



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

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Two hypotheses could explain the evolution of paternal care: caring males are more attractive to females and mate more often (sexual selection); males care when the benefits in terms of offspring survival exceed the costs (natural selection). To test these hypotheses we used *Phyllomorpha laciniata*: females can choose whether to lay eggs on plants or on conspecifics, and the extent to which males carry eggs varies between populations. Our results do not support the sexual selection hypothesis: females did not choose to mate with egg-carrying males in either natural populations or experimental contexts. We compared two populations that differ in the extent of male egg carrying and we show that in the population where male egg carrying was more prevalent, parasitism pressure was higher. Field experiments revealed that, in the population with high parasitism rate, egg mortality as a result of parasitoid attack was up to 10 times higher on plants than on conspecifics. Egg carrying is thus an effective strategy that protects eggs against parasitoids. We conclude that the main benefit derived by males from egg carrying is an increase in offspring survival, and that males are sensitive to interpopulation differences in egg mortality risks. Male care in this system has evolved despite intermediate levels of paternity certainty 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, although additional work on more populations is needed to verify this.

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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 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; Halfpeter et al. 1996), protecting offspring

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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, 1995; Scott 1990; Kudô & Nakahira 1993; 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 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 is important (Tallamy 2000, 2001). According to this hypothesis, caring males in these species benefit from female preferences to mate with males that show their willingness and ability to look after young. Thus, these males would not only avoid the costs of reduced mating opportunities, but also 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 selective forces favour the appearance of male care, one should ideally compare populations of the same species that differ in the extent to which care has spread in the population. 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.

We examined which selective forces explain egg carrying by males in *P. 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* (Hymenoptera: Scelionidae). The survival rates of eggs are low on plants (3%) but are much higher when the eggs are carried by an adult (25%; Reguera & Gomendio 2002). Given the benefits in terms of egg survival, females prefer to lay eggs on conspecifics if given the choice (García-González & Gomendio 2003a). Egg carrying, however, is costly for the individuals, which 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; Gomendio & Reguera 2001, for a reply). Kaitala et al. (2001) argued that natural selection acts on the ovipositing female, which lays eggs on both male and female conspecifics whenever the opportunity arises, such as when she encounters a pair engaged in copulation (Kaitala 1996). However, this hypothesis has not been supported by data from natural populations (García-González & Gomendio 2003b). In contrast, Gomendio & Reguera (2001) 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 that accepts to provide care. Thus, while it is clear that females would benefit from laying eggs on any conspecific (García-González & Gomendio 2003a), it remains to be explained why, at the peak of the reproductive season, all (95–100%) males carry eggs but only 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 opportunistic female 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; García-González & Gomendio 2003a, b; 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 (García-González & Gomendio 2004). Under such conditions, natural selection is expected to favour egg acceptance by males only when the impact in terms of offspring survival is very high, and the costs in terms of reduced mating opportunities low (García-González et al. 2003, 2005).

Our aim in this study was to disentangle the potential roles of sexual and natural selection in favouring egg acceptance by males. For this purpose we compared populations (in central and southern Spain) in which egg carrying by males has evolved to a different extent. A higher proportion of males carry eggs in central 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 noncarrying 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 around the Mediterranean, 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 in 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 oviposition either on plants or on other adults. Females mate with a number of males during the reproductive period and egg laying takes place throughout the active season (from March/April to August). Females lay eggs continually throughout the breeding season, producing one egg at a time, and 0–4 eggs daily on average. Eggs change colour as they develop: eggs are white for some hours after being laid, then they turn to yellow and hatch after about 10–12 days (Kaitala 1996; Reguera 1999). Hatched eggs remain solidly stuck to the backs of the individuals. Eggs parasitized by *G. bolivari* turn black. Nymphs start an independent life as soon as they hatch, and after five instars they become adults. The first adults in 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 sclerotized exoskeleton of the latter, newly emerging adults are pale and soft. These individuals do not copulate during the remainder 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).

Sexual Selection Hypothesis

Field sampling

To test whether male egg carrying influences female choice, we conducted field observations in five adjacent localities in central Spain (Villaviciosa de Odón, Robledo de Chavela, Colmenar del Arroyo, Valdemorillo and El Espinar) during 1998 and 1999 throughout the reproductive season. In addition, in 1998 we conducted field observations in southern Spain (Boca de los Frailes, Almería) during 3 days at the peak of the reproductive season in this locality (early April). In those sites that were sampled repeatedly, individuals were marked with unique combinations of typists' correction fluid spots to allow for individual identification. We recorded whether individuals were mating 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 the sample size.

Of particular interest for our predictions was whether eggs carried by mating individuals were white, since only

white eggs would have been laid recently (either just before the current copula or between successive copulations). Thus, we distinguished in the analyses white eggs, that is recently laid eggs, from old (yellow) eggs, that is 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 led to pseudoreplication. 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 whether 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 together in constant conditions (25°C, lights on from 0800 to 2100 hours) in plastic containers (29 × 51 cm and 32 cm high) to favour high rates of male egg carrying.

After a week, all the individuals were isolated for 2 days. Only males that were carrying eggs were used in the experiment. Males were weighed and 17 females were individually enclosed in a cage (18 × 18 cm and 10 cm high) with a pair of haphazardly chosen males that had been previously matched by size. This controlled for any effect of size on female choice, although evidence exists 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 with a pair of fine forceps). We then marked each male with a dot of blue typists' correction fluid; one male was marked on the right side of the pronotum and the other on the left side.

Once the experimental units were set up, we monitored the bugs every hour during the day (0800–2100 hours) and every 2 h under red light at night (2100–0800 hours). During this time, we recorded all copulations. Copulations last for a long time (in this experiment, $\bar{X} \pm SD = 32.5 \pm 6$ h), so the interval between observations was deemed sufficient to detect all of them. We separated individuals in each enclosure if they did not start mating during the 24 h after we set up the replicates.

Natural Selection Hypothesis

Field sampling

We sampled populations of *P. laciniata* in 2004 from two locations in Spain: Almería (Pozo de los Frailes, southern Spain) and Segovia (El Espinar, central Spain). We monitored both populations 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 appeared.

We carried out sampling in an identical way in both populations. For 2 consecutive days (8 and 9 May for Almería and 30 and 31 May for Segovia; the peak of reproductive activity differs between these populations owing 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 adult males, the number of eggs they were carrying and whether these eggs were unparasitized (yellow or whitish eggs) or parasitized (black eggs). To avoid counting the same male twice, we captured males and temporarily kept them in plastic boxes (30 × 20 cm and 8 cm high) with some *P. argentea* inflorescences. After the sampling was over, males were released back into the field. Given the large number of inflorescences in the sampling area, and the cryptic nature of eggs on the inflorescences, finding eggs on plants requires a considerable sampling effort and few are usually found (personal observation). For this reason, in this experiment we made no further attempt to find eggs laid on plants.

Egg manipulation experiments

Two experiments were carried out simultaneously 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 on 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: 3–13 May; Segovia: 21 June–1 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 2 consecutive days (days 1–2) and recorded their sex, the number of eggs they were carrying at the time of collection, and the status of these eggs (unparasitized or parasitized). Eggs carried by the bugs were removed with entomological forceps and the bugs were placed in plastic boxes (30 × 20 cm and 8 cm high) with some *P. argentea* inflorescences. Each box contained approximately 20 males and 10 females. Individuals were kept in the box for 48 h at ambient temperature to allow females to lay fresh eggs on 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). In Almería, the number of eggs on collection ($\bar{X} \pm \text{SE} = 2.46 \pm 0.16$) and release (2.48 ± 0.14) were similar (generalized linear model, GLM: $\chi^2_1 = 0.004$, $P = 0.94$), but in Segovia the number of eggs on release (3.47 ± 0.20) was significantly lower than the number on collection (5.59 ± 0.30 ; $\chi^2_1 = 54.44$, $P < 0.0001$).

On the day of release (days 3–4), we marked the bugs 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 we released them in the field in two haphazardly chosen *P. argentea*-containing areas. In each area, we marked three release points, separated by 4–10 m, with wooden sticks nailed to the ground. A total of 95 males and 34 females (Almería) and 70 males and 37 females (Segovia) were released in this way, 12–15 males and 5–6 females per release point.

We recaptured marked individuals over 2 consecutive days (days 10–11). For this purpose the release area was systematically searched within a radius of ca. 20 m 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. We removed the bags and the females 24 h later (day 4) and recorded the number and position of eggs laid in each inflorescence. On day 11, we revisited the plants and recorded the number of eggs that had been parasitized over the 7-day period.

Statistical Analyses

The data were analysed with generalized linear modelling techniques available in GLMStat version 6.0 (<http://www.glmstat.com>) and Statistica 6.0 (Statsoft, Tulsa, OK, U.S.A.) statistical packages. Proportional data (proportion of egg-carrying adults, proportion of eggs that disappeared from, or were parasitized on, the backs of individuals or plants) were analysed specifying binomial errors and the logit link (Crawley 1993). We analysed count data (number of eggs carried per individual) by 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 analysing 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, we tested its appropriateness by checking and correcting for overdispersion (Crawley 1993). The significant values given in the text are for the minimal model while nonsignificant values are those obtained prior to the deletion of the variable from the model.

Data from the mate choice experiment were analysed with a chi-square test between observed frequencies (number of females mating 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

Sexual Selection Hypothesis

Field sampling

In populations from central Spain, we analysed egg carrying in 344 single males and 76 mating pairs. If females chose to mate with egg-carrying males we should have found 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: 40/76 copulating males and 152/344 single males; $\chi_1^2 = 1.78$, $P = 0.18$) or in the number of old eggs carried ($\bar{X} \pm SE$ eggs carried: mating males: 3.33 ± 0.4 , $N = 40$; single males: 3.27 ± 0.18 , $N = 152$; GLM: $\chi_1^2 = 0.018$, $P = 0.89$). These results remained unaltered regardless of whether we considered 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 did not differ from the proportion of single males carrying old eggs (egg carriers: 21/48 copulating males; 49/119 single males; GLM: $\chi_1^2 = 0.09$, $P = 0.76$), and mating males did not carry more old eggs than single males ($\bar{X} \pm SE$ eggs carried: mating males: 3.67 ± 0.62 , $N = 21$; single males: 2.96 ± 0.34 , $N = 49$; $\chi_1^2 = 1.13$, $P = 0.29$). These results remained unaltered regardless of whether we considered hatched eggs as old eggs or not.

Mate choice experiment

In 14 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-square test: $\chi_1^2 = 0.29$, $P = 0.59$).

Natural Selection Hypothesis

Field sampling

In Segovia (central Spain) most of the males carried eggs (81/83 males captured), but in Almería (southern Spain) the proportion of egg-carrying males was significantly lower (91/139 males captured; GLM: $\chi_1^2 = 38.82$, $P < 0.0001$; Fig. 1a). The mean number of eggs carried per male was also significantly higher in Segovia than in Almería ($\bar{X} \pm SE$: Segovia: 6.46 ± 0.33 ; Almería: 2.05 ± 0.19 ; $\chi_1^2 = 262.5$, $P < 0.0001$; Fig. 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 (Fig. 2).

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

Egg manipulation experiments

In Almería (southern Spain) we recaptured 43 males and 13 females, or 43.41% of the total individuals released. In

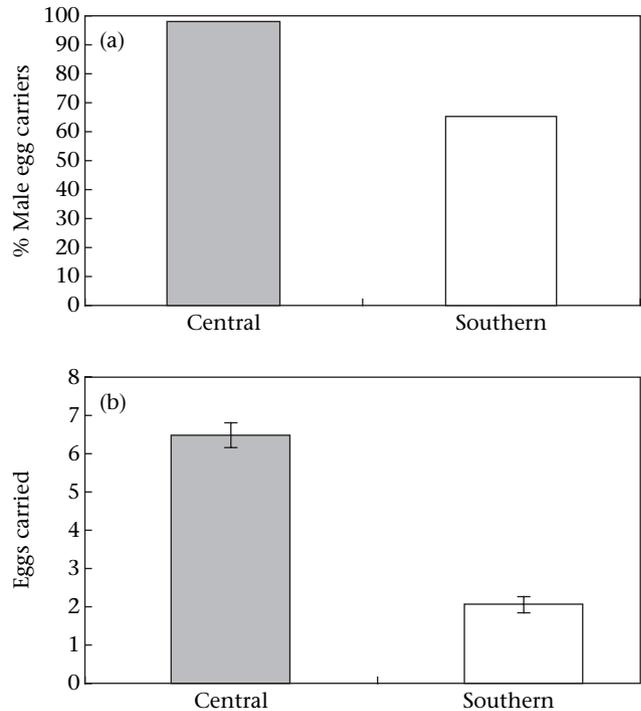


Figure 1. Comparison between central and southern Spanish populations in (a) the percentage of males carrying old eggs, and (b) the mean number of old eggs carried by these males (central Spain: $N = 83$; southern Spain: $N = 139$). Vertical lines represent SEs. Field sampling data from 2004.

addition, 41 of the 42 marked inflorescences were recovered. In Segovia (central Spain) we recaptured 13 males and five females, or 16.82% of the total individuals released. In addition, 18 of the 21 inflorescences were recovered.

As expected from the population sampling, in Almería no eggs were parasitized either on 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 on plants (total number of eggs parasitized

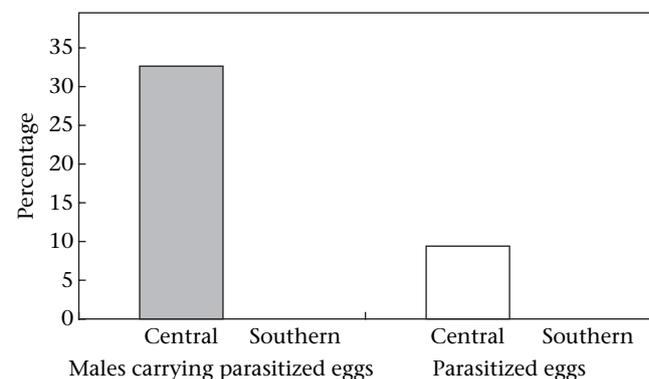


Figure 2. Comparison between central and southern Spanish populations in the percentage of males carrying parasitized eggs and in the percentage of total eggs in the population that were parasitized (central Spain: $N = 83$; southern Spain: $N = 139$). Field sampling data from 2004.

over total number of eggs laid on plants) were parasitized during the experimental week (Fig. 3). The statistical analysis was done with egg-carrying adult (males plus females) or inflorescence as a replication unit. Eggs suffered up to 10 times more parasitism when laid on the inflorescences ($\bar{X} \pm \text{SE}$ proportion of eggs parasitized per inflorescence = 66.67 ± 10.69) than when carried on the backs of individuals (6.31 ± 3.14 per individual; GLM: $\chi^2_1 = 28.81$, $P < 0.0001$).

DISCUSSION

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 did not carry more old eggs than single males, and this was the case in populations from both 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 (García-González & Gomendio 2003b). In the present study we focused 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 of mating with two males, one with and the other without eggs, they showed no preferences. These results are in agreement with an experiment by Kaitala (1998) carried out with females from a different population. Thus, sexual selection does not seem to have been important 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: (1) males should be more prone to accept eggs in populations where egg survival on plants is low, and (2) egg carrying by

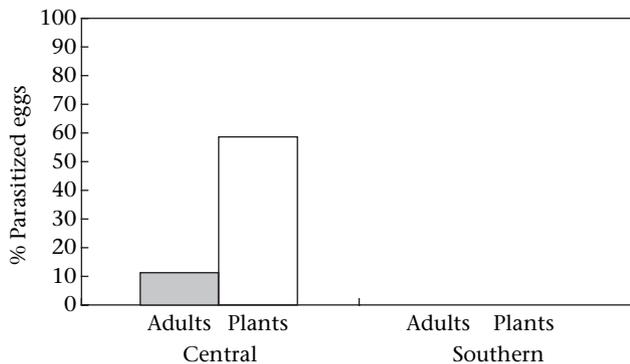


Figure 3. Comparison of the percentage of eggs parasitized in the egg manipulation experiment when carried by individuals (■) and when laid on *P. argentea* inflorescences (□) in central and southern Spain (total eggs carried by individuals: central Spain: $N = 365$; southern Spain: $N = 303$; total eggs laid on plants: central Spain: $N = 42$; southern Spain: $N = 81$).

males should result in a considerable improvement in survival rates. We have focused on the parasitoid wasp *G. bolivari* because 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 were significantly higher in the population where parasitoids were most abundant (central Spain). The results of the egg manipulation experiment provide an explanation for these results. In central Spain, eggs suffered up to 10 times more parasitism by *G. bolivari* when laid on the inflorescences than when carried on the backs of individuals. Egg carrying is thus effective in protecting 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 whether it is suitable for oviposition; Quicke 1997) before they can lay an egg on it. The movement of walking *P. laciniata* and the vibrations that they typically produce when they are disturbed (personal observation) 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, whereas in contexts in which offspring are not at such high risk males are less likely to accept eggs. We suggest that male acceptance behaviour is important because males from populations in southern Spain reject up to 70% of attempts by females to lay eggs, whereas 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 our conclusions.

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 the 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 found.

Male care is expected to evolve through natural selection only 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 be expected to provide care only 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 provide 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 with which they mate. 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 *P. 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 showed in this study, egg carrying does not decrease their mating chances. In natural populations males carry four eggs on average, so back space is not a limiting factor, as it seems to be in giant water bugs (Heteroptera: Belostomatidae) (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 (Reguera & Gomendio 1999) could also have favoured the evolution of care by males in this species.

Do our findings from *P. 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 can 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 in 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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