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Life history of the endangered ground beetle *Pterostichus isumiensis* Kasahara & Saito, 1997 (Coleoptera: Carabidae) deduced from laboratory rearing experiments

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The endangered ground beetle *Pterostichus isumiensis* Kasahara & Saito, 1997 was reared experimentally in a laboratory. Under rearing conditions that simulated seasonal temperature changes through autumn, winter, spring, and early summer, eggs were laid in October, and individuals hatched in November and reached the third (final) instar stage after January of the following year. Pupation occurred in late May, and the adult stage was reached in late June. These results provide definitive evidence for the autumn breeding of *P. isumiensis*. However, rearing conditions that induce sexual maturity in new adults, particularly temperature, remain to be elucidated.

key words：Chiba Prefecture, Ex situ conservation, Japan, *Nialoe*, Red List

Introduction

Pterostichus (*Nialoe*) *isumiensis* Kasahara & Saito, 1997 is a flightless carabid species endemic to Chiba Prefecture, eastern Japan, that has been found only in a hilly area of the Sotobô region. Due to its restricted distribution, it is listed as "Vulnerable" on the latest Red List of the Ministry of the Environment and as "Critically Endangered" on that of Chiba Prefecture (Sasakawa, 2011a). However, the distribution and life history of this beetle, which are essential for its conservation, remain poorly understood. Information on its distribution was recently updated after the discovery of a new population (Sasakawa, 2022); however, little new information on its life history has been discovered since reports of its oviposition behavior (Sasakawa, 2011b) and larval feeding habits (Sasakawa, 2016).

In this study, a sample of eggs obtained during oviposition experiments in 2009 (Sasakawa, 2011b) were used for larval rearing, and one individual was observed to reach adulthood. The findings are discussed in terms of the life history and conservation of this species.

Materials and methods

Larvae were obtained from two females collected at the known locality during September 21–25, 2009. All rearing experiments were performed at a laboratory of the University of Tokyo (Komaba, Meguro-ku, Tokyo; 35.661600°N, 139.683557°E). The females were reared individually in a plastic box (12.0×11.0×9.0 cm) housed in an incubator maintained at 15℃ under an 8 h/16 h light/dark photoperiod. Larvae of the grain beetle *Tenebrio molitor* were cut into pieces and provided as food. The females laid a total of 124 eggs. The experimental rearing of *P. isumiensis* females was described in greater detail in a previous study (Sasakawa, 2011b). Most hatchlings were processed as specimens for morphological examination at either of the larval instar stages (Sasakawa, 2016, 2023); however, nine individuals were further reared in an attempt to obtain adult beetles.

The nine larvae were reared individually in Petri dishes (diameter, 3.5 cm; height, 1.0 cm; filled to a depth of 0.5 cm with moistened garden soil) during the egg, first instar, and second instar stages, and in a plastic bottle (diameter, 6.5 cm; height, 7.5 cm; filled with moistened garden soil to a depth of 5 cm) after the third instar stage. Larvae were fed daily with *Tenebrio* larvae cut into pieces, and mortality and molting were checked at the same time. By definition, a larva that died during molting was considered as having died in the subsequent developmental stage. Eggs and hatchlings were initially reared in an incubator maintained at 15°C. To simulate seasonal changes in temperature, the rearing temperature was gradually lowered in autumn and increased again in spring, using incubator temperatures of 10°C, 12°C, 14°C, 15°C, 18°C, 22°C, and 25°C; in addition, some individuals were kept outdoors for short periods in winter. According to data obtained from the Japan Meteorological Agency (http://www.jma.go.jp/jma/index.html), the average temperatures in Ôtemachi (Tokyo) closest to the laboratory in late November; early, mid-, and late December 2009; and early, mid-, and late January 2010 during the outdoor rearing period were 12.0°C, 10.8°C, 8.1°C, 8.3°C, 6.6°C, 5.7°C, and 8.6°C, respectively. The incubator photoperi-

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od was set to short-day conditions (8 h light, 16 h dark) until May 7, 2010, and long-day conditions (16 h light, 8 h dark) thereafter. Outdoor rearing was conducted under the natural photoperiod. When individuals reached adulthood, their fertility was examined with subsequent rearing.

Results

The growth and development of the nine experimentally reared individuals (L#18, L#22, L#28, L#33, L#52, L#53, L#55, L#57, and L#58) are shown as a bar chart in Figure 1; more detailed data are available in Appendix 1. Of the nine individuals, one died in the first instar stage and another in the second instar; the remaining seven larvae reached the third instar stage after January 2010. Of these seven individuals, L#18 and L#52 died during molting, L#22 died 3 days after reaching the third instar stage, and the remaining four individuals survived for a relatively long period after reaching the third instar stage. Of these four larvae, L#33 and L#55 died as third instars for unknown reasons, and L#53 was killed as a specimen because it did not pupate in mid-June, by which time L#58 had already pupated. Larva L#58 became an adult (female) in late June. On July 5, the female was transferred to the same rearing box that was used for the parental females and fed *Tenebrio* larvae. On September 22, a male newly collected from the same collection site during September 21–22 was added to the rearing container of L#58 female, and they were allowed to mate. Once copulation was confirmed, the male was removed, and the female was allowed to oviposit. However, the female did not lay any eggs, and was therefore killed on October 9 and dissected for ovary examination. The ovaries were undeveloped and no eggs were found.

Discussion

The Carabidae are typically divided into two breeding types: spring breeders, in which mating and oviposition occur in spring or early summer and the offspring become adults before summer; and autumn breeders, in which mating and oviposition occur in autumn and the offspring overwinter and become adults in the following spring or early summer. A previous study showed that *P. isumiensis* mates and oviposits in autumn (Sasakawa, 2011b), suggesting that it is an autumn breeder. In this study, most larvae reached the third instar stage in winter and one individual became an adult in early summer of the following year, providing definitive evidence for autumn breeding in *P. isumiensis*. A similar finding was reported for *Pterostichus yokohamae* Nakane & Straneo, 1979, which is closely related to *P. isumiensis*, and similarly inhabits the lowlands of the Kanto Plain (riverbed of the Tsurumi River). Field surveys and laboratory rearing experiments showed that the seasonal life history of *P. yokohamae* is as follows: egg stage in mid-October, first instar stage from mid-October to mid-December, second instar from mid-November to mid-January, third instar from January to mid-April, pupal stage from mid-April to the end of May, and adult stage reached after mid-May. Autumn breeding may be widely common among lowland species of the *Pterostichus asymmetricus* species group, to which both *P. isumiensis* and *P. yokohamae* belong.

The *P. isumiensis* adult female obtained by laboratory rearing did not lay eggs. Sota (1987) examined the

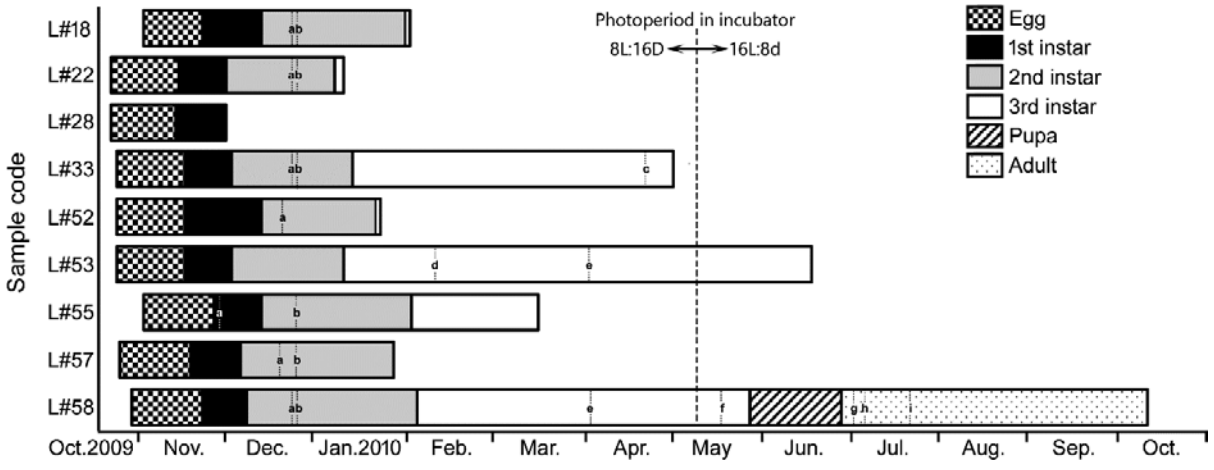

Figure 1. Growth and development of *Pterostichus isumiensis* under laboratory rearing conditions. Letters in bars indicate temperature changes, as follows: (a) 15°C to outdoors, (b) outdoors to 10°C, (c) 10°C to 14°C, (d) 15°C to 10°C, (e) 10°C to 12°C, (f) 12°C to 15°C, (g) 15°C to 18°C, (h) 18°C to 22°C, (i) 22°C to 25°C. Outdoor temperatures are provided in the text.

effects of temperature on egg laying in *Carabus* (*Leptocarabus*) *kumagaii* (Kimura & Komiya 1974), another autumn-breeding carabid species. His results showed that when females that had been maintained at 25°C were transferred to and maintained at 15°C and 20°C, they laid eggs, whereas females that had been maintained at 25°C during the reproductive season laid no eggs. Because the present study maintained the *P. isumiensis* female at 25°C, it is possible that rearing at 25°C is not suitable for sexual maturation in this species. To elucidate the effect of temperature on adult sexual maturation in *P. isumiensis*, a future study should conduct rearing experiments similar to that of Sota (1987). The results would provide useful information for future ex situ conservation of *P. isumiensis*.

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絶滅危惧種イスミナガゴミムシ（甲虫目：オサムシ科）の生活史：室内飼育実験からの推定

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千葉県固有の絶滅危惧種イスミナガゴミムシ*Pterostichus isumiensis* Kasahara & Saito, 1997の生活史を明らかにするために室内飼育実験を行なった。成虫は10月に産卵し，野外の気温変化を模した飼育下では，11月に孵化した幼虫は翌年１月以降に終齢（３齢）となり，５月下旬に蛹化，６月下旬に新成虫となった。これらの結果から，本種は秋繁殖型の生活史を持つと考えられた。一方で，新成虫の性成熟を誘発する飼育条件（特に温度条件）の解明は，今後の課題として残されている。

キーワード：千葉県，生息域外保全，日本，ベーツナガゴミムシ亜属，レッドリスト

Appendix 1. Dates of the first day of each developmental stage, death or sacrifice, and temperature change (YYMMDD).

Sample code	Eggs	1st instar	2nd instar	3rd instar	Pupa	Adult	Death or sacrifice	Temperature change†
L#18	091102	091122	091212	100130	—	—	100130	(a) 091224 (b) 091226
L#22	091022	091114	091130	100106	—	—	100109	(a) 091224 (b) 091226
L#28	091022	091113	—	—	—	—	091130	—
L#33	091024	091116	091202	100112	—	—	100501	(a) 091224 (b) 091226 (c) 100420
L#52	091024	091116	091212	100120	—	—	100120	(a) 091220
L#53	091024	091116	091202	100109	—	—	100617	(d) 100112 (e) 100401
L#55	091102	091126	091212	100201	—	—	100316	(a) 091126 (b) 091226
L#57	091025	091118	091205	—	—	—	100126	(a) 091218 (b) 091226
L#58	091029	091122	091207	100203	100527	100627	101009	(a) 091224 (b) 091226 (e) 100401 (f) 100517 (g) 100701 (h) 100705 (i) 100721

† (a) 15°C to outdoors; (b) outdoors to 10°C; (c) 10°C to 14°C; (d) 15°C to 10°C; (e) 10°C to 12°C; (f) 12°C to 15°C; (g) 15°C to 18°C; (h) 18°C to 22°C; (i) 22°C to 25°C.