Within-clutch egg-size variation in a subsocial bug: the positional effect hypothesis

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Abstract: If there are differences in predation risk among the offspring within a clutch, parents should allocate less resources to the offspring facing higher risk. Predation risk, and thus offspring size, may depend on the spatial position of individual offspring within a clutch. To test this positional effect hypothesis, I examined egg-size (egg-mass) variation in the subsocial bug *Elasmucha signoreti* Scott, 1874 (Hemiptera: Acanthosomatidae). In subsocial insects, including *Elasmucha*, in which females guard their clutches against predators by covering the clutch with their bodies, there are large differences in survival between offspring at the centre and at the periphery of the clutch. There was considerable variation in reproductive output among females; female body size was positively correlated with egg mass but not with clutch size. Females laid significantly lighter eggs in the peripheral, and thus more vulnerable, part of the clutch. No phenotypic trade-off between egg mass and clutch size was detected. Egg mass was positively correlated with hatched first-instar nymph mass. Thus, *E. signoreti* females seem to allocate their resources according to the different predation risks faced by the offspring within a clutch. I suggest that the positional effect hypothesis can generally be applicable to species whose females lay eggs in clutches and that the eggs suffer different mortality rates which depend on their spatial positions within the clutch.

Résumé : Lorsqu'il y a des différences dans le risque de prédation parmi les rejetons d'une même ponte, les parents devraient allouer moins de ressources aux petits qui courent un risque plus élevé. Le risque de prédation et donc la taille du rejeton peuvent donc dépendre de la position spatiale du rejeton en question dans la portée. Afin de vérifier cette hypothèse sur l'effet de la position, la variation de la taille (masse) des oeufs a été déterminée chez la punaise subsociale *Elasmucha signoreti* Scott, 1874 (Hemiptera: Acanthosomatidae). Chez les insectes subsociaux, tels qu'*Elasmucha*, chez lesquels la femelle protège sa ponte des prédateurs en la couvrant de son corps, il existe de fortes différences dans la survie des petits entre la périphérie et le centre de la ponte. Le rendement reproductif chez les femelles est très variable; il y a une corrélation positive entre la taille corporelle de la femelle et la masse des oeufs, mais pas avec l'importance de la ponte. Les femelles pondent des oeufs significativement plus légers en périphérie de la ponte, donc dans la zone la plus vulnérable. Il n'y a pas de compromis phénotypique entre la masse des oeufs et la taille de la ponte. La masse de l'oeuf est en corrélation positive avec la masse de la larve néonate de premier stade. Ainsi, les femelles d'*E. signoreti* semblent attribuer leurs ressources en fonction des risques de prédations courus par leurs rejetons au sein de la portée. L'hypothèse de l'effet de la position semble donc s'appliquer de façon générale aux espèces dont les femelles pondent leurs oeufs en masses et dont les oeufs subissent des taux de mortalité différents d'après leur position dans la masse d'oeufs.

[Traduit par la Rédaction]

Introduction

In arthropods, a large variation in maternal investment in terms of egg size has been reported among species or among populations within species (Fox and Czesak 2000). The variation can be explained by the selection of different optimal egg sizes in different environments (Smith and Fretwell 1974; Roff 2002). However, egg size often varies among females within populations and even among the eggs produced by single females, i.e., among clutches and (or) within clutches (e.g., Karlsson and Wiklund 1985; McLain and Mallard 1991; Schenk and Söndgerath 2005). The adaptive nature of such egg-size variation is very controversial (McGinley et al. 1987; Fox and Czesak 2000). In particular, the extrinsic and intrinsic factors leading to egg-size variation within clutches are poorly understood, although several theoretical models have attempted to explain them (reviewed by Clutton-Brock 1991; Stearns 1992; Forbes 1999; Roff 2002; Koops et al. 2003). There have been few empirical studies to test explicit hypotheses on the adaptive significance of the intraclutch egg-size variation in arthropods (see below).

Offspring may differ in quality, and thus have different expectations of fitness, given the same parental investment. If females can detect such differences, they should allocate less resources to offspring that have lower expectations (Temme 1986; Haig 1990). The difference in offspring quality is not necessarily genotypic (Haig 1990). Predation risk often varies among individuals within a group (reviewed by Krause and Ruxton 2002). This can be a factor leading to the different fitness expectations and, consequently, unequal parental investment among offspring in species where females lay eggs in clutches; when the predation risk varies in a consistent manner among eggs within clutches, egg size should also vary in response to the variation in predation risk (Kudo 2001).

The offspring of many subsocial insects suffer potentially

Received 24 May 2006. Accepted 4 October 2006. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 8 December 2006.

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doi:10.1139/Z06-163

severe predation and (or) parasitism pressure, and parents provide effective protection against enemies (Tallamy and Wood 1986; Trumbo 1996). Although parental defence is usually effective, parents sometimes lose a portion of their offspring during parental care (e.g., Eberhard 1975; Mappes et al. 1997). In such cases, the effectiveness of parental defence often depends on the spatial position of the offspring within the clutch (e.g., Eberhard 1975; Mappes et al. 1997). Recently, Mappes et al. (1997) and Kudo (2001) showed position-dependent intraclutch egg-size variation, which should be related to predation risk, in several subsocial hemipteran species. However, to determine whether or not such maternal resource allocation is an adaptive strategy commonly adopted in insects, further evidence is be needed.

In this paper, I clarify patterns of resource allocation associated with female phenotype and maternal care in *Elasmucha signoreti* Scott, 1874 and test the positional effect hypothesis.

Materials and methods

Reproductive history

Like other species of *Elasmucha* Stål, 1864 (e.g., Kaitala and Mappes 1997), *E. signoreti* is a subsocial species (Kudo and Nakahira 1993). Females lay eggs in single compact clutches on the underside of leaves of the host plant. First-instar nymphs are quite inactive and remain in tight aggregations on natal leaves, but second-instar or later instar nymphs move to feed on host fruit while maintaining the aggregations. Female parents straddle and shield their offspring on natal leaves from the egg stage until they moult to the second instar (Kudo and Nakahira 1993). It is most likely that *E. signoreti* is a semelparous species that has two generations per year, each of which depends on different plants (Kudo and Nakahira 1993; S. Kudo, unpublished data).

Measurements

Eggs

I collected clutches guarded by females in mid-June 2002 at Mt. Soranuma, Hokkaido, Japan. The clutches were laid on the underside of leaves of the currant *Ribes japonicum* Maxim. Damaged clutches and those in which embryos had developed and had visible structures were excluded from the analysis.

Eggs were divided into two categories: peripheral eggs (from the outermost row of the clutch) and central eggs (from the innermost row). Ten eggs randomly chosen from the outermost or innermost eggs of each clutch were carefully removed from the leaf using fine forceps under a stereomicroscope and weighed using an electronic balance (Sartorius AG) to an accuracy of 0.01 mg. Egg samples were kept under 4 °C conditions until they were weighed.

Nymphs

I examined the relationship between egg mass and nymph mass. Ten eggs, from either the centre or the periphery of the clutch, that had been weighed were placed on a moistened filter paper in a plastic petri dish. First-instar nymphs within 24 h after hatching were killed by freezing and quickly weighed in the same way as the eggs. Hatchability was sometimes low, probably because of the handling of the eggs. Thus, samples in which less than five eggs had hatched were excluded from the analyses. Although hatchability of eggs might differ between the centre and the periphery of a clutch (Mappes et al. 1997), I did not compare hatchability between egg samples.

Females

I measured body length and prothorax width of females under a stereomicroscope and used the product of these two as the body-size measurement of the females. This measurement reflects the clutch area that can be covered with the bodies of females.

Evaluation of measurement errors

Differences in mass between offspring samples were small (see the Results). Thus, I checked the repeatability of the measurements (r; Falconer 1989) by measuring samples repeatedly. The offspring sample (from the centre or the periphery of the clutch) was chosen at random. Each sample was sequentially weighed three times for eggs and twice for nymphs. The mean mass were highly repeatable for eggs from the centre or the periphery of the clutch (central: $r = 0.996$, $F_{[23,48]} = 251.46$, $P < 0.001$; peripheral: $r = 0.997$, $F_{[23,48]} = 393.97$, $P < 0.001$). High repeatabilities were also obtained from measurements of nymphs in the two categories (central: $r = 0.933$, $F_{[13,14]} = 13.95$, $P < 0.001$; peripheral: $r = 0.986$, $F_{[13,14]} = 68.37$, $P < 0.001$), indicating that the measurement of samples was sufficiently precise. The mean of repeated measurements for an individual sample was used for subsequent analyses.

All statistical analyses were conducted using StatView® version 5.0 (SAS Institute Inc. 1998).

Results

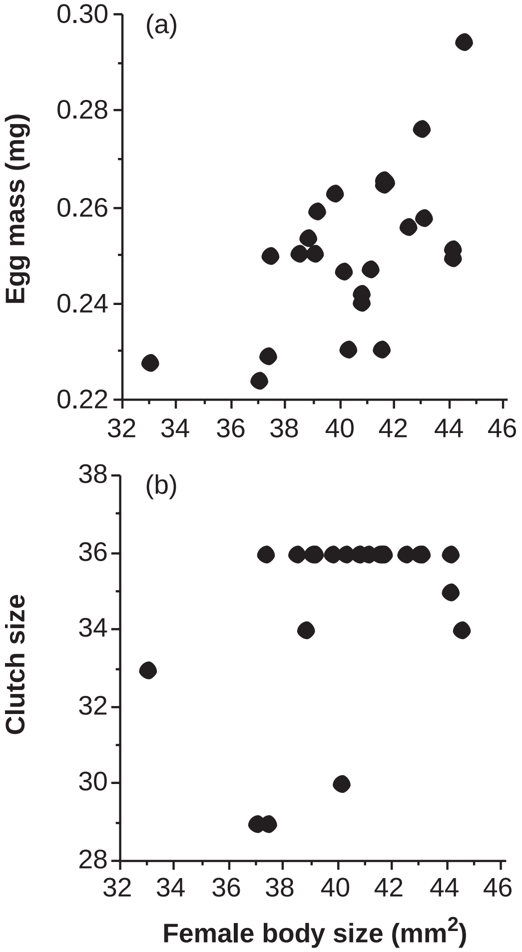
There was little variation in clutch size (34.83 ± 2.28 (mean ± SD), range 29–36). The body size of females affected their reproductive output; it was positively correlated with egg mass ((central egg mass + peripheral egg mass)/2: Spearman's correlation (r_S) = 0.570, $N = 24$, $p = 0.006$; Fig. 1*a*), but not with clutch size ($r_S = 0.324$, $N = 24$, $p = 0.12$; Fig. 1*b*). No significant phenotypic trade-off between egg mass and clutch size was detected even when the effects of female body size on egg mass were statistically controlled (partial $r = -0.038$, $p = 0.86$). Relative constant clutch-size suggests that decreasing investment in some eggs could induce increasing investment in the other eggs.

Eggs from the centre of the clutch were significantly heavier than those from the periphery (Table 1). Heavier nymphs hatched from heavier eggs from either the centre ($r_S = 0.768$, $N = 16$, $p = 0.006$; Fig. 2) or the periphery of the clutch ($r_S = 0.844$, $N = 14$, $p = 0.002$; Fig. 2). First-instar nymphs hatched from the central eggs also tended to be heavier than those from the peripheral eggs (Table 1), although the difference was not statistically significant, possibly because of the small sample size.

Discussion

Elasmucha females guard egg masses and hatched

Fig. 1. Relationships between female body size and egg mass (*a*) or clutch size (*b*) in *Elasmucha signoreti*. Female body size (mm^2) is calculated as body length × prothorax width and egg mass (mg) is calculated as (central egg mass + peripheral egg mass)/2.



nymphs, which form tight aggregations on leaves, by shielding them with their bodies (Melber and Schmidt 1975*a*; Kudo et al. 1989; Kudo 1990; Mappes et al. 1997). The females show specific defensive responses against predators that attack their offspring (Melber and Schmidt 1975*a*; Kudo 1990). Although the maternal defence is usually effective, it is not always perfect and it is often the case that some of the offspring are killed by predators (Melber and Schmidt 1975*b*; Kudo et al. 1989; Mappes and Kaitala 1994; Mappes et al. 1997; Kudo 2002). It has been shown that predation risk of offspring during maternal care depends on their spatial position within clutches in *Elasmucha* species (Mappes and Kaitala 1994; Mappes et al. 1997), as well as in other subsocial hemipterans (Eberhard 1975, 1986; Tallamy and Horton 1990; Cocroft 2002); offspring at the periphery of the clutch (or brood) are more vulnerable than those at the centre. It is most likely that this is also the case for *E. signoreti*, the offspring of which suffer potentially high predation pressure in the field (Kudo and Nakahira 1993).

In *E. signoreti*, eggs at the periphery of the clutch were lighter than those at the centre (Table 1). There were significant relationships between mass of eggs and hatched nymphs, with lighter nymphs hatching from lighter eggs (Fig. 2). These results support the positional effect hypothesis, i.e., the skewed distribution of egg size within clutches is the result of adaptive allocation of maternal investment in response to the differences in predation risk.

Fig. 2. Relationships between egg mass and hatched nymph mass in *E. signoreti*. ●, eggs from the centre of clutches and nymphs that hatched from them; ○, eggs from the periphery of the clutch and nymphs that hatched from them. Nymphs were weighed within 24 h after hatching.

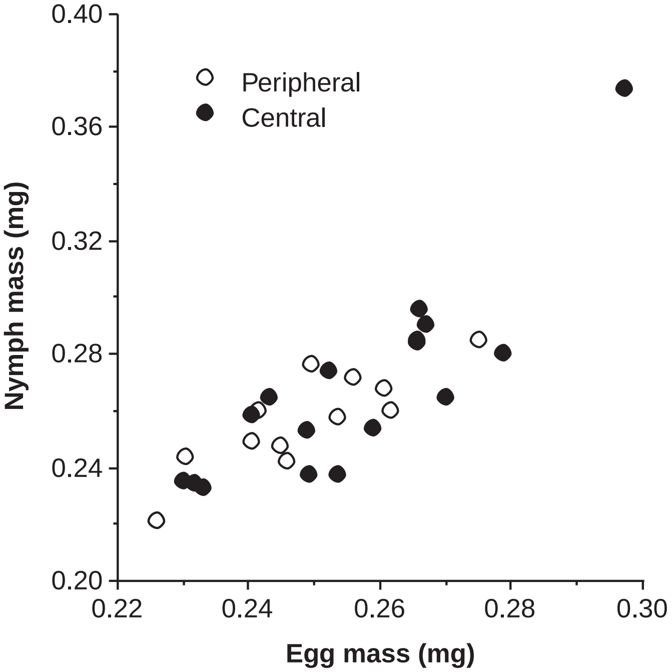


Table 1. Comparison between the mass of offspring from the centre and from the periphery of *Elasmucha signoreti* clutches.

		Position (mg; mean ± SD)			
Stage	*N*	Central	Peripheral	Paired *t*	*P*
Egg	24	0.252±0.017	0.250±0.016	2.93	0.008
Nymph*	14	0.258±0.020	0.255±0.020	1.47	0.17

*From eggs at each position within 24 h after hatching.

The positional effect hypothesis may also be associated with the origin of trophic eggs, inviable eggs supplied as food for siblings, in some subsocial cydnid bugs (Kudo and Nakahira 2004; Hironaka et al. 2005; Kudo et al. 2006). In *Parastrachia japonensis* Scott, 1880 and *Adomerus triguttulus* (Motschulsky, 1886), females lay trophic eggs on the surface of spherical mass of viable eggs, with the former eggs being smaller than the latter eggs (Hironaka et al. 2005; Kudo et al. 2006), which indicates less material investment. It is most likely that eggs situated on the surface of spherical egg masses are more vulnerable than those internally located.

The fundamental assumption underlining the adaptive explanation of egg-size variation is that the expected fitness of the offspring increases with increasing egg size (e.g., Smith and Fretwell 1974). Larger eggs have a variety of fitness advantages (e.g., higher developmental rates of immatures, higher survival, and larger adult size) in hemipteran insects (e.g., Solbreck et al. 1989; McLain and Mallard 1991; Toda et al. 1995; Mohaghegh et al. 1998), as well as in other insect taxa (Fox and Czesak 2000). This is also the case for the genus *Elasmucha* and the closely related genus *Elasmostethus* Fieber, 1860 (Mappes et al. 1996, 1997). In addition, larger *Elasmucha* nymphs that hatched from larger eggs would be superior in sibling competition for the safest area, i.e., the centre of the brood guarded by the female (Mappes et al. 1997). As in other *Elasmucha* spp., first-instar nymphs of *E. signoreti* that form tight aggregations always direct their heads to the centre, suggesting such competition (S. Kudo, personal observation). The size of eggs often affects the rate of embryonic developments in arthropods (e.g., Schenk and Söndgerath 2005). If eggs differing in size within single clutches have different developmental periods and result in hatching asynchrony, the hatching order might also affect sibling competition. In *E. signoreti*, however, eggs within a given clutch hatched synchronously (S. Kudo, personal observation), and thus the intraclutch difference in egg size does not seem to lead to an apparent hatching asynchrony.

On the other hand, it has often been suggested that the size variation among eggs produced by single females may be an adaptive strategy according to spatially or temporally changing environments of the offspring (Kaplan and Cooper 1984; Koops et al. 2003) or might be due to physiological constraints in females on making each offspring the same size (discussed in McGinley et al. 1987; Koops et al. 2003). The magnitude of egg-size variation within a clutch (|central egg mass – peripheral egg mass| / egg mass) was, on average, 0.016 in *E. signoreti*. This value is within the range of those observed in other subsocial acanthosomatid species and is also close to that in an asocial species (Kudo 2001). It is difficult to evaluate the magnitude of egg-size variation per se. Even if the variation is due to physiological constraints in oogenesis, the female may accomplish adaptive allocation of egg resources by biasing the placement of relatively small eggs using simple behavioural rules during oviposition (McLain and Mallard 1991).

Individuals within a group are generally expected to suffer different predation risks that depend on their spatial positions (Hamilton 1971; Krause and Ruxton 2002), and thus, different maternal investment according to predation risk would not be restricted to species with parental care, such as the genus *Elasmucha*. McLain and Mallard (1991) have reported intraclutch egg-size difference similar to that observed in subsocial acanthosomatid bugs for the pentatomid bug *Nezara viridula* (L., 1758). *Nezara viridula* females produce large clutches, and offspring at the periphery of the clutches may be more vulnerable to environmental threats. I suggest that the positional effect hypothesis explaining skewed distributions of offspring size can be generally applicable to species in which females lay eggs in clutches and that eggs suffer differential mortality which is dependent on their spatial positions within the clutch. There is little available data on intraclutch variation in offspring mortality, as well as offspring size, in arthropods. Further empirical studies using different taxa, in particular those without parental defensive behaviour, will be needed to test the generality of the positional effect hypothesis.

Acknowledgements

I thank L. Filippi for her useful comments on the manuscript. This study was supported in part by Grant-in-Aids for Scientific Research (KAKENHI: 11740430, 13640628) from The Ministry of Education, Culture, Sports, Science and Technology and the Japan Society for the Promotion of Science.

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