

COMMUNICATIONS ARISING

The adaptive significance of male egg carrying in the golden egg bug: defining research avenues. A reply to Härdling *et al.*

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Härdling *et al.* (2007) present a commentary on our recent paper (García-González *et al.*, 2005) in which we analysed the paternity of the eggs carried by male golden egg bugs (*Phyllomorpha laciniata* Villers; Heteroptera, Coreidae) and determined whether males enclosed with females carried true genetic offspring with a higher frequency than that expected from random oviposition on conspecifics.

Phyllomorpha laciniata females exhibit a very flexible pattern of oviposition behaviour: they can lay eggs on host plants (*Paronychia argentea*), where they develop unattended, or on the body of conspecific males and females, where they are carried until hatching (Mineo, 1984; Kaitala, 1996; Reguera, 1999; Gomendio & Reguera, 2001; García-González, 2002). Because most of the eggs carried by conspecifics are carried by males (García-González & Gomendio, 2003a), and males carry genetic offspring as well as unrelated eggs (Tay *et al.*, 2003; García-González *et al.*, 2005), the adaptive significance of male egg-carrying behaviour has been the subject of controversy (Gomendio & Reguera, 2001; Kaitala *et al.*, 2001).

Härdling *et al.* (2007) challenged our conclusions based largely on a re-analysis of the data presented in our original paper (García-González *et al.*, 2005) and made some further comments on this model system that warrant discussion. Härdling *et al.* (2007) made three major propositions: (a) the data cannot be used as evidence that females preferentially lay eggs on males with whom they have mated or as evidence of non-random acceptance of eggs by males with respect to paternity, (b) the data cannot be used as evidence that females preferentially lay eggs on males, and (c) the experimental set-up does not strengthen the null hypothesis of no paternal care. We will discuss each of these in turn before presenting some counter-arguments to the general picture drawn by Härdling *et al.* (2007) for male egg-carrying behaviour in *P. laciniata*.

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(a) *Acceptance of eggs by males with respect to paternity.* Härdling *et al.* (2007) main concern arises following a re-analysis of the simulations based on random allocation of eggs that we performed in our original paper. They conclude that the rates at which males carry genetic offspring does not differ significantly from the random expectation. In other words, that the results in the experiment can be explained by females laying eggs randomly and males accepting eggs randomly regardless of whether they are the sires or not.

However, we believe that Härdling *et al.*'s (2007) re-analysis does not serve the purpose of calculating the probability of carrying fertilised eggs under the real (observed) egg-carrying and egg-laying scenarios. In order to do so one must take into account not only the number of eggs that the males sired and the total number of males in the group, but also the number of eggs carried by each male and the number of eggs not fertilised by males in the experimental group. Härdling *et al.* (2007) seem to ignore this fact in their simulations of random egg allocation as they simply dispensed the eggs carried by males in the group 'taking into account that the males had sired different numbers of eggs'. In the simulations it is crucial to bear in mind that the question to address is whether or not the rates at which males carry true genetic offspring is the result of random egg allocation, and for this reason the number of eggs 'carried' by each male has to be considered. In addition, one must consider that females also laid eggs fertilised by males outside the experimental groups, and consequently that males were carrying not only eggs sired by them and other experimental males but also by non-experimental males. The re-analysis by Härdling *et al.* (2007) neglects this information, and consequently the probabilities arising from their re-analysis are not relevant for the events occurring in the experiment described in García-González *et al.* (2005).

The question to be addressed with the simulations is not to find out the probability of N_m males carrying their own offspring when N_e eggs sired by these males are randomly dispersed between them. The question is: What is the probability of male i carrying X genetic offspring when this male has sired Y eggs

and he is carrying Z number of eggs under the conditions that there are N_m males in the group that sired N_e eggs and the females also laid another N_e eggs sired by other males with whom they copulated before? In our paper we carried out simulations to specifically address the events occurring in the experiment with regard to male egg carrying. We calculated for each simulation and for each male the ratio between the number of carried eggs that were fertilised by the male and the number of eggs carried by him, under the conditions stated above, which were the real conditions. A distribution of frequencies for this ratio was obtained after a reasonable number of simulations (2000). The distribution of frequencies allowed us to calculate whether the observed event for every male was likely to be obtained by random egg allocation and random male acceptance of eggs. Our calculations were further confirmed by obtaining the probabilities of our real events occurring by chance using permutations with repetition.

We believe that the questions behind the simulations must address real events, and the simulations themselves must be adjusted to the real conditions under which the experimental output was obtained. This is not accomplished by Härdling *et al.*'s (2007) re-analysis because it fails to calculate probabilities of obtaining the real events observed in the experiment.

(b) *Egg-laying preferences.* Härdling *et al.*'s (2007) re-analysis of the number of eggs allocated to males and females indicates that the hypothesis that eggs are allocated randomly with respect to the sex of the egg recipient cannot be rejected. We agree with these authors on this point because it confirms what we showed in our original paper. In our paper we aimed to comment on the existing trend that males tended to carry more eggs than females. This tendency is further supported by Härdling *et al.*'s (2007) analysis: although there are no statistically significant differences, in all the groups the observed number of eggs carried by males is higher than that expected under the null hypothesis of random allocation, whereas the observed number of eggs carried by females is always lower than that expected (table 1 in Härdling *et al.*, 2007). Katvala and Kaitala (2001) documented that sex affected the probability of receiving eggs and that males receive significantly more eggs than females, leading to an unequal distribution of eggs between the sexes. Data from natural populations show that a higher proportion of males than females carry eggs, and that males tend to carry more eggs, and this result is consistent over years and between populations (Gomendio & Reguera, 2001; García-González & Gomendio, 2003a; Gomendio *et al.*, 2007). Thus, the reasons why statistically significant differences were not obtained in the experiment are probably that sample size was low and that under high density conditions individuals are less able to reject egg-laying attempts by simply moving away as they do in the field.

(c) *Experimental conditions.* Härdling *et al.* (2007) are concerned that our experimental conditions did not strengthen the null hypothesis of no paternal care. We agree that the likelihood of eggs being placed on the sires depends on the number of individuals in each replicate. However, as pointed out in our paper, the high densities in our experimental groups, compared

with those occurring in natural populations, most likely restricted the opportunities for males to reject egg-laying attempts, which probably increased the rates at which males carried unrelated eggs. Previous work has shown that high densities in experimental set-ups result in an artificial increase in the number of eggs laid on conspecifics, resulting in egg-carrying frequencies much higher than those found in natural populations (Gomendio & Reguera, 2001). The view that high densities facilitate female exploitation is further supported by the results of Katvala and Kaitala (2005) who found that when two females were placed in small enclosures they laid a higher number of eggs on conspecifics than when two females were placed in larger enclosures. In addition, in our study a high proportion of the eggs laid by the experimental females were sired by males with whom the females had mated in the field before the experiment began. Thus, the experimental conditions (i.e. high densities and high proportion of eggs sired by non-experimental males) reduced the chances that males would carry their genetic offspring, not the opposite as Härdling *et al.* (2007) argue.

General comments to Härdling et al. (2007): the paternal care hypothesis cannot be ruled out. The main argument presented by Härdling *et al.* (2007) is that 'egg carrying can be viewed as a female strategy to increase offspring survival'. In addition, they state that 'Although egg-carrying may not have evolved for the purpose of parental care, any male relatedness to the carried eggs will certainly "help" egg-carrying behaviour increase in frequency by natural selection'.

We believe that these arguments are flawed. Females benefit from laying eggs on conspecifics because it increases egg survival (Kaitala, 1996; Gomendio & Reguera, 2001; Reguera & Gomendio, 2002). Thus, females prefer to lay eggs on conspecifics rather than on plants (Reguera & Gomendio, 2002; García-González & Gomendio, 2003b; Katvala & Kaitala, 2003), but they do not show preferences in terms of the sex of the carrier or the degree of relatedness between egg and the carrier because the chances that eggs will survive are the same; there is no disagreement in this respect. This, however, does not explain why conspecifics accept eggs and, more importantly, why most eggs are carried by males (77% of eggs are carried by males in natural populations; García-González & Gomendio, 2003a). In other words, patterns found in natural populations are the result of selective forces operating both on laying females and on egg carriers; to focus exclusively on laying females is to miss part of the story. Thus, it is necessary to understand the costs and benefits for laying females, as well as the costs and benefits for individuals who accept eggs. Contrary to Härdling *et al.* (2007) statement, traits do not evolve for a 'purpose', but rather they are favoured by natural selection when they improve lifetime reproductive success. Thus, we do not understand the distinction that these authors draw between 'evolving for a purpose' and 'being helped by natural selection'. In addition, Härdling *et al.* (2007) state that there is no evidence suggesting that carrying eggs is beneficial for the carriers, and mention that egg carrying does not increase male attractiveness or mating frequency. However, they fail to mention that males benefit through improved offspring survival (see Gomendio & Reguera, 2001; Reguera & Gomendio, 2002).

The reason why they do not consider this obvious possibility seems to be that males carry genetic offspring as well as unrelated eggs. Again, this argument is incorrect. The literature on male parental care is full of examples of species in which males care for eggs/young despite of the fact that a proportion are unrelated, and this proportion can reach high values (Dixon *et al.*, 1994; Mulder *et al.*, 1994; Griffith *et al.*, 1999; Double & Cockburn, 2000; Griffith *et al.*, 2002). The reasons why parental care can evolve despite a low confidence of paternity are well understood and include whether mean paternity and confidence of paternity change over time or remain the same over successive matings, whether males can discriminate their own offspring, how high the costs of paternal behaviour are in terms of reduced reproductive residual value for the carers, and whether paternal care is crucial for offspring survival or not (see for instance Westneat & Sherman, 1993; Wright, 1998). Based on our own findings (which are not cited by Härdling *et al.*, 2007) we have argued that parental care has evolved in this system, despite intermediate levels of confidence of paternity, because sperm competition mechanisms are such that males are unable to predict when their own eggs will be laid (García-González *et al.*, 2003; García-González & Gomendio, 2004), males do not suffer mating costs when they carry eggs (García-González *et al.*, 2003), and egg survival on plants is extremely low (3%) but it improves considerably when eggs are carried by adults (25%) (Reguera & Gomendio, 2002). Thus, care by males has a great impact on egg survival.

Sperm competition mechanisms may influence male parental care because they determine confidence of paternity and how it changes over time. Our work indicates that the mechanism of sperm competition in the golden egg bug is one of sperm mixing, and that in natural populations the proportion of eggs sired by the last male to copulate with a female is around 43% on average and does not change significantly over a period of several days (García-González *et al.*, 2003). Males attempt to increase their paternity share by transferring more spermatozoa for a longer period of time when there are rival males (García-González & Gomendio, 2004). On the basis of this evidence we have argued that mechanisms of sperm competition have favoured the evolution of male care in this system for two reasons. First, males cannot choose to care for their offspring exclusively because they can neither discriminate between their own eggs and unrelated eggs, nor can they predict when their own eggs will be produced as a result of the degree of uncertainty created by sperm mixing. Second, males have nothing to gain from decreasing their parental effort in a given reproductive event because sperm mixing makes it difficult for males to reach high paternity levels, so they are unlikely to improve paternity levels in future reproductive events. In light of this, and since the survival of eggs laid on plants is virtually zero, a male must balance the cost of caring for unrelated eggs against the extremely high mortality cost for its own young if no care is provided.

Sperm competition mechanisms are determined to a great extent by female reproductive physiology and morphology (Birkhead & Møller, 1998), so it is expected that females will promote sperm competition mechanisms that will favour their reproductive interests. Female *Phyllomorpha laciniata* do not

lay clutches, but rather lay one egg at a time many times throughout the breeding season (0–10 eggs per day for 4–5 months), so they benefit from promoting a sperm competition mechanism that maximises the number of males that will accept eggs and the contexts in which they are likely to accept them. Thus, females maximise the chances that several males will accept eggs for long periods of time by promoting a mechanism of sperm mixing which ensures that all males that have copulated with a female have some chance of fathering offspring, that this probability remains constant with time, and that males have no cues as to when their own offspring will be produced. We suggest that sperm mixing allows females to manipulate males into accepting eggs not just after copulation, but at different times. Härdling *et al.* (2007) argue at length about whether males accept eggs before or after mating, assuming that if males accept eggs before mating it cannot constitute a form of parental care, and if they accept eggs immediately after mating it always will. There are no conclusive data in this respect since, as mentioned before, patterns of egg laying and egg acceptance under experimental conditions in which densities are artificially high and space restricted bear no resemblance whatsoever to patterns found in natural populations. Thus, patterns of egg laying and egg acceptance should be studied under natural conditions if their adaptive significance is to be understood. However, it is worth pointing out that female promiscuity and sperm mixing imply that only a proportion of the eggs laid after copulation are fathered by the last male to copulate, and that males will continue to have a share of paternity for a long time after copulating. Thus, males will not be selected to accept eggs only immediately after copulation, since paternity will be no higher then than later on. There is indirect evidence from the field indicating that males may mate repeatedly with the same female and accept eggs between successive copulations (García-González & Gomendio, 2003a). It is also possible that males may accept eggs before copulation, if this improves the chances of copulating and then receiving more eggs, but how often this happens in the field is unknown.

If males care when the benefits in terms of offspring survival exceed the costs, then males should be sensitive to differences between populations in egg mortality risks. Our recent work has shown that parasitoid wasps are a main cause of egg mortality on plants (Reguera & Gomendio, 2002), and that parasitoid pressure varies between populations (Gomendio *et al.*, 2007). As predicted by the parental care hypothesis, in populations where parasitoid pressure is higher, male egg carrying is more prevalent. In these populations, egg mortality as a result of parasitoid attack is up to 10 times higher on plants than on conspecifics. Thus, males carry eggs more often when the benefits in terms of protection against parasitoids are high (Gomendio *et al.*, 2007). Evidence that differences between populations are the result of differences in the behaviour of males comes from a study that showed that in southern Spain (where parasitoid pressure is expected to be low or absent) males resisted 65–73% of the oviposition attempts by their partners, while males from a population in northern Spain (where parasitoid pressure is expected to be higher) resisted 0–20% oviposition attempts (Miettinen & Kaitala, 2000).

In conclusion, we believe that field data and experimental evidence gathered over the last years lends support to the hypothesis that males carry eggs because in this way they protect them from the attack of parasitoids and enhance offspring survival. The reproductive interests of males and females are likely to be in conflict, since females would benefit from laying eggs on any conspecific, while males must balance the costs of caring for unrelated eggs against the benefits in terms of the survival of their own offspring. In addition, the benefit/cost ratio differs between populations given that parasitoid pressure varies. Thus, the golden-egg bug is a challenging model system to work with and offers exciting new avenues of research. However, the arguments developed by Härdling *et al.* (2007) in their recent commentary are very similar to those developed by Kaitala and collaborators years ago in a previous critique to the paternal care hypothesis (Kaitala *et al.*, 2001). We hope that this new exchange of ideas will stimulate further work on this fascinating species, rather than polarise the debate.

References

- Birkhead, T.R. & Møller, A.P. (1998) *Sperm Competition and Sexual Selection*. Academic Press, San Diego.
- Dixon, A., Ross, D., O'Malley, S.L.C. & Burke, T. (1994) Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. *Nature*, **371**, 698–700.
- Double, M. & Cockburn, A. (2000) Pre-dawn infidelity: females control extra-pair mating in superb fairy-wrens. *Proceedings of the Royal Society of London. Series B*, **267**, 465–470.
- García-González, F. (2002) *La evolución del transporte de huevos en Phyllomorpha laciniata (Het., Coreidae): una aproximación comportamental, fisiológica y molecular para explicar su significado adaptativo en machos y hembras*. PhD thesis, Universidad Complutense de Madrid, Madrid.
- García-González, F. & Gomendio, M. (2003a) A field test of the intraspecific brood parasitism hypothesis in the golden egg bug (*Phyllomorpha laciniata*). *Behavioral Ecology and Sociobiology*, **53**, 332–339.
- García-González, F. & Gomendio, M. (2003b) Oviposition site selection and oviposition stimulation by conspecifics in the golden egg bug (*Phyllomorpha laciniata*): implications on female fitness. *Behavioral Ecology and Sociobiology*, **53**, 385–392.
- García-González, F. & Gomendio, M. (2004) Adjustment of copula duration and ejaculate size according to the risk of sperm competition in the golden egg bug (*Phyllomorpha laciniata*). *Behavioral Ecology*, **15**, 23–30.
- García-González, F., Núñez, Y., Ponz, F., Roldán, E.R.S. & Gomendio, M. (2003) Sperm competition mechanisms, confidence of paternity and the evolution of paternal care in the golden egg bug (*Phyllomorpha laciniata*). *Evolution*, **57**, 1078–1088.
- García-González, F., Núñez, Y., Ponz, F., Roldán, E.R.S. & Gomendio, M. (2005) Paternity analysis in the golden egg bug using AFLPs: do the males preferentially accept their true genetic offspring? *Ecological Entomology*, **30**, 444–455.
- Gomendio, M., García-González, F., Reguera, P. & Rivero, A. (2007) Male egg carrying in *Phyllomorpha laciniata* is favoured by natural not sexual selection. *Animal Behaviour* (in press).
- Gomendio, M. & Reguera, P. (2001) Egg carrying in the golden egg bug (*Phyllomorpha laciniata*): parental care, parasitism, or both? Reply to Kaitala *et al.* *Behavioral Ecology*, **12**, 369–373.
- Griffith, S.C., Owens, I.P.F. & Burke, T. (1999) Environmental determination of a sexually selected trait. *Nature*, **400**, 358–360.
- Griffith, S.C., Owens, I.P.F. & Thuman, K.A. (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Molecular Ecology*, **11**, 2195–2212.
- Härdling, R., Borg, Å., Carrasco, D., Katvala, M., Kaitala, A. (2007) Male golden egg bugs (*Phyllomorpha laciniata* Vill.) do not preferentially accept their true genetic offspring: comment on the paper by García-González *et al.* (2005, *Ecological Entomology*, **30**, 444–455). *Ecological Entomology*, **32**, in press.
- Kaitala, A. (1996) Oviposition on the back of conspecifics: an unusual reproductive tactic in a coreid bug. *Oikos*, **77**, 381–389.
- Kaitala, A., Härdling, R., Katvala, M., Macías Ordóñez, R. & Miettinen, M. (2001) Is nonparental egg carrying parental care? *Behavioral Ecology*, **12**, 367–368.
- Katvala, M. & Kaitala, A. (2001) Egg performance on an egg-carrying bug. Experiments in the field. *Oikos*, **93**, 188–193.
- Katvala, M. & Kaitala, A. (2003) Conspecifics enhance egg production in an egg-carrying bug. *Behavioral Ecology*, **14**, 897–901.
- Katvala, M. & Kaitala, A. (2005) The effect of conspecific density on female reproduction in an egg-carrying bug. *Animal Behaviour*, **69**, 269–273.
- Miettinen, M. & Kaitala, A. (2000) Copulation is not a prerequisite to male reception of eggs in the golden egg bug *Phyllomorpha laciniata* (Coreidae; Heteroptera). *Journal of Insect Behavior*, **13**, 731–740.
- Mineo, G. (1984) Notizie biologiche su *Phyllomorpha laciniata* (Vill.) (Rhynchota, Het., Coreidae). *Phytophaga*, **2**, 117–132.
- Mulder, R.A., Dunn, P.O., Cockburn, A., Lazenbycohen, K.A. & Howell, M.J. (1994) Helpers liberate female fairy-wrens from constraints on extra-pair mate choice. *Proceedings of the Royal Society of London. Series B*, **255**, 223–229.
- Reguera, P. (1999) *Cuidado parental en Phyllomorpha laciniata (Het.: Coreidae): implicaciones para la evolución del cuidado por parte de machos y hembras*. PhD thesis, Universidad Complutense de Madrid, Madrid.
- Reguera, P. & Gomendio, M. (2002) Flexible oviposition behavior in the golden egg bug (*Phyllomorpha laciniata*) and its implications for offspring survival. *Behavioral Ecology*, **13**, 70–74.
- Tay, W.T., Miettinen, M. & Kaitala, A. (2003) Do male golden egg bugs carry eggs they have fertilised? A microsatellite analysis. *Behavioral Ecology*, **14**, 481–485.
- Westneat, D.F. & Sherman, P.W. (1993) Parentage and the evolution of parental behavior. *Behavioral Ecology*, **4**, 66–77.
- Wright, J. (1998) Paternity and paternal care. *Sperm Competition and Sexual Selection* (ed. by T. R. Birkhead and A. P. Møller), pp. 117–145. Academic Press, San Diego.

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