

**Maternal care under large clutches with small eggs: The evolution of life history traits in shield bugs**

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## Abstract

Patterns in the correlated evolution of parental care and life history traits are long established but controversial. Although parental care is related to large egg size in many taxa, conflicting results have also been reported. To test the evolutionary relationships between parental care and life history traits, we performed phylogenetic comparative analyses using shield bugs (Heteroptera: Acanthosomatidae), in which maternal guarding of eggs and young has repeatedly evolved. Our analyses revealed that female body size affected reproductive resource allocation. Contrary to the expectations of current theories, the acquisition of maternal care was associated with small eggs, large clutches, and large egg-resource allocation. There was a greater trade-off between egg size and clutch size in caring species than in non-caring species. Egg and hatchling developmental rates were not correlated with egg size, but were slower in caring species than in non-caring species. Analyses of evolutionary transitions suggest that the establishment of large clutches, small eggs and large egg-resource allocation preceded the evolution of maternal care. To our knowledge, this is the first study clarifying the evolution of parental care linked with small eggs in invertebrates.

## Introduction

The relationship between parental care and other life history traits such as egg size has attracted much attention among evolutionary biologists. In a variety of ectothermic taxa, both in vertebrates (e.g., Gross and Sargent 1985; Nussbaum 1987; Summers et al. 2006; Vági et al. 2019; Vanadzina et al. 2021; Furness et al. 2022) and invertebrates (e.g., Iwata and Sakagami 1966; Strathmann and Strathmann 1982; Kutschera and Wirtz 2001; Machado and Raimundo 2001), it has repeatedly been shown that the evolution of parental care is related to large egg size and consequently often to small clutch size.

Despite these correlated patterns, there are important exceptions. Some comparative studies, using cichlid fishes (Kolm et al. 2006) and spiders (Simpson 1995), showed a positive relation between parental care and clutch size. Kolm et al. (2006) suggested that an initial increase in clutch size may have driven parental care because the large clutches will be of increased reproductive value (Coleman et al. 1985; Sargent and Gross 1985). Although it is possible that the evolution of large clutches results in small eggs due to trade-offs between size and number, no study has detected a clear association between small eggs and parental care (Simpson 1995; Kolm et al. 2006, but see Furness et al. 2022). On the other hand, neither egg size nor clutch size correlate to parental care in insects (Gilbert and Manica 2010) or salamanders (Vági et al. 2022).

Thus, we currently lack a general explanation for the different associations between parental care and reproductive traits among taxa.

Causal relationships between life history traits such as egg size and parental care could be different among taxa when the relationships of these traits to fitness differ in different environments. In aquatic taxa, for example, larger eggs may suffer oxygen deficiency due to diffusion constraints and may require parental oxygenating behavior (Nussbaum 1987; Smith 1997; Kolm and Ahnesjö 2005). By contrast, larger young that hatch from larger eggs may enjoy fitness benefits, such as higher competitive abilities and survival (Fox and Czesak 2000). However, when larger eggs require a longer time for development until hatching, they will be exposed to environmental threats during this period. In this case, parental care provides “a safe harbor” for eggs, and consequently egg size will shift to be larger (Shine 1978; Sargent et al. 1987).

When parents provide food to the offspring, potential reduction of parental fitness due to sibling competition can select for smaller clutches to reduce the competition (Godfray et al. 1991), which can result in larger eggs. On the other hand, harsh environments for offspring, e.g., low food availability for hatchlings (Ito 1980; Ito and Iwasa 1981) or small size-specific predation (Shine 1989), can select for large eggs and parental care independently.

Shine (1989) arranged explanations for the establishment of a correlation between parental care and egg size (applicable to other traits) into four types: parental care selects for egg size (Shine 1978; Sargent et al. 1987), egg size selects for parental care (Nussbaum 1987), parental care and egg size coevolve (Nussbaum and Schultz 1989), and some third factor simultaneously favors both of the traits and thus leads to an association between them (Ito 1980; Ito and Iwasa 1981; Kolm and Ahnesjö 2005). One way to discriminate such alternative hypotheses is to estimate the temporal order of trait evolution within a lineage, including species with and without parental care (Shine 1989). Such attempts, however, have been limited in studies on vertebrates such as amphibians (Summers et al. 2006; Furness and Capellini 2022).

Recently, large-scale comparative analyses, sometimes dealing with more than one thousand species, have been applied to the study of life history trait evolution in animal taxa (e.g., Stoddard et al. 2017; Church et al. 2019; Vagi et al. 2019; Vanadzina et al. 2021; Furness and Capellini 2022). Such analyses could have advantages in increasing the explanatory power of a model to find general trends and to disentangle influences of explanatory variables included in the model (e.g., caring, body size and others). However, they would inevitably be accompanied by confounding factors (which are not included in the model) derived from diversified ecology and behavior and/or different

constraints among the distant taxa included (e.g., Montgomerie et al. 2021). Moreover, such studies always analyze the data gathered from the literature, but the data quality is often inconsistent among studies, and this might provoke confusion regarding the results obtained (e.g., Vitt and Seigel 1985). One way to avoid such potential problems may be to choose a lineage consisting of closely related species for the analysis, in which there is sufficient variation in traits of interest, but others are uniform, and the trait data are obtained in a consistent manner.

Shield bugs (Acanthosomatidae: Heteroptera) are an appropriate system for comparative testing of the hypotheses on correlated evolution between parental care and life history traits. Although there is a large interspecific variation in the life history traits of interest in the above-mentioned theories and analyses, the species included in this monophyletic taxon (Grazia et al. 2008; Schuh and Weirauch 2020) show rather uniform conditions in other traits of reproduction and lifestyles; females lay eggs in discrete clutches in a single layer on host plant leaves, and nymphs grow on the host plant while feeding on fruit (Tachikawa 1991; Kobayashi and Tachikawa 2004; Kudo et al. 2019a, b; Kudo 2023). Tsai et al. (2015) showed that maternal care evolved repeatedly within this lineage (Figure 1). In species with care behavior, the female always stays (in a straddling posture) with an egg clutch and the first instar nymphs that make a tight

aggregation (without feeding) on the natal site (Melber and Schmidt 1975b; Kudo et al. 1989; Tachikawa 1991; Kudo and Nakahira 1993; Mappes et al. 1997; Kudo and Ogasa 2017; Kudo and Yano 2019). In some species, the caring female continues to attend an aggregation of the second or later instar nymphs that leave the natal leaf to seek host fruit (Melber and Schmidt 1975a; Kudo et al. 1989; Kudo 1996). The function of maternal care is restricted to attend and guard eggs and young against arthropod predators (Melber and Schmidt 1975b; Melber et al. 1980; Honbo and Nakamura 1985; Kudo et al. 1989; Kudo and Nakahira 1993; Mappes et al. 1997; Kudo 2002; Nishimura et al. 2023). Comparative analyses using a lineage including species possessing a single simple form of parental care may help to avoid difficulties in untangling complexities in correlated evolution among life history traits and test explicitly the hypotheses on it (Furness and Capellini 2022).

In the present study, we analyze evolutionary correlations between maternal care and other life history traits, and estimate transition sequences between maternal care and life history traits in shield bugs using phylogenetic comparative methods. We address the following questions on this account: Is the evolution of maternal care correlated with that of egg size, clutch size, or reproductive output? Is the trade-off between egg size and clutch size different between caring and non-caring species? Is the evolution of



egg (and hatchling) developmental rates correlated with that of egg size and maternal care? Finally, do the evolutionary changes of egg size, clutch size and reproductive output precede the evolution of maternal care or vice versa?

## Materials and methods

### Collection of life history data

Overwintered females were collected in the breeding season of each species from 1988 to 2022 (see Supplementary Table S1). The females were maintained individually in a plastic case (9 cm in diameter, 3 cm in height for small species or 14 cm in diameter, 6.5 cm in height for large species) with host plant shoots bearing leaves and fruit at  $20.0 \pm 0.5^\circ\text{C}$  under 16-h light:8-h dark (or 15-h light:9-h dark) conditions. Females laid clutches of eggs on the leaf of the host plants (Yamamoto et al. 2009). In some species, the female produces multiple clutches during her lifetime (e.g., Mappes et al. 1996). In such cases, we used the first clutch produced for the following measurement. We recognized maternal care when the female stayed on an egg mass after oviposition and showed aggressive responses against disturbance (e.g., Kudo 1990). After counting the number of eggs in the clutch, five to ten eggs were randomly selected and weighed together to the nearest 0.01 mg to obtain average egg weight (referred to as “egg size”). In some species, particularly caring species, clutch size and egg size data were also obtained from clutches found in the field.

The weight of the fresh egg specimens was not available for two species, namely *Elasmotethus rotundus* and *Ditomotarsus hyadesi* (although data for egg weight in *D.*

*hyadesi* are described in Carvajal and Faúndez, [2015], these have apparently low measurement accuracy). Using egg specimens preserved in 80% alcohol, which had been randomly chosen from clutches of these species, we measured the length and width of eggs and estimated the egg volume proxy as a spheroid ( $= \text{length} * \text{width}^2$ ). Then, mean egg weight for each species was estimated using a linear regression constructed by data for other shield bug species:  $\text{mean egg weight} = 0.01 + \text{mean egg-volume proxy} * 0.543$  ( $R^2 = 0.997$ ,  $P < 0.0001$ ).

When testing the safe harbor hypothesis (Shine 1978), the relationship between egg size and egg developmental periods is crucial. Furthermore, recent theoretical models suggest that a low developmental rate for eggs is one of the key traits leading to the origin of parental care (Klug and Bonsall 2010; Klug et al. 2013). In shield bugs, hatchlings (the first instar nymphs) take no nutrients (but absorb bacterial symbionts: Kikuchi et al. 2009), are inactive and stay on natal sites in tight aggregations until molting (Kobayashi and Tachikawa 2004). Therefore, instead of egg developmental periods, we adopted the developmental period of both eggs and hatchlings as a target trait in this study. To measure the developmental period, egg clutches were kept at  $20.0 \pm 0.5^\circ\text{C}$  under 16-h light:8-h dark (or 15-h light:9-h dark) conditions. Egg hatching and molting to the second instar were usually synchronized within clutches and ended

within a day. Even when the day when oviposition had occurred was not identified (e.g., in cases where egg clutches were found in the field), we measured the first instar period in the laboratory. For *Elasmucha grisea*, *E. fieberi* and *E. ferrugata*, the data for developmental periods of eggs and hatchlings were extracted from Figure 1 in Kaitala and Mappes (1997) and those for *D. hyadesi* were obtained in Carvajal and Faúndez (2015), in which the eggs and nymphs were kept under the same mean temperature as that in this study.

Generally, female body size in arthropods corresponds closely to the total amount of reproductive resources and affects their allocation, such as egg size and clutch size (Simpson 1995; Fox and Czesak 2000; Gilbert and Manica 2010; Church et al. 2019). Thus, to examine trade-offs between the size and number of eggs, female body size must be controlled (Fox and Czesak 2000). We measured the abdomen length of adult female specimens (referred to as “female body size”) as a proxy for body size and egg resource storage (see Kudo et al. 2019b; Kudo 2023), using a digital sight imaging controller (Nikon DS-L2; Nikon, Tokyo, Japan) integrated into a binocular microscope.

When data for life history traits were available from the literature (Kudo et al. 1989; Kudo and Nakahira 1993; Kaitala and Mappes 1997; Kudo 2001, 2002, 2006; Hanelová and Vilímová 2013; Carvajal and Faúndez 2015; Kudo and Ogasa 2017; Kudo and Yano

2019; Kudo et al. 2019a, b; Kudo 2023), we also used them for the analyses. Newly obtained original data were combined with the literature data when raw data were available. When data for life history traits for a single species were published in different papers, we used the data from the paper describing both egg weight and clutch size (or more traits analyzed in this study) for the species or combined the data in different papers when raw data were available.

Caring status of each species was assigned a binary value: caring = 1 or non-caring = 0. Other traits, i.e., female body size, egg size, clutch size, reproductive output (i.e., the product of egg size and clutch size), and developmental periods of eggs and hatchlings, were averaged for each species (Supplementary Table S1). These mean values were natural-log-transformed before they were used in comparative analyses. As a result, the mean trait values (Supplementary Table S1) were organized for 33 species (including one subspecies) from all three subfamilies of the Acanthosomatidae, which accounts for about 12% of the species described in this family (Schuh and Weirauch 2020).

#### Evolutionary associations between traits

Throughout the phylogeny-based comparative analyses conducted in this study, we used the molecular phylogeny of Acanthosomatidae by Tsai et al. (2015), which contains all

species analyzed in this study (Figure 1).

We tested evolutionary associations between parental care and life history traits using phylogenetic generalized least squares (PGLS) models (Grafen 1989; Martins and Hansen 1997). Pagel's lambda, a measure of the phylogenetic signal, was estimated and this value was utilized to scale the phylogenetic correlation structure in the PGLS models (Pagel 1999; Freckleton et al. 2002). We separately built a PGLS model including caring status and female body size as independent variables and one of the life history traits, i.e., egg size, clutch size, or reproductive output as a dependent variable. When each PGLS model was run, species with missing data on the traits subjected to analysis were removed and thus pruned from the phylogenetic tree shown in Figure 1.

To examine a trade-off between egg size and clutch size and the influence of maternal care on the trade-off (i.e., on the slope), we constructed a PGLS model including clutch size as a dependent variable and caring status, egg size, female body size, and an interaction term between the former two variables, as independent variables. When a significant interaction term between the effects of egg size and caring status is detected, it indicates the influence of maternal care on the trade-off.

Finally, to examine associations between developmental periods of eggs and hatchlings and egg size (Shine 1978) or maternal care (Klug and Bonsall 2010), we

constructed a PGLS model including the developmental period of eggs and hatchlings as a dependent variable and caring status and egg size as independent variables.

All PGLS analyses were performed using the `phylolm` function in the *phylolm* package (Ho and Ané 2014) in R version 4.2.2 (R Development Core Team 2022).

### Transitions of trait evolution

To infer the temporal order of evolutionary changes in maternal care and other life history traits, the pattern of evolutionary transitions among character states was examined using the Discrete dependent model implemented in BayesTraits version 3.0.5 (Meade and Pagel 2021). The dependent model can adopt four states, one for each combination of two binary traits (State 1 = 0,0; State 2 = 0,1; State 3 = 1,0; State 4 = 1,1) and assumes that the rate of change in one trait is dependent on the state of the other. This model considers all possible forward and reverse transitions between the states, with the assumption that transitions involving simultaneous changes in both traits do not occur. These transitions are defined by eight transition rate parameters,  $q_{12}$ ,  $q_{13}$ ,  $q_{21}$ ,  $q_{24}$ ,  $q_{31}$ ,  $q_{34}$ ,  $q_{42}$ , and  $q_{43}$ , in which the parameter  $q_{ij}$  denotes the rate of change from state  $i$  to state  $j$ .

The dependent models were run separately for the combinations of maternal care

(care = 1 and no care = 0) and one of the three life history traits, i.e., egg size, clutch size, and reproductive output, which were correlated with maternal care (see the Results section). Egg size, clutch size, and reproductive output were converted from continuous variables to binary categories because the Discrete dependent model exclusively handles binary traits. These conversions were performed while controlling for differences in female body size, given that female body size affects these traits (see the Results section). Specifically, each egg size, clutch size, and reproductive output was regressed on female body size, and a residual value was calculated for each species. Positive residuals were categorized as “large” (= 1), while negative residuals were categorized as “small” (= 0). These regression analyses were conducted using PGLS models, in which the estimated lambda values were incorporated. The statistics are shown in Supplementary Table S2.

The transition rate parameters were estimated using reversible-jump Markov Chain Monte Carlo (MCMC) methods. Three independent MCMC chains were run for 11,000,000 iterations with the first 1,000,000 iterations discarded as burn-in and a thinning interval of 1000 iterations. Convergence was assessed by the Gelman–Rubin statistic (Gelman and Rubin 1992) using the `gelman.diag` function in the *coda* package (Plummer et al. 2006) in R version 4.2.2 (R Development Core Team 2022). The



Gelman–Rubin statistic values were less than 1.04 for all parameter estimates, indicating convergence across the three MCMC chains, as values  $<1.1$  are generally indicative of convergence. The results for three chains were combined to obtain the posterior distribution. The Z-score for each parameter was calculated as the proportion of iterations in which the transition rate had a value of zero (Pagel et al. 2006) in the posterior distribution. The Z-score indicates the probability of a transition between states not occurring. Z-scores were used to identify potential transitions between states that have occurred, allowing for the determination of the most likely temporal order of trait evolution.

## Results

*Is the evolution of maternal care correlated with that of egg size, clutch size or reproductive output?*

Female body size greatly affects reproductive output and its allocation in shield bugs (Table 1). Larger females invest more egg resources (Table 1C) and allocate them in a manner such that they produce larger (Table 1A) and more eggs (Table 1B). When female body size effects were controlled, it was revealed that resource allocation depends on the caring status of females; caring females produced smaller (Table 1A, Figure 2A) and more (Table 1B, Figure 2B) eggs and invested more resources in a single breeding event (Table 1C, Figure 2C) than non-caring females.

*Is the trade-off between egg size and clutch size different between caring and non-caring species?*

When the effect of female body size was controlled, a significant interaction between egg size and caring status, i.e., an effect of maternal care on a trade-off between egg size and clutch size was detected (Table 2). In both analyses conducted separately in caring and non-caring species, significant negative relationships between egg size and clutch size were detected (Supplementary Table S3), but the slope of the

trade-off in caring species (PGLS regression estimate  $\pm$  SE =  $-0.888 \pm 0.167$ ; Supplementary Table S3A) was steeper than that in non-caring species ( $-0.431 \pm 0.164$ ; Supplementary Table S3B).

*Is the evolution of egg (and hatchling) developmental rates correlated with that of egg size and maternal care?*

There was no significant relationship between egg size and developmental periods of eggs and hatchlings (PGLS with the lambda value of 0.948: Intercept, estimate  $\pm$  SE =  $2.637 \pm 0.127$ ,  $t = 20.830$ ,  $P < 0.001$ ; Egg size, estimate  $\pm$  SE =  $0.134 \pm 0.097$ ,  $t=1.389$ ,  $P=0.177$ ). On the other hand, irrespective of their size, eggs of species with maternal care took a longer time to hatch and molt to the second instar than those of non-caring species (Care status: estimate  $\pm$  SE =  $0.236 \pm 0.095$ ,  $t = 2.482$ ,  $P = 0.020$ ) (Figure 3).

*Do evolutionary changes of egg size, clutch size and reproductive output precede the evolution of maternal care or vice versa?*

Using three traits, namely egg size, clutch size, and reproductive output, which have been shown to be evolutionarily correlated with maternal care, we reconstructed historical transitions of the states of maternal care (caring or non-caring) and those traits

(small or large) (Figure 4). Our transition rate analyses indicated that egg size was evolutionarily labile under the non-caring state and bidirectional transitions between the states of egg size (small or large) often occurred (q12 and q21 in Figure 4A, see also Supplementary Figure S1A). Maternal care was very unlikely to evolve from an ancestral state of large eggs (q24) but likely to evolve from an ancestral state of small eggs (q13). These results support the scenario in which the evolution of maternal care followed egg size reduction.

Clutch size was also labile in the non-caring state (q12, q21 in Figure 4B, see also Supplementary Figure S1B). Maternal care was very unlikely to evolve from an ancestral state of small clutches (q13) but likely to evolve from an ancestral state of large clutches (q24). These results suggest that the evolution of maternal care followed clutch size enlargement.

For the transition of the states of reproductive output and maternal care, the results were like those for egg size and clutch size (Figure 4C, see also Supplementary Figure S1C). Maternal care was not likely to evolve from an ancestral state of small reproductive output (q13) but likely to evolve from an ancestral state of large reproductive output (q24).

## Discussion

Our results offer evidence of an evolutionary correlation between parental care and the production of large clutches with small eggs. Although a few studies showed an association between parental care and large clutch size (Simpson 1995; Kolm et al. 2006), no studies indicated an association between parental care and small eggs until recently. Furness et al. (2022) showed that although parental care is generally associated with large eggs, small eggs have also evolved with a particular type of parental care in amphibians (see below). The results presented here are, to the best of our knowledge, the first evidence of an evolutionary association between parental care and large clutches with small eggs in invertebrates.

As pointed out by Furness et al. (2022), in which an association between small eggs and female tadpole attendance was detected, the coevolutionary model by Nussbaum and Schultz (1989) predicted that “at any given level of parental care, egg size may decrease if environmental conditions for juvenile survival improve”. Furness et al. (2022) explained that the association between small eggs and maternal care (i.e., tadpole attendance) can be due to breeding habitats, such as ponds and terrestrial burrows, where the tadpoles can be protected from predators. However, this may not be the case for shield bugs, because nymphs in both species with and without maternal care live in

similar environments throughout their life, i.e., on host plants where they are exposed to environmental threats, such as predation and parasitism. Furness et al. (2022) also indicated that in amphibians, provisioning tadpoles with trophic eggs is associated with smaller eggs, as well as smaller clutches (see also Vági et al. 2019). However again, this is not the case for shield bugs. Although in other true bug taxa, females provision nymphs with trophic (or specialized) eggs as food (Nakahira 1994; Kudo and Nakahira 2004; Hironaka et al. 2005; Filippi et al. 2008; Lopez-Ortega and Williams 2018), shield bug females do not provide such trophic eggs, but instead only guard against predators (Melber and Schmidt 1975b; Melber et al. 1980; Honbo and Nakamura 1985; Kudo et al. 1989; Kudo and Nakahira 1993; Mappes et al. 1997; Kudo 2002; Nishimura et al. 2023).

We thus need a novel hypothesis for evolutionary associations between maternal care and small eggs, as well as large clutches and large reproductive output, in shield bugs.

Parental care is expected to evolve under conditions where the fitness benefits, in terms of increased offspring survival and growth, to a parent providing care outweigh the costs to the parent in terms of decreased survival and future reproduction (Clutton-Brock 1991; Alonso-Alvarez and Velando 2012). Parental care, therefore, will be favored under conditions where the benefits of providing care are high and/or the

costs are low. The former conditions have traditionally been stressed in the arguments on the origin of parental care (e.g., Wilson 1975; Clutton-Brock 1991).

Recent theories have specified the life history conditions working at the origin of parental care, e.g., stage-specific mortality and maturation rates, and reproductive rates, and those for each sex (Klug and Bonsall 2010, Klug et al. 2013). The baseline high mortality rate of eggs is the most important and consistently favors the evolution of parental care in any combination of other conditions (Klug and Bonsall 2010, Klug et al. 2013). The results of female-removal experiments using shield bugs in past studies support this prediction; when the mortality of eggs and hatchlings in the absence of caring females was measured under field conditions, high mortality rates caused by arthropod predation have always been shown (Melber and Schmidt 1975b; Melber et al. 1980; Honbo and Nakamura 1985; Kudo and Nakahira 1993; Kudo 2002; Nishimura et al. 2023).

On the other hand, parental care will also be favored under the condition that the costs of providing care are low, as mentioned above. This condition has not received much attention, although for male parental care, it has often been addressed (i.e., the cost of caring on mating success: e.g., Tallamy 2000; Manica and Johnstone 2004). The semelparity hypothesis, which states that semelparous reproduction promotes the

evolution of maternal care (Tallamy and Brown 1999), can be categorized under this condition (see also Kudo et al. 1989; Kudo et al. 1995). Under an interclutch trade-off between current and future reproductive efforts (Clutton-Brock 1991; Stearns 1992), selection increasing the former will decrease the latter and consequently can limit breeding opportunity. However, this causal relationship can be reversed. When intrinsic or extrinsic factors, which are independent of current reproductive effort, inhibit the future breeding opportunities of females, selection should favor females that invest in the current breeding opportunity as much as possible. Such enforced semelparity could release the females from the imposed costs of reproduction including post-ovipositional investment. Consequently, maternal care, as well as larger reproductive output, will be favored (Tallamy and Brown 1999; but see Trumbo 2013).

Although the information is still fragmentary, in non-caring acanthosomatid taxa, i.e., *Elasmotethus*, *Cyphostetus*, *Ditomotarsus*, *Lindbergicoris*, and some *Acanthosoma*, females lay egg clutches repeatedly (Mappes et al. 1996; Hanelová and Vilimová 2013; Carvajal and Faúndez 2015; Kudo et al. 2019a, b; Kudo unpublished data). By contrast, females of caring species lay eggs only once (or probably twice in *Elasmucha ferrugata*; Kaitala and Mappes 1997) under field conditions (Kudo et al. 1989; Tachikawa 1991; Kudo and Nakahira 1993; Kaitala and Mappes 1997; Kudo 2002).



Even in such semelparous species, females sometimes produce more than one clutch, particularly when they lose the first one under laboratory conditions (Kaitala and Mappes 1997; Kudo 1994). However, this probably does not occur (or the offspring never survive even if it does occur) under field conditions due to ecological constraints (functional semelparity, *sensu* Tallamy and Brown 1999). The limited period during which food is available to young can work as such a constraint (Kudo et al. 1995), and in shield bugs, the dependence of nymphs on the ephemeral fruit of hosts may be responsible for functional semelparity (Kudo et al. 1989; Kudo 1994).

The shift from iteroparous to semelparous breeding can result in larger clutch size than iteroparous breeding, even when egg size is constant. Large clutch size can enhance the benefit of parental care because the large clutch will be of high reproductive value (Sargent and Gross 1985; Coleman et al. 1985; Kolm et al. 2006). The benefit that comes from lowering egg mortality rates might also be enhanced if larger clutches are more susceptible to predation (Krause and Raxton 2002) in the absence of maternal care.

Large clutches resulting from semelparity may be further enlarged by selection based on the group size of young after hatching. Nymphs of acanthosomatid bugs, particularly, those of early instars live in groups (Kobayashi and Tachikawa 2004). The benefits of

group formation have been explored from a variety of aspects, e.g., avoiding predation, finding food, reducing heat and water loss, and moving efficiently (Krause and Raxton 2002). Heteropteran nymphs emit chemical defensive substances from their scent glands when attacked (Schuh and Weirauch 2020). It has been suggested that large aggregations of distasteful young, such as those reported for many heteropteran species, are likely to evolve as an anti-predator adaptation when eggs have already been deposited as a clutch (Sillen-Tullberg and Leimar 1988). Although the survival of immature insects is often affected by egg size, which determines their initial size (Fox and Czesak 2000), the survival of heteropteran nymphs may be compensated by aggregation formation, and thus may depend strongly on aggregation size rather than on egg size. When predation pressure is elevated in breeding habitats of semelparous species, large clutch size (thus large nymphal aggregations) will be selected, and consequently small eggs may result through size-number trade-offs. Such enlarged clutches under high predation pressure will also favor maternal guarding behavior. The results of our transition rate analyses imply this scenario; the state of large clutches and small eggs, as well as large reproductive output, preceded the evolution of maternal care in shield bugs.

Once maternal guarding behavior has originated, selection can reinforce downsizing

of eggs with keeping (or even enhancing) clutch size in shield bugs. In species with maternal guarding of clutches deposited on leaves, eggs placed on the peripheral area of the clutch suffer high predation due to the guarding posture of the female, covering the clutches with her body (Eberhard 1975; Mappes and Kaitala 1994). Such selection may lead to not only small peripheral eggs due to low fitness return per resource investment (Mappes et al. 1997; Kudo 2001, 2006), but also small eggs on average to accomplish clutch area fitted to the female body area, particularly when egg size does not tightly affect the survival of young due to compensatory effects of their aggregation.

We also show that the development rate of eggs and hatchlings is not correlated with egg size but is slower in species with maternal care than in those without care. The former result is consistent with a general trend across diverse insect taxa (Church et al., 2019) and does not meet a key prediction of the safe harbor hypothesis, which postulates that larger eggs develop slower (Shine 1978). It has been theoretically indicated that the egg developmental rate is an important component of life history conditions at the origin of parental care, and a low egg developmental rate will favor parental care (Klug and Bonsall 2010). This prediction is supported in shield bugs. The slow development of eggs and hatchlings, in combination with high predation pressure, can result in low survival during their development, and consequently will increase

benefits of maternal guarding.

Our analysis found that the trade-off between egg-size and number was different between caring and non-caring species. The steep trade-off in caring species suggests that the resource limitation on egg production was strict. It is likely that such effects are not direct but indirect through possible differences in parity between species with and without maternal care (Fox and Czesak 2000). In semelparous species (with care), females invest stored egg resources into the first clutch production and retain just undeveloped ovaries after oviposition (Kudo et al. 1989; Kudo and Nakahira 1993; Ogorzałek and Trochimczuk 2009). On the other hand, in iteroparous species (without care), females feed on host fruit to obtain nutrients for serial egg production and possess a series of developing oocytes within ovaries during their reproductive life (Kudo 1994; Ogorzałek and Trochimczuk 2009). Thus, the resource limitation in a single oviposition, and consequently the trade-off between egg size and clutch size, can be weakened by serial nutrient intake in iteroparous females without maternal care.

## Conclusion

In summary, we clarified the relationships between maternal care and other life history traits in shield bugs; the maternal care is associated with large clutches with

small eggs, large reproductive output, a steep egg-size-clutch-size trade-off, and low egg (and hatchling) development rates. The evolution of small egg size, large clutch size and large reproductive output is most likely to precede that of maternal care. Then, we provided an explanation of selective forces leading to the pattern, focusing on a possible shift from iteroparous to semelparous reproduction (or an extremely limited number of breedings) and predation pressure at the origin of the care. We showed here that the correlated evolutionary patterns of the traits and their transitions are in line with implications from the semelparity hypothesis (Tallamy and Brown 1999), and highlighted that some of the reproductive ecology traits are not consequences but are drivers of the evolution of maternal care (see also Furness and Capellini [2022] for parental care in amphibians). To evaluate the generality of the semelparity hypothesis for understanding the evolution of life history traits, it is required to accumulate the data on the parity states realized in the field for species with and without care in a variety of taxa.

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#### Statement of Authorship

SK conceived and designed the study; SK and JFT collected the data and identified the species; TH, NK and KY analyzed the data; SK and TH wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

#### Data and Code Accessibility

Data and code used in this study are available on Figshare (<https://doi.org/10.6084/m9.figshare.25217828>; Kudo et al. 2024).

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Table 1. Estimates from phylogenetic generalized least squares (PGLS) models for evolutionary associations between egg size (A), clutch size (B) or reproductive output (C) and maternal care in shield bugs.

Independent variable	Estimate	SE	t	P
(A) Egg size (lambda = 0.958)				
(Intercept)	-3.065	0.537	-5.703	< 0.001
Caring status	-0.548	0.131	-4.193	< 0.001
Female body size	1.355	0.297	4.560	< 0.001
(B) Clutch size (lambda = 0.000)				
(Intercept)	0.888	0.307	2.897	0.007
Caring status	1.233	0.099	12.427	< 0.001
Female body size	1.161	0.163	7.126	< 0.001
(C) Reproductive output (lambda = 0.591)				
(Intercept)	-2.620	0.487	-5.382	< 0.001
Caring status	0.675	0.132	5.115	< 0.001
Female body size	2.717	0.269	10.111	< 0.001

Note. Caring status is a binary variable (caring = 1 or non-caring = 0). Female body size refers to mean female abdomen length (mm). Egg size is provided as mean weight (mg). Reproductive output (mg) is calculated as egg size  $\times$  mean clutch size. Trait values (excluding caring status) are natural-log-transformed before the analyses. The lambda values estimated were used to scale the phylogenetic correlation structure in the PGLS model. See the text for details.



Table 2. Estimates from phylogenetic generalized least squares (PGLS) models for an evolutionary trade-off between egg size and clutch size and effects of maternal care on it in shield bugs.

Independent variable	Estimate	SE	t	P
(Intercept)	-1.369	0.540	-2.538	0.017
Caring status	0.637	0.210	3.037	0.005
Female body size	2.218	0.254	8.739	< 0.001
Egg size	-0.447	0.114	-3.907	< 0.001
Caring status × Egg size	-0.373	0.163	-2.283	0.031

Note. The lambda value = 0.000. See Table 1 legend for details.

## Figure legends

Figure 1. Molecular phylogeny and the evolution of maternal care in shield bugs.

Reconstructed ancestral states of maternal care mapped onto the molecular phylogeny by Tsai et al. (2015). Ancestral states were reconstructed using the maximum likelihood method using the ace function in the ape package (Paradis and Schliep, 2018) in R version 4.2.2 (R Development Core Team, 2022). Pie diagrams at nodes show the probability of the absence or presence of maternal care (white: non-caring, black: caring). The results of Tsai et al. (2015) were revised using additional information for *Acanthosoma forficula* (Kudo and Ogasa, 2017) and *Acanthosoma crassicaudum* (this study).

Figure 2. Relationship between maternal care and egg size (A), clutch size (B) and reproductive output (RO) (C).

Raw species data (natural-log-transformed) are shown. Caring: closed circles, non-caring: open circles.

Figure 3. Relationship between egg size and developmental periods of eggs and hatchlings.

Raw species data (natural-log-transformed) are shown. Caring: closed circles, non-caring: open circles.

Figure 4. Evolutionary transitions among character states of maternal care and life history traits, egg size (A), clutch size (B) and reproductive output (C).

The character states are represented as the combination of maternal care (caring = 1 and non-caring = 0) and egg size, clutch size, and reproductive output, which are correlated with maternal care. Egg size, clutch size, and reproductive output after controlling for differences in female body size were converted to binary variables, large (= 1) or small (= 0), using the residuals of phylogenetic generalized least squares (PGLS) regression for each trait on the female body size (see the text for details). Transitions among the states are defined by eight transition rate parameters,  $q_{12}$ ,  $q_{13}$ ,  $q_{21}$ ,  $q_{24}$ ,  $q_{31}$ ,  $q_{34}$ ,  $q_{42}$ , and  $q_{43}$ . Arrows representing transitions between states are accompanied by Z-scores, reflecting the proportion of iterations assigned as zero in the posterior distribution shown in Supplementary Figure S1. When a Z-score was  $> 0.8$ , transitions were considered very unlikely to occur and are depicted by dashed arrows. Bold arrows indicate transitions with a Z-score  $< 0.05$ , which were considered most likely to occur, and thin arrows indicate other cases.

Figure 1

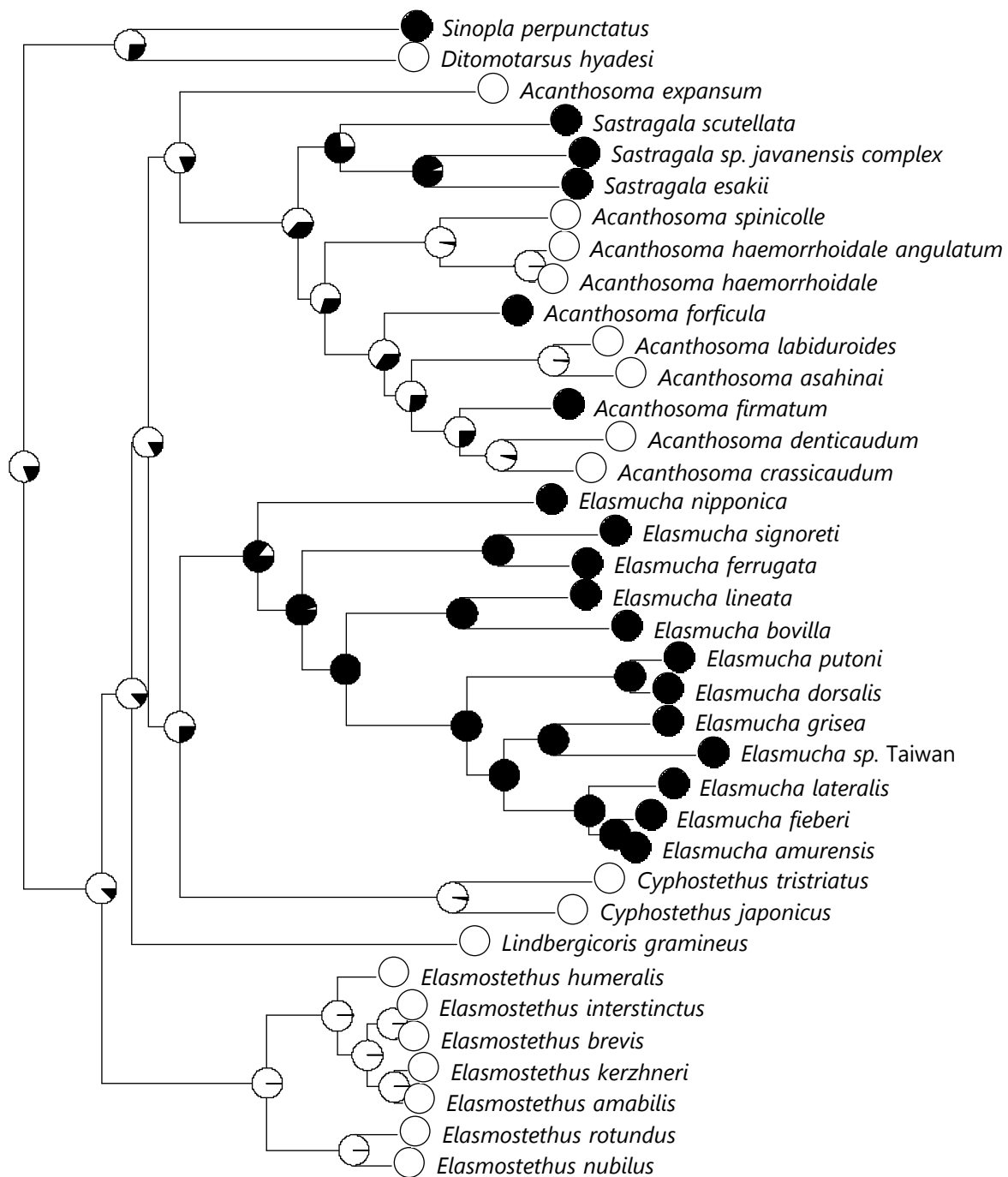


Figure 1

Figure 2

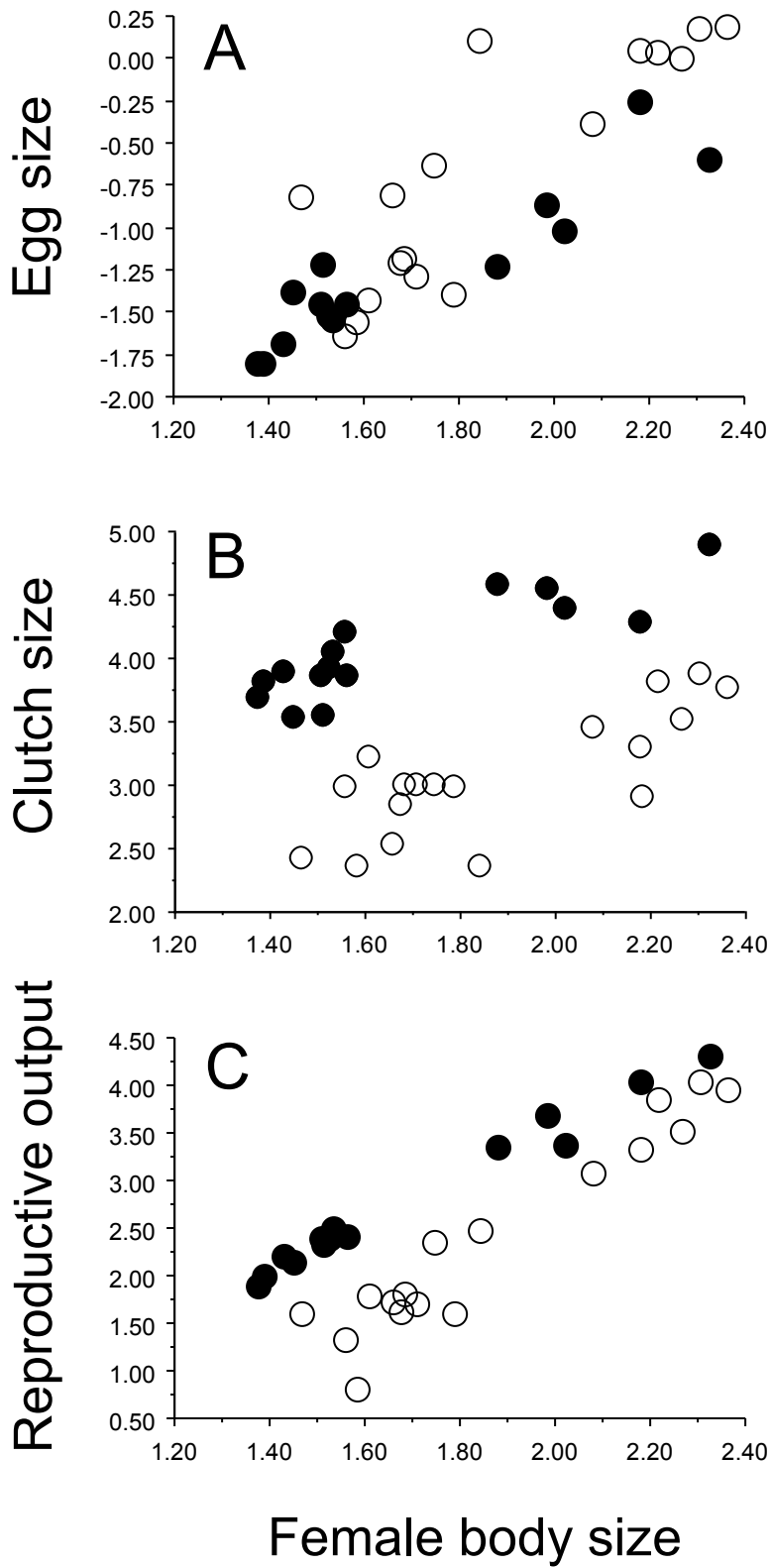


Figure 2

Figure 3

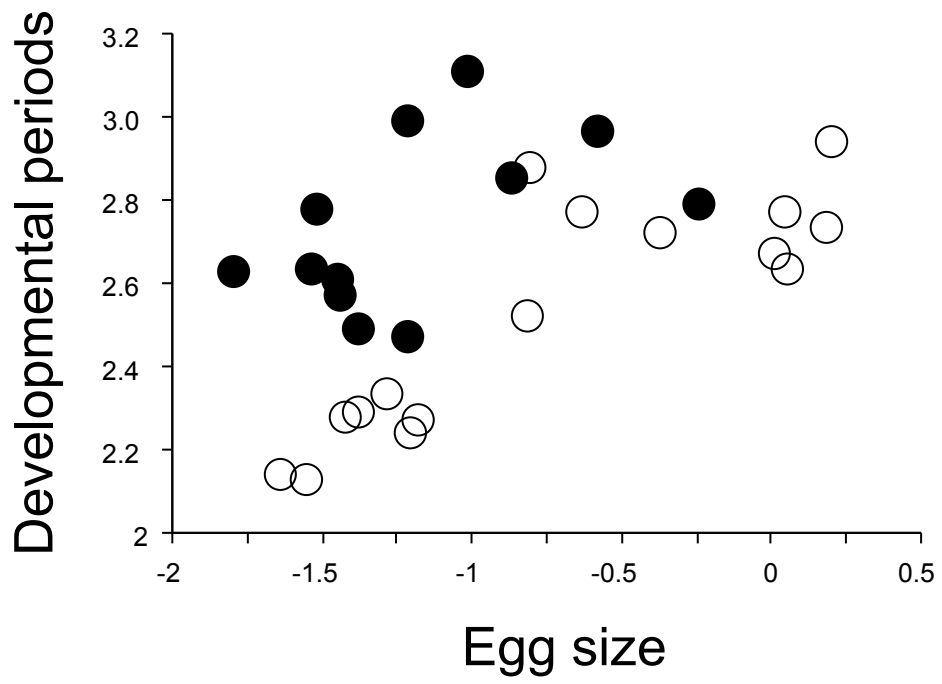


Figure 3

Figure 4

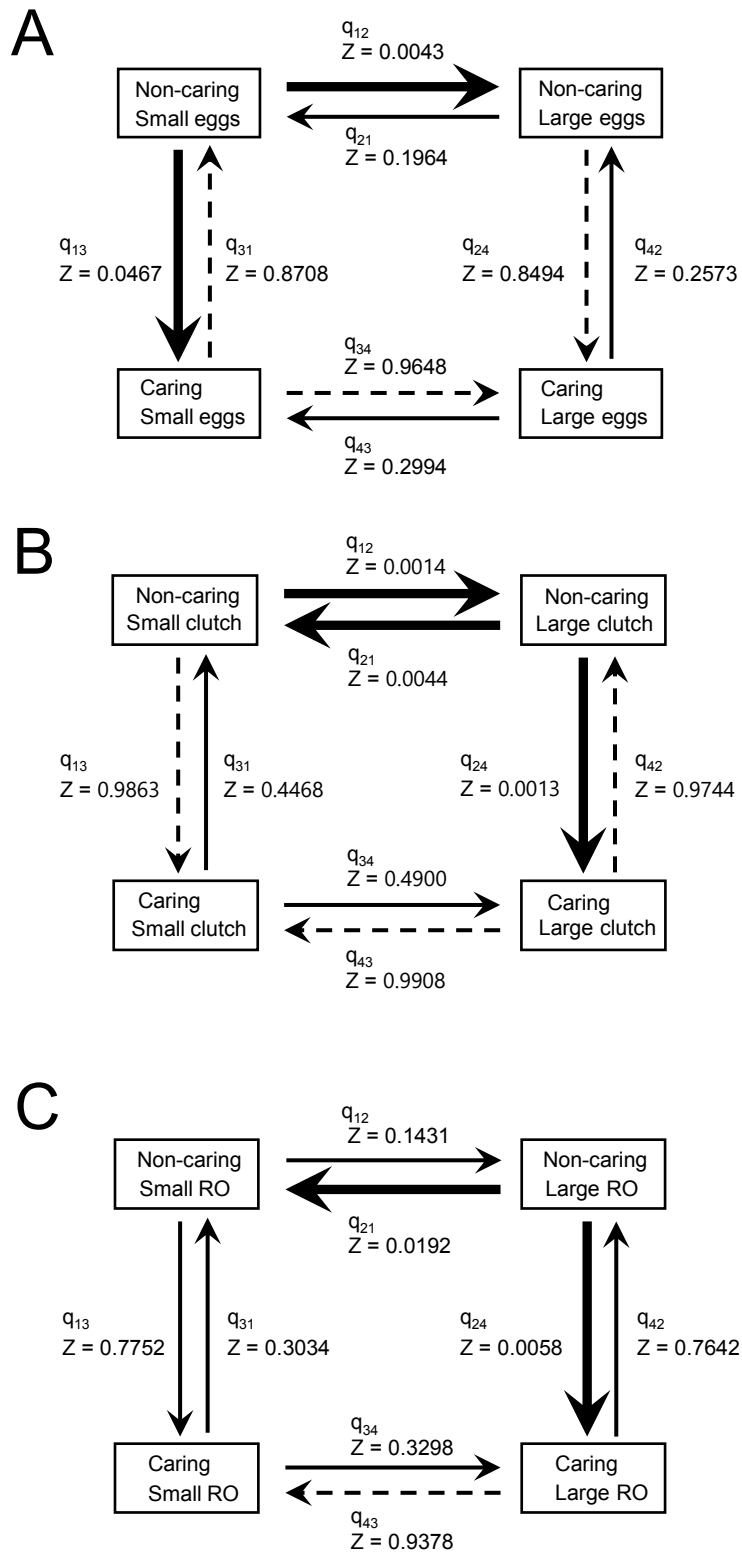


Figure 4

1 Life history evolution in shield bugs.

**Maternal care under large clutches with small eggs: The evolution of life history traits in shield bugs**

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## 2 Life history evolution in shield bugs.

### Supplementary tables and figures

Table S1. Data for comparative analyses of life history traits in shield bugs.

Caring status is a binary variable (caring = 1 or non-caring = 0). Female body size refers to mean female abdomen length (mm). Egg size is provided as mean weight (mg). Reproductive output (mg) is calculated as the egg size  $\times$  mean clutch size. Mean value and sample size are shown. The literature source for data of egg size and clutch size is indicated for some species, but the data were often combined with newly obtained original data, or the data from different literature sources were combined when possible. In cases of such modification, the literature cited are indicated as “revised” and the mean values have been re-calculated. Data for the developmental period of eggs and hatchlings are original (sample sizes of the former and the latter are shown separately), except those for *Elasmucha grisea*, *E. fieberi*, *E. ferrugata* (Kaitala & Mappes, 1997) and *Ditomotarsus hyadesi* (Carvajal & Faúndez, 2015). Data for female body size are also original, except those for some *Elasmotherus* (Kudo et al., 2019a) and *Acanthosoma* species (Kudo, 2023). The locality is indicated for species in which life history data were newly obtained (for localities of others, see the literature cited). See the text for details.

Species	Caring status (caring = 1, non-caring = 0)	Egg size	N	Female body size	N	Clutch size	N	Reproductive output (egg size $\times$ clutch size)	Developmental period for eggs and hatchlings (days)	N (eggs, hatchlings)	Data source (sampling locality for original data)
<i>Acanthosoma spinicolle</i>	0	1.048	3	8.838	23	27.00	2	28.29	14.00	2, 2	Original (Sapporo, Hokkaido, Japan)
<i>Acanthosoma crassicaudum</i>	0	1.218	1	10.633	6	43.00	1	52.37	19.00	1, 1	Original (Kamiyama, Shikoku, Japan)
<i>Acanthosoma haemorrhoidale angulatum</i>	0	1.007	12	9.650	30	33.61	23	33.85	14.47	10, 12	Kudo (2023)
<i>Acanthosoma haemorrhoidale</i>	0			8.869	14	18.33	6				Hanelová & Vilimová (2013)
<i>Acanthosoma labiduroides</i>	0	1.197	22	10.038	32	48.05	20	57.53	15.42	19, 19	Kudo (2023)
<i>Acanthosoma forficula</i>	1	0.779	10	8.829	31	72.41	17	56.40	16.33	1, 6	Kudo & Ogasa (2017), Kudo (2023)
<i>Acanthosoma denticaudum</i>	0	1.038	47	9.175	46	45.48	98	47.22	16.08	26, 26	Kudo (2023)
<i>Acanthosoma expansum</i>	0	0.686	9	7.994	25	31.57	14	21.64	15.31	13, 13	Kudo (2023)
<i>Acanthosoma firmatum</i>	1	0.556	12	10.230	26	133.27	11	74.04	19.50	3, 4	Kudo (2023)
<i>Elasmotherus humeralis</i>	0	0.250	25	5.964	40	19.79	271	4.95	9.89	45, 45	Kudo et al. (2019a)
<i>Elasmotherus interstinctus</i>	0	0.277	14	5.527	28	20.06	17	5.55	10.33	6, 8	Kudo et al. (2019a) revised

### 3 Life history evolution in shield bugs.

<i>Elasmotethus brevis</i>	0	0.299	25	5.328	28	17.11	37	5.11	9.40	10, 10	Kudo et al. (2019a)
<i>Elasmotethus amabilis</i>	0	0.239	15	5.001	23	24.95	19	5.97	9.77	5, 6	Kudo et al. (2019a)
<i>Elasmotethus kerzhneri</i>	0	0.306	14	5.385	30	20.12	34	6.15	9.71	14, 14	Kudo et al. (2019a)
<i>Elasmotethus nubilus</i>	0	0.194	38	4.760	41	19.73	90	3.82	8.55	27, 26	Kudo et al. (2019a)
<i>Elasmotethus rotundus</i>	0	0.212	1	4.865	11	10.67	21	2.26	8.42	12, 12	Original (Sapporo, Hokkaido, Japan)
<i>Lindbergicoris gramineus</i>	0	1.113	9	6.315	18	10.67	9	11.87			Original (Kumakogen, Shikoku, Japan)
<i>Elasmucha dorsalis</i>	1	0.165	22	3.952	40	39.97	77	6.60	13.86	7, 15	Kudo et al. (1989), Kudo (2001) revised
<i>Elasmucha putoni</i>	1	0.234	48	4.529	465	47.37	573	11.08	13.67	9, 9	Kudo (2001, 2002) revised
<i>Elasmucha signoreti</i>	1	0.251	24	4.269	35	33.94	33	8.52	12.09	5, 7	Kudo & Nakahira (1993), Kudo (2007) revised
<i>Elasmucha amurensis</i>	1	0.214	21	4.631	30	57.35	31	12.28	14.01	10, 19	Original (Sapporo, Hokkaido, Japan)
<i>Elasmucha nipponica</i>	1	0.295	5	6.544	30	97.00	1	28.61	20.00	4, 4	Kudo & Ogasa (2017) revised
<i>Elasmucha fieberi</i>	1	0.236	18	4.762	19	47.20	18	11.14	13.13	13, 13	Kaitala & Mappes (1997)
<i>Elasmucha grisea</i>	1	0.218	44	4.601	21	50.40	44	10.99	16.12	22, 22	Kaitala & Mappes (1997)
<i>Elasmucha ferrugata</i>	1	0.296	10	4.533	20	34.80	24	10.30	11.88	14, 14	Kaitala & Mappes (1997)
<i>Elasmucha lineata</i>	1	0.166	21	4.009	15	44.95	21	7.441			Original (see footnote 1)
<i>Elasmucha sp. Taiwan</i>	1	0.185	26	4.174	32	48.86	36	9.037			Original (see footnote 2)
<i>Sastragala esakii</i>	1	0.420	19	7.254	35	94.48	31	39.67	17.41	18, 17	Kudo (2023)
<i>Sastragala scutellata</i>	1	0.362	12	7.554	30	80.54	26	29.14	22.50	2, 2	Kudo (2023)
<i>Cyphostethus tristriatus</i>	0	0.446	5	5.253	8	12.65	17	5.64	17.79	8, 12	Kudo et al. (2019b)
<i>Cyphostethus japonicus</i>	0	0.531	1	5.723	7	20.00	1	10.62	16.00	1, 1	Kudo et al. (2019b)
<i>Ditomotarsus hyadesi</i>	0	0.439	1	4.326	7	11.20	10	4.92	12.50	8, 8	Carvajal & Faúndez (2015) revised
<i>Sinopla perpunctatus</i>	1			4.759	9	67.00	1				Original (personal communication by M. Carvajal & E. Faúndez)

1: Xindian (Erbazi Botanical Garden, Tutang Vill. and Guangxin), Sanxia (Rd. 113), and Pinglin (Cuku Vill.), New Taipei City, Taiwan; Fanlu (Gongxing Vill.), Chiayi County, Taiwan.

2: Renai (Jingying Vill.) and Meifeng (Mt. Living), Nantou County, Taiwan; Xiulin (Bilu Sacred Tree), Hualien County, Taiwan.

#### 4 Life history evolution in shield bugs.

Table S2. Phylogenetic generalized least squares (PGLS) models used to determine whether egg size (A), clutch size (B), and reproductive output (C) is small or large relative to female body size.

See Table S1 legend for details. All variables were natural-log-transformed before the analyses. The lambda values estimated were used to scale the phylogenetic correlation structure in the PGLS model.

Independent variable	Estimate	SE	t	P
(A) Egg size (lambda = 0.975)				
Intercept	-3.483	0.670	-5.202	< 0.001
Female body size	1.534	0.372	4.125	< 0.001
(B) Clutch size (lambda = 0.941)				
Intercept	1.968	1.154	1.705	0.098
Female body size	0.771	0.653	1.182	0.246
(C) Reproductive output (lambda = 0.983)				
Intercept	-1.551	0.775	-2.001	0.055
Female body size	2.175	0.430	5.059	< 0.001

## 5 Life history evolution in shield bugs.

Table S3. Phylogenetic generalized least squares (PGLS) models used to estimate the partial regression slope of egg size against clutch size in non-caring species (A) or that in caring species (B) when effects of female body size were controlled.

See Table S1 legend for details. All variables were natural-log-transformed before the analyses. The lambda values estimated were used to scale the phylogenetic correlation structure in the PGLS model.

Independent variable	Estimate	SE	t	P
(A) Non-caring species (lambda = 0.000)				
(Intercept)	-1.274	0.821	-1.553	0.143
Female body size	2.173	0.387	5.615	< 0.001
Egg size	-0.431	0.164	-2.623	0.020
(B) Caring species (lambda = 0.000)				
(Intercept)	-1.002	0.610	-1.643	0.129
Female body size	2.325	0.241	9.633	< 0.001
Egg size	-0.888	0.167	-5.314	< 0.001

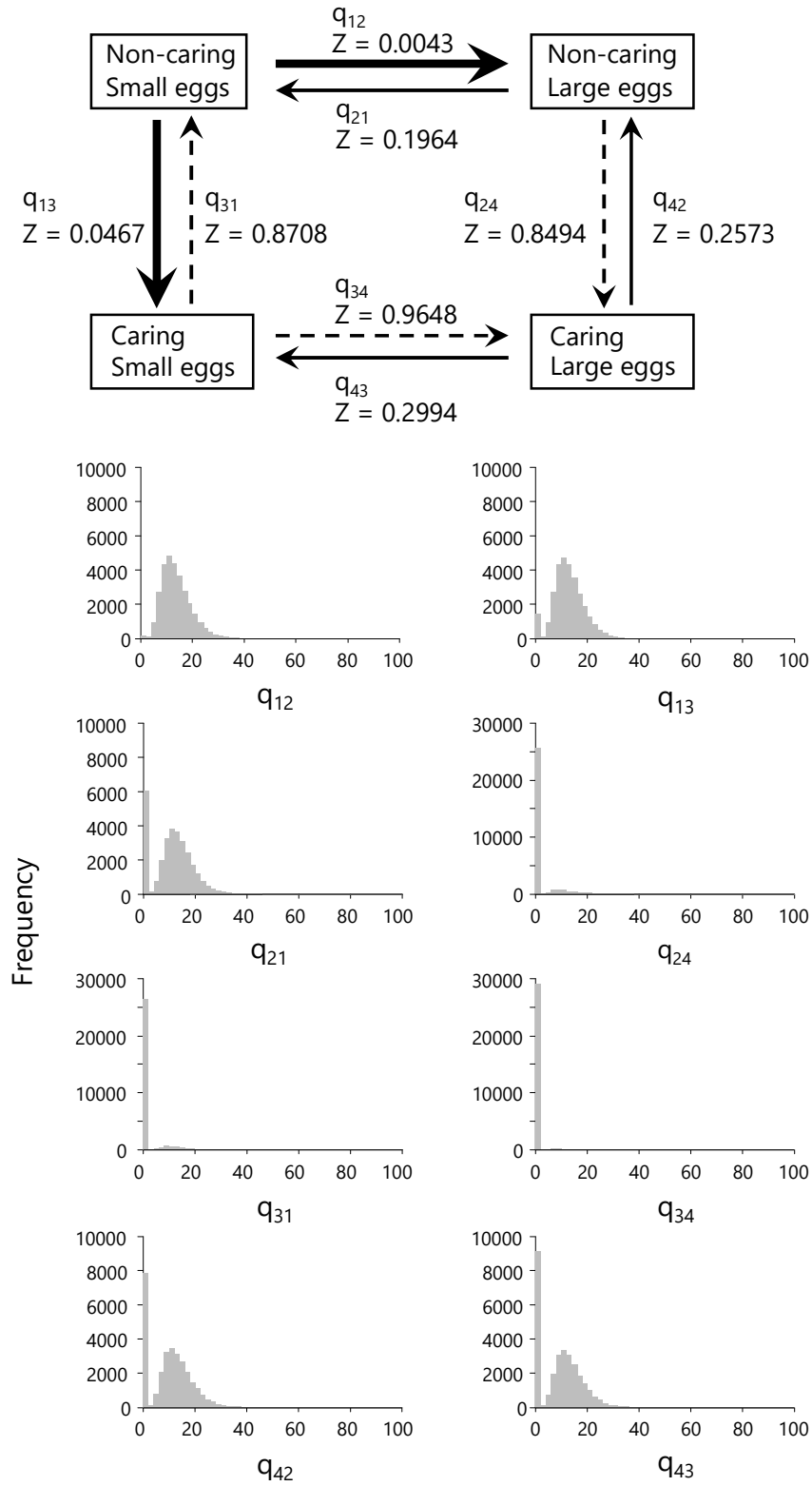
## 6 Life history evolution in shield bugs.

Figure S1. Evolutionary transitions and posterior distributions of transition rate parameters among states of maternal care (non-caring or caring) and other traits (small or large), egg size (A), clutch size (B) and reproductive output (C).

The Z-score for each parameter was calculated as the proportion of zero values in the posterior distribution. Transitions with a Z-score  $> 0.8$  are represented by dashed arrows, which were considered very unlikely to occur, while transitions with a Z-score  $< 0.05$  are represented by bold arrows, which were considered most likely to occur.

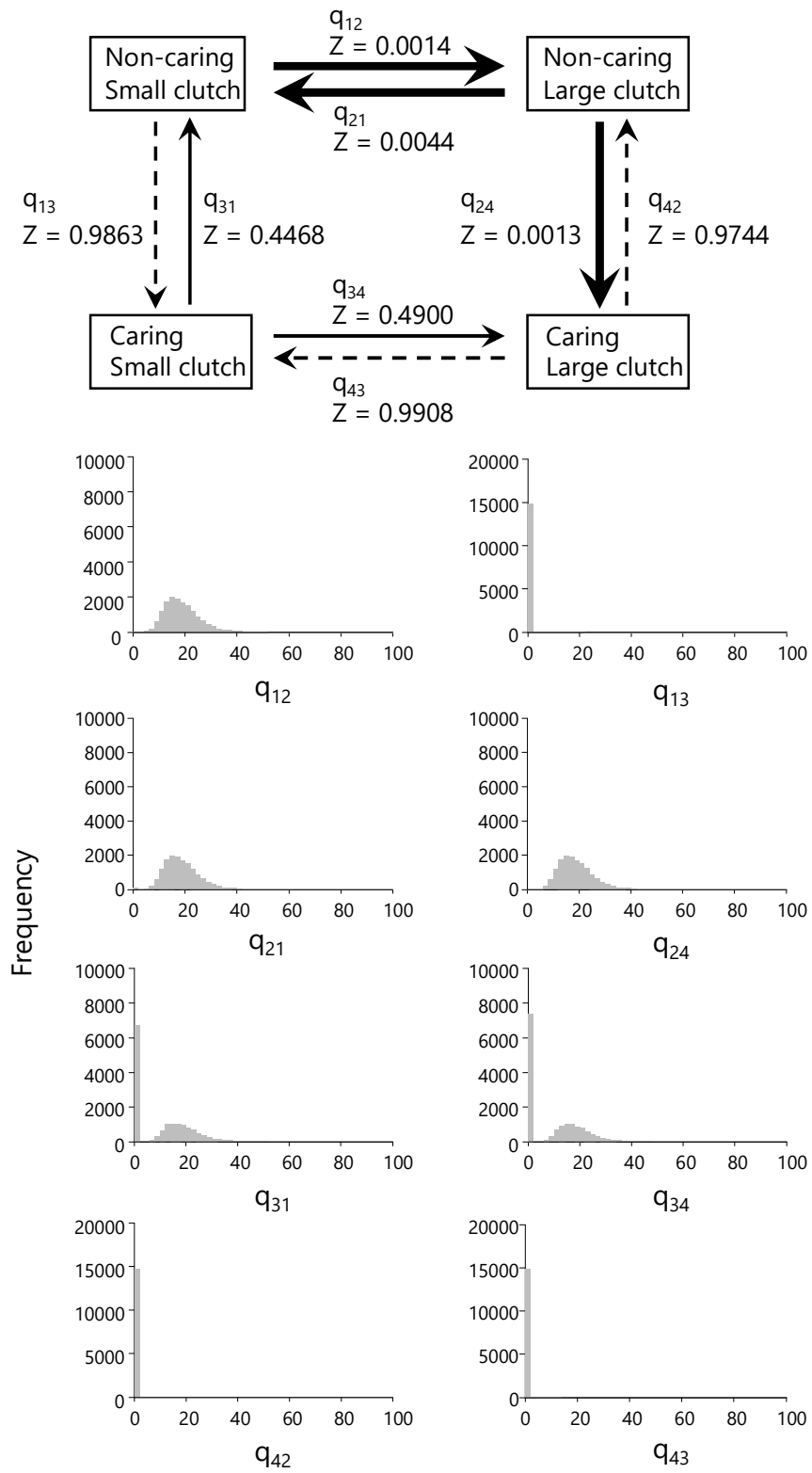
7 Life history evolution in shield bugs.

Figure S1A.



8 Life history evolution in shield bugs.

Figure S1B.



9 Life history evolution in shield bugs.

Figure S1C.

