El Espinar) during the years 1998 and 1999 throughout the reproductive season. In addition, in 1998 field observations were conducted in Southern Spain (Boca de los Frailes, Almeria) during three days at the peak of the reproductive season in this locality (early April). We recorded whether individuals were mating or not and the number of eggs carried by each individual. Since no significant differences were found in the patterns of egg carrying among the Central Spain populations, we pooled the data to increase sample size.

Of particular interest to the predictions that will be tested, is whether eggs carried by mating individuals are white or not, since this would mean either that they have been laid recently (either just before the current copula or between successive copulations), or some time before the current copula, respectively. Thus, we distinguished in the analyses white eggs, i.e. recently laid eggs, from old (yellow) eggs, i.e. eggs that individuals were carrying long before the start of the current copula, which includes parasitized eggs. Hatched eggs were also counted as old eggs. We did not include in the analyses individuals that were recaptured, because this could have lead to pseudo-replication. We also excluded from the

analyses individuals born during the reproductive season, because these individuals do not mate and do not accept eggs until the following year.

Mate choice experiment

To find out if females prefer to mate with egg-carrying males we also carried out female choice experiments using individuals from El Espinar (Central Spain). Adults were captured during the first week of June 1998 and were kept at constant conditions (25C°, lights on from 8:00 to 21:00 h) together in plastic containers (29 x 51 x 32 cm) to favour high rates of male egg-carrying.

After one week, all the individuals were isolated for two days. Only males that were carrying eggs were selected to be used in the experiment. Males were weighed and 17 females were individually enclosed in a cage (18 x 18 x 10 cm) with a pair of haphazardly chosen males that had been previously matched by size. This controlled for the effect of size on female choice, although it is worth noting that further evidence exists showing that male size does not influence female choice (García-González & Gomendio 2004). Before the female was introduced into the cage, one of the males was haphazardly chosen and the eggs on his back removed (although eggs are firmly glued to the back of males, these are easily removed using a pair of fine forceps). Each male was then marked with a dot of blue typists' correction fluid; one male was marked on the right side of the pronotum whereas the other was marked on the left side.

Once the experimental units were set up, we monitored the bugs every hour during the day (8:00 to 21:00) and every two hours under red light during night (21:00 to 8:00). During this time, all copulations were recorded. Copulations last for a long time (in this experiment copulations lasted for 32.5 ± 6 hours on average), thus the interval between observations was deemed sufficient to detect all of them. Individuals in each enclosure were separated if they did not start mating during the 24 hours following the setting up of the replicates.

Tests of the natural selection hypothesis

Field sampling

Populations of *P.laciniata* were sampled in 2004 from two different locations in Spain: Almería (Pozo de los Frailes, Southern Spain) and Segovia (El Espinar, Central Spain). Both populations were monitored on a regular basis from the beginning of the reproductive season (appearance of first adults in the spring). Population sampling was carried out in each population to coincide with the peak of the reproductive activity of the bugs in the field, defined as the period with the highest number of first generation adults carrying eggs, before the nymphs and second generation adults made an appearance.

Sampling was carried out in an identical way in both populations. For two consecutive days (8th and 9th May for Almería and 30th and 31st May for Segovia; the peak of reproductive activity differ between these populations due to latitudinal and climatic differences) two or three people systematically searched an area of ca. 1500 m² looking for *P. laciniata* males. We recorded the number of *P. laciniata* adult males, the number of eggs they were carrying and whether these eggs were unparasitised (yellow or whitish eggs) or parasitized (black eggs). In order to avoid counting the same male twice, males were captured and temporarily kept in plastic boxes (30 x 20 x 8 cm) with some *P. argentea* inflorescences. After the sampling was over, males were released back to the field. Given the high number of inflorescences in the sampling area, and the cryptic nature of eggs on the inflorescences, finding eggs on plants requires a considerably sampling effort and usually renders very few eggs (pers. obs.). For this reason, in this experiment no further attempt was made at finding eggs laid on plants.

Egg manipulation experiments

Two experiments were simultaneously carried out in each population: a mark and recapture experiment (to determine the fate of eggs carried by adults in the field) and a plant experiment (to determine the fate of eggs laid in plants in the field). The experiments took place in both populations to coincide with the period of maximum reproductive activity of the bugs (maximum number of individuals carrying eggs, Almería: 3rd-13th May, Segovia: 21st June - 1st July) in an area adjacent to the sampling area, but separated from it by at least 20 m. The experimental methodology was identical in both populations.

For the mark and recapture experiment, we collected individuals from the experimental area for two consecutive days (days 1-2). Their sex, the number of eggs they were carrying at the time of collection, and the status of these eggs (unparasitized or parasitized) were recorded. Eggs carried by the bugs were removed using entomological forceps before placing the bugs in plastic boxes (30 x 20 x 8 cm) with some *P. argentea* inflorescences. Each box contained approximately 20 males and 10 females. Individuals were kept in the box for 48h at ambient temperature to allow females to lay fresh eggs in the backs of conspecifics. The purpose of this exercise was to ensure that all eggs carried by individuals were roughly at the same stage of development at the time of release (as parasitoid attack is known to be highly dependent on the stage of development of the host, Quicke 1997). We tried to ensure that the mean number of eggs carried by individuals at the time of release was roughly representative of the mean number of eggs they were carrying at the time of collection as artefacts may arise when numbers are artificially high (Gomendio & Reguera 2001). While in Almería the number of eggs on collection (mean $\pm$ s.e. : 2.46 ± 0.16) and release (mean $\pm$ s.e: 2.48 ± 0.14) were similar ($\chi^2_1 = 0.004$, $p = 0.94$), in Segovia the

number of eggs on release (mean $\pm$ s.e: 3.47 ± 0.20) was significantly lower than the number of eggs on collection (mean $\pm$ s.e: 5.59 ± 0.30 , $\chi^2_1 = 54.44$, $p < 0.0001$).

On the day of release (days 3-4), the bugs were marked with coloured correction fluid (Reguera & Gomendio 2002). The number of freshly laid eggs and their position on the backs of each individual were recorded before releasing them in the field in 2 haphazardly chosen *P. argentea*-containing areas. In each area, 3 release points separated by 4-10 metres were marked using wooden sticks nailed to the floor. A total of 95 males and 34 females (Almería) and 70 males and 37 females (Segovia) were released in this way, between 12-15 males and 5-6 females per release point.

Recapture of marked individuals was carried out over two consecutive days (days 10-11). For this purpose the release area was systematically searched within a radius of ca. 20 metres from the release points. We noted the number of eggs that had been parasitized on the backs of individuals during the release period.

For the plant experiment, 42 (Almería) and 21 (Segovia) females were collected (days 1-2) and kept in plastic boxes (as above) at ambient temperature. On day 3, each female was individually bagged around a haphazardly selected *P. argentea* inflorescence (for details see Reguera & Gomendio 2002). The plant was marked by nailing a small, numbered wooden stick in its proximity. 24 hours later (day 4), the bags and the females were removed and the number and position of eggs laid in each inflorescence recorded. On day 11, the plants were revisited and the number of eggs that had been parasitized over the 7-day period were recorded.

Statistical analyses

The data were analyzed using generalized linear modelling techniques available in the GLMStat (v.6.0, 2005, <http://www.glmstat.com>) and Statistica (6.0) statistical packages. Proportional data (proportion of egg carrying adults, proportion of total eggs disappeared or parasitized from the backs of individuals or from plants) were analyzed specifying binomial errors and the logit link (Crawley 1993). Count data (number of eggs carried per individual) were analysed using Poisson errors and the log link. In both cases, the *full model* was built by fitting in all potential explanatory variables and their interactions. The significance of each element was assessed by removing it from the model and analyzing the resulting change in deviance (Crawley 1993). With both binomial and Poisson errors the change in deviance attributable to a given factor is distributed as χ^2 . After the *minimal model* (the model including only significant terms and interactions) was obtained, its appropriateness was tested by checking and correcting for overdispersion (Crawley 1993). The significant values given in the text are for the minimal model while non-significant values are those obtained prior to the deletion of the variable from the model.

Data from the mate choice experiment were analysed using a Chi-squared test between observed frequencies (number of females mating to either egg-carrying or non egg-carrying males) and expected frequencies under the null hypothesis of absence of female choice based on egg-carrying.

Results

Tests of the sexual selection hypothesis

Field sampling

In populations from Central Spain, we analysed egg carrying in 344 single males and 76 mating pairs. If females choose to mate with egg-carrying males we should find differences in the number of old eggs (yellow eggs) carried by single males and by males in copula, since those are the ones that males were already carrying before the current mating. No differences were found between mating and single males either in the proportion of males carrying old eggs (egg carriers: 40 out of 76 copulating males and 152 out of 344 single males, $\chi^2_1 = 1.78$, $p = 0.18$) or in the number of old eggs carried (mean $\pm$ s.e eggs carried: 3.33 ± 0.4 and 3.27 ± 0.18 for 40 mating and 152 single egg-carrying males respectively, $\chi^2_1 = 0.018$, $p = 0.89$). These results remain unaltered regardless of whether we consider hatched eggs as old eggs or not.

In Southern Spain we found 119 single males and 48 mating pairs. The proportion of mating males carrying old eggs does not differ from the proportion of single males carrying old eggs (egg carriers: 21 out of 48 copulating males and 49 out of 119 single males $\chi^2_1 = 0.09$, $p = 0.76$), and mating males do not carry more old eggs than single males (mean $\pm$ s.e eggs carried: 3.67 ± 0.62 and 2.96 ± 0.34 for 21 mating and 49 single egg-carrying males respectively, $\chi^2_1 = 1.13$, $p = 0.29$). Likewise, results remain unaltered regardless of whether we consider hatched eggs as old eggs or not.

Mate choice experiment

In 14 out of 17 trials the female copulated with one of the two males. Eight of the females copulated with egg carrying males and six with males without eggs. The difference between the observed and expected frequencies under the null hypothesis did not show statistical significance ($\chi^2_1 = 0.29$, $p = 0.59$).

Tests of the natural selection hypothesis

Field sampling

While in Segovia most of the males carried eggs (81 out of 83 males captured), in Almería the proportion of egg carrying males was significantly lower (91 out of 139 males captured, $\chi^2_1 = 38.82$, $p < 0.0001$, Figure 1a). The mean number of eggs carried per male was also significantly higher in Segovia than in Almería (mean $\pm$ standard error: 6.46 ± 0.33 and 2.05 ± 0.19 respectively, $\chi^2_1 = 262.5$, $p < 0.0001$, Figure 1b).

In Almería none of the eggs carried by males were parasitized. In contrast, in Segovia 32.53% of the males carried at least one parasitized egg, and 9.31% of the total eggs carried by males were parasitized (Figure 2).

In both populations sex ratio was 1:1. A total of 262 adults were found in Almería (139 of which were males, 53.05%), while 153 adults were found in Segovia (83 of which were males, 54.24%); thus, densities were higher in the former.

Egg manipulation experiments

In Almería we recaptured 43 males and 13 females, or 43.41% of the total individuals released. In addition, 41 out of the 42 marked inflorescences were recovered. In Segovia we recaptured 13 males and 5 females, or 16.82% of the total individuals released. In addition, 18 out of the 21 inflorescences were recovered.

As expected from the population sampling, in Almería no eggs were parasitized either in the plants or on the backs of conspecifics. In Segovia, however, parasitism was high: 12.69% of the total eggs carried by recaptured adults (total number of eggs parasitized over total number of eggs on release, only for recaptured adults) and 64.70% of the total eggs laid in plants (total number of eggs parasitized over total number of eggs laid on plants) were parasitized during the experimental week (Figure 3). The statistical analysis was done using egg carrying adult (males plus females) or inflorescence as a replication unit. Eggs suffered up to ten times more parasitism when laid on the inflorescences (mean $\pm$ s.e. proportion of eggs parasitized per inflorescence: 66.67 ± 10.69) than when carried on the backs of individuals (mean $\pm$ s.e. proportion of eggs parasitized per individual 6.31 ± 3.14 , $\chi^2_1 = 28.81$, $p < 0.0001$).

Discussion

Phyllomorpha laciniata males accept eggs from females, even though carrying eggs makes them more vulnerable to predators (Reguera & Gomendio 1999). In this paper, we examine which are the benefits for males that could compensate such costs. We test whether egg-carrying by males has been favoured by sexual or natural selection. To do this we compare populations from Central Spain, where almost all males carry eggs, with populations from Southern Spain, where a lower proportion of males carry eggs.

The sexual selection hypothesis suggests that males care for offspring because they become more attractive to females and gain increased mating opportunities. Our findings from the field sampling do not support this hypothesis since mating males in natural populations were not found to carry more old eggs than single males, and this was the case in both populations from Central and Southern Spain. Previous work has already shown that mating males do have more recently laid eggs (white eggs) and that these are probably laid by the mating female between successive copulations (Garcia-Gonzalez & Gomendio 2003b). In the present study we focus on old (yellow) eggs because they were already carried by the mating male before the beginning of the current copula, and are therefore the only cue that females could have used when choosing mating partners. The results from the mate choice experiment also fail to support the sexual selection hypothesis: when females were given the choice to mate with two males, one with and the other without eggs, they showed no preferences. These results are in agreement with a previous experiment by Kaitala (1998) carried out with females from a different population. Thus, sexual selection does not seem to have played an important role in the evolution of egg-carrying by males.

The natural selection hypothesis suggests that males care for offspring because of the benefits in terms of offspring survival. This hypothesis has two predictions: (a) males should be more prone to accept eggs in populations where egg survival on plants is low, and (b) egg carrying by males should result in a considerable improvement in survival rates. We have focused on the parasitoid wasp *Gryon bolivari* because previous work has shown that it has a major impact on mortality rates of eggs on plants (Reguera & Gomendio 2002). Our results support both predictions. The number of egg-carrying males, and the mean number of eggs carried per male, are significantly higher in the population where parasitoids are most abundant (Central Spain). The results of the egg manipulation experiment provide an explanation for these results. In Central Spain, eggs suffer up to ten times more parasitism by *G. bolivari* when laid on the inflorescences than when carried on the backs of individuals. Egg carrying is thus an effective strategy that protects eggs against parasitoid attack. Most parasitoids require a long host handling process (a time during which the parasitoid explores and probes the host to determine if it is suitable for oviposition, Quicke 1997) before they can lay an egg on a host. The movement of a walking *P. laciniata* and the vibrations that they typically produce when they are disturbed (pers. obs.) are probably sufficient to disrupt the egg laying process, thus protecting the eggs against parasitoid attack. Thus, males seem to accept eggs only when the benefits for offspring survival are substantial, while in contexts in which offspring are not at such high risk males are less likely to accept eggs. We suggest that male behaviour plays an important role because it has already been shown that males from populations in Southern Spain reject up to 70% of female attempts to lay eggs, while rejection rates are lower in populations from other regions (Miettinen & Kaitala 2000). The

sensitivity of males to variation in parasitism pressure thus supports the view that care by males is favoured when offspring face high mortality risks and the benefits of care thus exceed the costs to males. These findings are based on a comparison of two populations; thus, data from more populations are needed to confirm the conclusions of this study.

Differences in levels of egg-carrying could also result from differences in sex ratio, so that biases in favour of females could result in more eggs being produced and laid on males, or from differences in population densities, since females could lay more eggs on males at high densities because encounter rates are higher (Gomendio & Reguera 2001). The first possibility can be excluded since sex ratio was 1:1 in both populations. The second possibility can also be excluded because densities were slightly higher in the population in which a smaller proportion of males carried eggs. Finally, differences in female fecundity could also influence the results. Inter-population differences in fecundity are possible because in Southern Spain females are heavier than in Central Spain, and female body weight is correlated with female fecundity (Reguera 1999). However, if differences in fecundity were responsible for differences in levels of egg-carrying we would expect higher levels of egg-carrying in Southern Spain, which is the opposite of what we find.

Male care is only expected to evolve through natural selection when a certain degree of paternity has been achieved, since the benefit is measured in terms of survival of true genetic offspring. Females mate with several males through the season and our own results from previous studies show that the last male to copulate with a female has an intermediate level of paternity (García-González *et al.* 2003). Females tend to lay more eggs after copulation, so males accepting eggs after they have copulated with a female have a 40% chance of carrying their offspring, and this probability does not change with time (García-González *et al.* 2003). When levels of paternity are intermediate, males would only be expected to provide care when the impact on offspring survival is high. Our findings show this to be the case since eggs laid on plants suffer high mortality rates as a result of parasitoids, and egg carrying by males protects them from this cause of mortality. Thus, males readily accept providing care to eggs, even if only a proportion of them are their genetic offspring, when egg carrying is needed to improve offspring survival. However, in populations where parasitism is low, and eggs have better survival prospects on plants, males are less likely to carry eggs.

Male reproductive success depends to a great extent on the number of females they mate with. When males care for offspring one of the main costs is a reduction in the time and energy that they can spend looking for other females and mating with them. This, however, does not seem to be a significant cost for *Phyllomorpha laciniata* males since, once the eggs are glued to their backs, they are free to move and they continue to mate with other females. Furthermore, as we show in this study, egg carrying does not decrease their mating chances.

Because in natural populations males carry on average 4 eggs, back space is not a limiting factor, as it seems to be in giant water bugs (Smith 1997). Similarly, egg-carrying does not seem to decrease foraging efficiency. The fact that egg-carrying seems to convey a cost only in terms of vulnerability to predators, could also have favoured the evolution of care by males in this species.

Do our findings from *Phyllomorpha laciniata* have general implications for the evolution of paternal care in other insects? Taken together the results from our long-term study show that male care is favoured when offspring survival is considerably improved, and costs to males in terms of loss of mating opportunities are low. This is consistent with other studies that show that male care is most likely to occur when either males do not suffer a marked loss in mating opportunities or such opportunities are scarce (Zeh & Smith 1985; Clutton-Brock 1991). In those species in which males carry the eggs glued to their body, males can continue to mate with other females while they look after offspring. In species in which males have to stay at a particular site looking after eggs laid on the substrate, the loss in mating opportunities may be small when local densities of receptive females are high, males can mate with several females, and males have the ability to look after several clutches simultaneously (Reynolds *et al.* 2002). In both cases, the main benefits for males may be in terms of offspring survival. It has been suggested that among birds, male care has evolved when extra mating opportunities for males are scarce (reviewed by Owens 2002). Experimental evidence also shows that males are more likely to desert offspring when the opportunities for remating are high (Keenleyside 1983; Clutton-Brock 1991; Webb *et al.* 2002).

In conclusion, care by males is most likely to evolve when the benefits in terms of offspring survival are high, and costs to males in terms of loss of mating opportunities are low. Males are sensitive to variations between populations in egg mortality risks, intensifying care when eggs are unlikely to survive without it. Males seem willing to provide care under conditions of intermediate certainty of paternity when the impact on offspring survival is high.

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Figure legends

Figure 1. Comparison between Central and Southern Spanish populations in the percentage of males carrying old eggs (a), and in the mean number of old eggs carried by these males (b)(Central Spain: n = 83, Southern Spain: n = 139). Bars represent standard errors. Field sampling data from 2004.

Figure 2. Comparison between Central and Southern Spanish populations in the percentage of males carrying parasitized eggs and in the percentage of total eggs in the population which are parasitized (Central Spain: n = 83, Southern Spain: n = 139). Field sampling data from 2004.

Figure 3. Comparison of the percentage of eggs parasitized in the egg manipulation experiment when carried by individuals (grey bars) and when laid on *P. argentea* inflorescences (open bars) in Central and Southern Spain (Total eggs carried by individuals: Central Spain n = 365, Southern Spain n = 303; Total eggs laid in plants: Central Spain n = 42, Southern Spain n = 81).

Figure 1

Figure 2

Figure 3