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THE COLLAPSED EGGS FOUND IN THE BURSA COPULATRIX OF A PLUM MOTH, *ILLIBERIS ROTUNDATA* JORDAN (ZYGAENIDAE: PROCRIDINAE): AN UNUSUAL EGG RESORPTION SYSTEM?

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ABSTRACT. In a plum moth, *Illiberis rotundata* Jordan (Zygaenidae), collapsed eggs and empty chorions were usually found in the bursa copulatrix. Effects of the number of these eggs on female longevity were analyzed with female body weight, body weight of her mate, and female fecundity. The results of multiple regression analysis showed that females with more eggs in the bursa copulatrix lived longer. It is most likely that eggs are resorbed in the bursa copulatrix and used for the survival of the females. This moth seems have an unusual egg resorption system.

Additional key words: oocyte, longevity.

Egg resorption, a specific type of reproductive tactic in which oocytes degenerate instead of being laid as eggs, has been reported in many insects (Bell & Bohm 1975), including lepidopteran species from groups such as Heliconiinae (Dunlap-Pianka et al. 1977) and other Nymphalidae (Boggs & Ross 1993).

Illiberis rotundata Jordan (Zygaenidae) is a univoltine and diurnal moth. Larval host plants include cherries, plums and other Rosaceae trees. Adults emerge from May to June in western Japan, and copulate and oviposit repeatedly almost for a month, taking only water (CK unpublished data). During the process of a study on mating behavior of *I. rotundata*, we found many collapsed eggs in the bursa copulatrix of females. In this paper, we describe this unusual phenomenon and suggest a possible function of these eggs.

MATERIALS AND METHODS

We collected pupae of *Illiberis rotundata* in Naruto City (34°11′N, 134°35′E) in May 2000. Each individual was kept separately in a paper cup (7 cm diameter, 7 cm depth) placed in a constant condition room at 21 ± 1°C with a 15L:9D photoperiod. After emergence, all adults were weighed using an electric balance (Sartorius AG) with an accuracy of 0.01 mg. Nineteen females were allowed to copulate once one day after emergence and five females were kept unmated through their lives (the laboratory females). Each mated female was moved to and kept in a plastic case (9 cm diameter, 5 cm depth) supplied with a single fresh cherry leaf (*Prunus* × *yedoensis*) and water. These females were allowed to lay eggs until death. Each cherry leaf was renewed every evening after the number of eggs laid by the female on that day had been checked. We dissected these females soon after their deaths and examined the contents of the bursa copulatrix. In 2001, we also collected and dissected females from the field (the wild females).

In order to reveal the function of the eggs in the bursa copulatrix, we examined their effects on female longevity. We selected four factors that might affect female longevity: female body weight (body weight of each female when she emerged), male body weight (body weight of her mate when he emerged), fecundity (total number of eggs each female laid during her lifetime) and the number of eggs (in any condition, see results) in the bursa copulatrix. Before further analyses, each value of female body weight, male body weight, fecundity and the number of eggs (+1) in the bursa copulatrix was log-transformed.

We analyzed correlation structures between these factors and longevity. Multiple regression analysis was also used to estimate only the direct effects of each factor on female longevity, using female longevity as the dependent variable, and the four factors as the independent variables. Data were analyzed using the StatView 5.0 (SAS Institute Inc.).

RESULTS

We dissected 22 laboratory females and 21 of them (95.5%) had eggs in their bursa copulatrix (Fig. 1). We found a few spheroid shaped eggs, several collapsed eggs, and many empty eggs including fragmented chorions (Fig. 1b). The spheroid eggs were observed near the ductus bursa, whereas the empty eggs were at the bottom of the bursa. In one female, a spheroid egg was also observed in the ductus seminalis. We could not recognize any distinct spermatophore. The fat body had almost been depleted at death.

The number of eggs, including spheroid, collapsed and empty eggs, in the bursa copulatrix varied from zero to 52 in the laboratory females (Mean ± SD = 11.4 ± 12.8, N = 19). In many cases, the bursa contained some other small fragmented chorions, thus the counted number of eggs seems to be underestimated. Among those 19 females, longevity was 23.6 ± 6.0 days (Mean ± SD), fecundity was 461.1 ± 177.3, body weight was 53.7 ± 8.8 mg, and body weight of their mates was 31.6 ± 3.4 mg.

The number of eggs in the bursa copulatrix and female body weight were positively correlated with female longevity (Table 1, Fig. 2), but fecundity was neg-

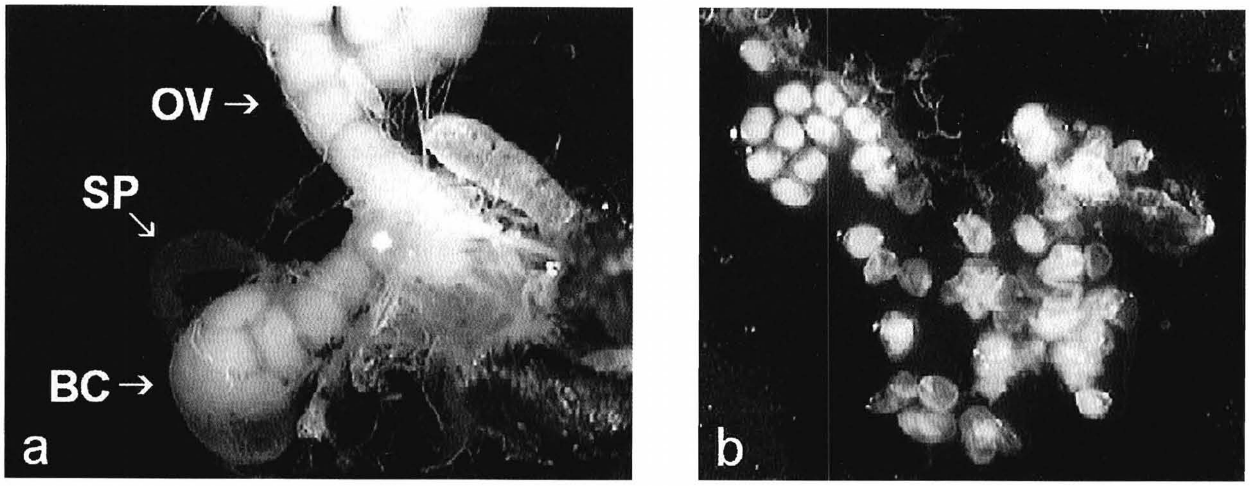

FIG. 1. The bursa copulatrix containing eggs in the laboratory females. (a) Many eggs contained are visible through the translucent wall of the bursa (BC, bursa copulatrix; OV, oviduct; SP, spermatheca). After dissection of this bursa, 9 collapsed and 18 empty eggs were counted. (b) Spheroid, collapsed or empty eggs from the bursa of another female.

atively correlated with female longevity. Fecundity was also positively correlated with female body weight (r = 0.46, P = 0.046). Fecundity and number of eggs in the bursa, however, showed no significant correlation (r = –0.36, P = 0.13).

The total multiple regression model was highly significant ($R^2 = 0.82$, F = 15.5, $P < 0.0001$; Table 1). Both the number of eggs in the bursa and female body weight positively influenced female longevity. Fecundity negatively affected female longevity, suggesting a phenotypic cost of reproduction (see Reznick 1985). Male body weight had no effect on female longevity.

There is no significant correlation between fecundity and the number of eggs in the bursa, partialling out longevity (partial r = 0.129, P = 0.59, N = 20).

Nine out of 16 wild females that had been collected in the field also had collapsed eggs or chorions in their bursa copulatrix. Seven of the nine females had one or two eggs, while two females had highly degenerated chorions that could not be counted. These observations indicate that retention of eggs in the bursa copulatrix is not caused artificially by keeping females in the laboratory for long periods. However, the number of retained eggs found in wild females (Mean ± SD = 0.64 ± 0.75, N = 14) was smaller than that of the laboratory females (Mann-Whitney's *U*-test, *U* = 13.5, $P < 0.0001$).

DISCUSSION

Resorption usually occurs in immature eggs (oocytes) within the ovarioles (Bell & Bohm 1975). On the other hand, the resorption of mature eggs (chorionated eggs) has been reported in Heliconiine butterflies (Dunlap-Pianka et al. 1977) and other insects (see Bell & Bohm 1975).

In most laboratory females of *I. rotundata*, collapsed eggs with chorions were found in their bursa copulatrix at death. To the best of our knowledge, such a phenomenon has never been documented in Lepidoptera. At this point we might ask, what is the function of these eggs? Eberhard (2000) found a mature egg or a larva just hatched from the egg in the bursa copulatrix in some female *Microsepsis armillata* (Diptera: Sepsidae) flies, and he also reported the same phenomenon in other flies. He suggested that the egg or larva would prevent intromission by a male, even though in these species females are immune to

TABLE 1. Results of correlation and multiple regression analysis for female longevity and factors potentially affecting the longevity. The total multiple regression model was highly significant (see text). r: correlation coefficient. β: standardized partial correlation coefficient.

	Correlation		Multiple regression		
Factor	r	P	β	*t*	P
Female body weight	0.29	0.232	0.49	3.55	0.003*
Male body weight	0.21	0.391	0.06	0.53	0.607
Female fecundity	–0.52	0.023	–0.55	–3.68	0.003*
Number of eggs in bursa	0.76	<0.001*	0.50	3.81	0.002*

* Significant after using the sequential Bonferroni correction.

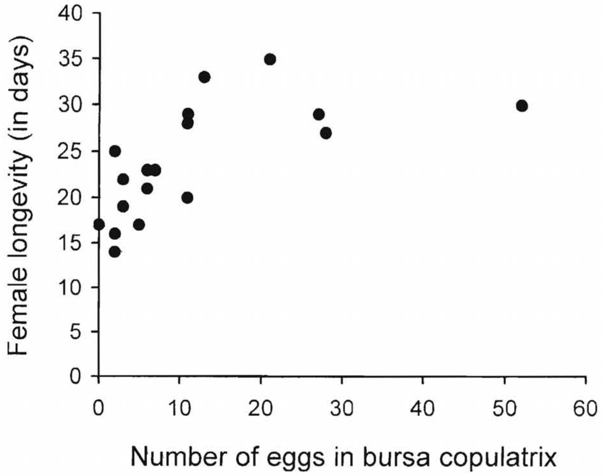

FIG. 2. The relationship between the number of eggs in the bursa copulatrix and female longevity.

rape. In *I. rotundata*, females are usually polyandrous and these eggs in the bursa copulatrix would not prevent intromission by males.

The result of multiple regression analysis shows that females which had more eggs in their bursa copulatrix lived longer. It is most likely that females consume eggs in the bursa copulatrix and use them to survive, that is, they re-allocate resources from reproduction to survival. There are two pieces of indirect evidence to support our hypothesis for *I. rotundata*. First, most of the eggs in the bursa copulatrix were highly degenerated. We could see many empty eggs or fragmented chorions at the bottom of the bursa, whereas some spheroid shaped eggs were observed near the ductus bursa. These suggest that egg contents were digested and resorbed during retention in the bursa copulatrix. The fact that a spheroid egg was in the ductus seminalis of a female suggests that eggs were transferred from the ovary to the bursa copulatrix via the ductus seminalis. Second, it is reported that many lepidopteran females can consume male spermatophores in their bursa copulatrix as nutrition for their eggs and/or themselves (Boggs & Gilbert 1979, Boggs 1981). In *I. rotundata*, no distinct spermatophores were detected in the bursa copulatrix of the laboratory females. It is possible that not only spermatophores but chorionated eggs are also degenerated in the bursa.

Bell and Bohm (1975) listed many factors promoting oosorption. For example, the restriction of the ovipositional site sometimes increases resorption of eggs. In our experiment, each female was restrained in a small plastic case with a leaf of the host plant. These unnatural conditions might inhibit their oviposition, although they laid eggs readily on the leaf or the wall of the case.

In some insects oocytes are quickly resorbed in the absence of mating. *Illiberis rotundata* females usually mate repeatedly under field conditions (CK unpublished data). In our experiment, however, females were allowed to mate only once. When females are prevented from mating, they may consume some of their eggs in order to live longer and possibly achieve additional matings. Through multiple matings, these females might gain additional nutritional and/or genetic benefits (Arnqvist & Nilsson 2000, Jennions & Petrie 2000).

Resorption can occur in Lepidoptera in response to qualitative or quantitative nutrient deficiencies (Dunlap-Pianka et al. 1977, Boggs & Ross 1993). Adults of *I. rotundata* take only water, and thus no additional nutrients from food are available for survival. Nevertheless they can live for relatively long periods, sometimes more than three weeks. Egg resorption in this species seems to be an effective system for obtaining additional nutrients at the expense of reproduction.

Wild females also had collapsed eggs or degenerated chorions in their bursa copulatrix, but the number of these eggs and chorions was very low. Two reasons for this are to be considered: first, the age of the wild females is uncertain and they may have been dissected at a younger age than the laboratory females, which were dissected after living out their lives. If there is a positive correlation between the number of eggs in bursa copulatrix and female age, younger wild females would have less eggs in the bursa than older laboratory females. Second, wild females may have copulated repeatedly until the point of collection, while the laboratory females mated only once. If males provide nutritional investment to females during copulation, mating frequency will have an effect on egg resorption by females.

Why, then, do *I. rotundata* females resorb eggs in their bursa copulatrix not in ovarioles like other insects? One possible reason could be to eliminate remains after egg resorption. If resorption of chorionated eggs occurs in ovarioles, the remains of resorbed eggs, such as chorions, should be eliminated to facilitate the passage of the following eggs. Some wasps oviposit empty chorions to solve this problem (see Bell & Bohm 1975). Disposal of the remains in the bursa copulatrix seems to be less costly than in ovarioles, because remains can be left in the bursa copulatrix. But it is still unknown why they resorb chorionated eggs.

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