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Altitudinal life-cycle and body-size variation in ground beetles of the genus *Carabus* (subgenus *Ohomopterus*) in relation to the temperature conditions and prey earthworms

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ABSTRACT

The body size of univoltine insect species generally decreases with increasing altitude or latitude. This pattern may have arisen from adaptations to multiple factors that potentially affect body-size variation, such as temperature, food, and interspecific interactions. We examined altitudinal variations in life history and body size, and their relationships to temperature and food resources in two ground beetle species of the genus *Carabus* (subgenus *Ohomopterus*; *C. tosanus* and *C. japonicus*) in a mountainous area (altitude 860–1730 m) of Shikoku Island, Japan. Larvae of these species are specialist predators of earthworms. The body size of *C. tosanus* decreased with an increase in altitude. *Carabus japonicus*, which is much smaller than *C. tosanus*, exhibited similar sizes across altitudes, although it was not abundant at high altitudes. Available cumulative temperatures for larval development were limited at higher altitudes, and *C. tosanus* started reproducing 1 month earlier at higher than at lower altitudes. Earthworms (larval food) were less abundant at higher than at lower altitudes. This may imply that food resources also restrict the optimal body size of *C. tosanus* at higher altitudes.

Introduction

Insect species that live along a climatic gradient experience less favorable conditions for reproduction and larval development at climatic extremes. As a component of the life-history evolution of univoltine insects along a climatic gradient, optimal body size is expected to decrease in cooler habitats (Masaki 1967, 1978; Roff 1980). Although this predicted pattern (the converse of Bergmann's rule) has been demonstrated in many univoltine insects (Park 1949; Masaki 1967; Mousseau 1997; Sota et al. 2000a), seasonal life-history patterns in the field and other factors that potentially affect body-size variation, such as food resources, have not been examined in most cases. Geographic variation in body size within a species can result not only from adaptation to environmental clines, but also from biotic interactions with prey or closely related species. Therefore, studying seasonal population dynamics as well as biotic and abiotic environmental conditions in the field is essential in determining if temperature is the major factor affecting clinal variation in body size.

Flightless ground beetle species of the subgenus *Ohomopterus* in the genus *Carabus* typically exhibit a decrease in body size along temperature gradients with increasing latitude or altitude (Sota et al. 2000b). This body-size variation is at least partly attributable to climatic adaptations, as noted above, but the coexistence of two or three species with different body sizes indicates the role of additional factors (Sota et al. 2000b). Several authors have suggested that differences in body size influence mechanical reproductive isolation between species and can lead to the coexistence of different populations/species (Nagata et al. 2007; Okuzaki et al. 2010a). Therefore, identifying the factors affecting body size differences among populations is important in understanding the factors promoting speciation and assemblage formation in this beetle group.

Field observations (Sota 1985a), breeding experiments (Sota 1985b; Y. Tsuchiya et al. unpublished data) and the stable isotopic profile of larvae (Okuzaki et al. 2010b) have shown that the larvae of *Ohomopterus* species feed exclusively on megascolecid earthworms. In addition, the seasonal life cycle of *Ohomopterus* is synchronized with the seasonal abundance of earthworms (Sota 1985a). As exothermic animals, earthworms also must cope with seasonal conditions at different altitudes and are expected to

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exhibit altitudinal changes in abundance and seasonal life cycles. However, seasonal trends in earthworm populations among altitudes are largely unknown in Japan.

In this study, we focused on altitudinal variation in body size in two *Ohomopterus* species occurring in a mountainous area of central Shikoku Island, Japan: *Carabus japonicus* Motschulsky and *Carabus tosanus* (Nakane, Iga et Uéno). Of these, *C. tosanus* is endemic to Shikoku, is larger than *C. japonicus* and shows marked size variation (Sota et al. 2000b), which may lead to speciation by mechanical reproductive isolation due to body-size differences between adjacent populations. The body sizes of *C. tosanus* and *C. japonicus* are significantly reduced with decreasing annual mean temperature and increasing altitude, although *C. japonicus*, which has a wide distribution range, exhibits different regional patterns in altitudinal body size variation (Sota et al. 2000b). We conducted a year-round field study of the populations of these species. Specifically, we examined how beetle phenology and body size as well as the altitudinal distribution of the beetles are related to temperature and earthworm abundance (the food of beetle larvae). With increasing altitude, temperature and larval food resources (earthworm populations) may become limiting factors in the reproduction and larval development of *Carabus* species. Our purpose in comparing altitudinal life-history and body-size variations between the two *Ohomopterus* species was to gain insight into the interactive effects of abiotic and biotic factors on body-size evolution in these beetles. We hypothesized that low temperatures and the scarcity of earthworms at high altitudes are important factors leading to the small body size of *Carabus* adults at high altitudes, as a result of adaptation to environmental conditions restricting larval developmental rates.

Materials and methods

Study beetle species and study sites

Carabus (*Ohomopterus*) *tosanus* is endemic to Shikoku Island, Japan. This species is polytypic, with three subspecies: *C. t. kawanoi*, *C. t. ishizuchianus*, and *C. t. tosanus* (Ishikawa 1985). Of these, all but *C. t. tosanus* occur in the upper regions of the four mountain ranges on Shikoku, typically with small, coppery bodies, in contrast to the large, black bodies of *C. t. tosanus*. A mitochondrial gene genealogy indicates that *C. tosanus* is a monophyletic lineage with no clear genetic distinction among subspecies (Sota and Nagata 2008; Y. Tsuchiya et al. unpublished data). Nevertheless, recent studies have revealed genetic differences in larval development and associated adult body size between subspecies from low and high altitudes (Y. Tsuchiya et al. unpublished data). *Carabus* (*Ohomopterus*) *japonicus* Motschulsky is smaller than *C. tosanus* and occurs in most of the range of *C. tosanus*. This species is not polytypic on Shikoku.

We selected five sampling sites at altitudes of 860–1730 m in the Ishizuchi mountain range (Table 1, Fig. 1) as representative of the life-history and environmental conditions at different altitudes. Air temperatures at the five sites during the field collection were recorded at 1-h intervals using data loggers (Thermo Recorder TR51S; T&D, Matsumoto, Japan) attached to trees at a height of 50 cm.

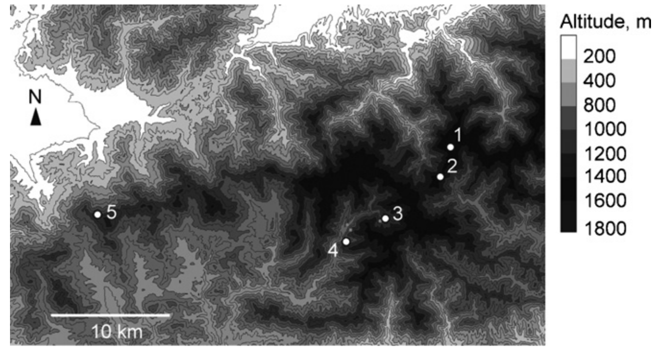

Fig. 1. Locations of the study sites. Numerals indicate site numbers given in Table 1.

Pitfall sampling

Continuous pitfall sampling of adult beetles was conducted at each site from 23 April to 14 November 2009. The pitfall system consisted of a plastic cup (opening 8 cm, 12.5 cm high) that was buried in the ground and covered by an iron roof (9 cm × 9 cm) with four legs to prevent input of rainfall and litter. Each cup contained 150 ml of 80% ethanol to preserve trapped beetles. Twenty to 30 pitfalls were set at 3-m intervals at each site (Table 1). At site 5, a few traps were fouled by soil erosion and were not included in the capture statistics. Pitfalls were emptied every 2 weeks, and ethanol was replaced. Collected beetles were stored in freezers in our laboratory.

Altitudinal trends

The thermal conditions available to beetles at each study site were evaluated by using accumulated temperature data above the developmental zero temperature. The relative abundance (activity density) of *Carabus* species at each site was indicated by the annual total capture per trap, which was the sum of the number of trapped beetles per trap throughout the sampling period. The body length (from the front margin of the labrum to the apical part of the elytra) of each trapped beetle was measured using a digital caliper.

To determine the reproductive condition of field-collected beetles, we dissected females and checked for the presence of mature eggs. Beetles with soft exoskeletons or a weakly chitinized aedeagus (male) were considered to be newly emerged adults.

Analysis of temperature conditions for larval development

To determine whether temperature conditions in the field could restrict the larval period, we estimated the relationship between oviposition day and the expected day of adult emergence based on the daily air-temperature data at each altitude and larval developmental parameters in terms of the sum of the effective temperature (K, °C day) above the developmental zero temperature (T_0, °C). The larval developmental parameters were determined in the laboratory (Y. Tsuchiya et al. unpublished data) by rearing two subspecies of *C. tosanus* from sites 2 and 5, and *C. japonicus* from

Table 1
Latitude, longitude, sampling period, and the number of pitfall traps at each study site.

Site no./name	Latitude (°N)	Longitude (°E)	Altitude (m)	Trap period	No. traps [a]
1 Mt. Kamegamori	33.7874	133.1892	1660–1730	Apr. 24–Nov. 14	20
2 Mt. Ibukiyama	33.7661	133.1817	1420–1480	Apr. 24–Nov. 14	30
3 Kanayamadani	33.7338	133.1349	1010–1030	Apr. 23–Nov. 14	20
4 Skyline 2.9 km	33.7148	133.1060	860–880	Apr. 23–Nov. 14	20
5 Mt. Saragamine	33.7224	132.8861	980–1000	Apr. 23–Nov. 14	22

[a] In April and May, the number of traps was 20 at sites 2 and 10 each at sites 3, 4 and 5.