Early Leaf Abscission Has Little Effect on Larval Mortality of *Ectoedemia cerviparadisicola* (Lepidoptera, Nepticulidae) Associated With *Quercus gilva*

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ABSTRACT There has been an argument as to whether early leaf abscission substantially affects larval mortality of leafminers because of leaf senescence. Recently, a study reported that leaf abscission considerably increased the mortality of a leafminer (*Ectoedemia cerviparadisicola* Sato, sp. nov.) associated with *Quercus gilva* (Blume) in Nara Park, central Japan, where sika deer (*Cervus nippon* (Temminck)) have been protected for 1,200 yr, because deer consumed many abscised leaves containing living larvae. The study, however, did not investigate the life history of the leafminer or survey the leaf-fall pattern of *Q. gilva* through the season, so that it failed to quantify larval mortality because of deer predation. To test whether deer have a substantial effect on larval mortality of this leafminer, we regularly collected abscised and nonabscised leaves of *Q. gilva* through the season, examining mines and larvae in those leaves. Over 90% of mined leaves abscised in the period of peak leaf fall, when almost all larvae had already emerged from mines to pupate. Most dead larvae in abscised leaves were judged to have already died at the time of abscission from their instars and body features. The proportion of living larvae in abscised leaves was estimated to be <1.8%. Thus, even when deer preyed upon all living larvae in abscised leaves, they would hardly contribute to the increase in larval mortality. These results indicate that the effect of leaf abscission on leafminer mortality via deer predation and other causes is limited. The leafminer was described as new to science in the Appendix.

KEY WORDS deer predation, description of new species, life history, mortality factor, plant-insect interaction

Early leaf abscission is often induced by the presence of leafminers; mined leaves are apt to abscise earlier than unmined leaves in many leafminer-plant systems. In some systems, moreover, leaf abscission is positively correlated with the number of mines per leaf (Maier 1983, 1989; Stiling et al. 1991; Mopper and Simberloff 1995; Kagata and Ohgushi 2004; Wagner et al. 2008).

Many studies have mentioned that early leaf abscission is an important source of mortality for leafminers because of leaf desiccation and senescence (Naruse 1978; Owen 1978; Faeth et al. 1981; Faeth 1985, 1986, 1990; Potter 1985, 1992; Bultman and Faeth 1986; Williams and Whitham 1986; Stiling et al. 1987; Simberloff and Stiling 1987; Auerbach and Simberloff 1989; Stiling and Simberloff 1989; Preszler and Price 1993; Connor et al. 1994; Auerbach 1991; Mopper et al. 1995; Kagata and Ohgushi 2004). This hypothesis is based on two assumptions: 1) leafmining larvae are still feeding on leaf tissue within the mine at the time of abscission and 2) they are unable to develop into mature instars in the abscised leaves. In some species, however, most larvae complete their feeding or pupate by the time of abscission (Prichard and James 1984a,b; Maier 1989; Stiling and Simberloff 1989; Kahn and Cornell 1989; Oishi and Sato 2007) or can complete their development in the abscised leaves by making "green islands," that is, photosynthetically active green patches in otherwise senescent leaves (Engelbrecht et al. 1969, Kaiser et al. 2010). Furthermore, miners in abscised leaves could escape from parasitism, because adult parasitoids hardly search abscised leaves (Kahn and Cornell 1983). This means that early leaf abscission could not only be irrelevant but even adaptive in terms of enemy avoidance, especially, for leafminers that are able to make green islands. Thus, early leaf abscission does not always greatly contribute to leafminer mortality.

Recently Yamazaki and Sugiura (2008) reported fascinating interactions among a lepidopteran leafminer (*Ectoedemia cerviparadisicola* Sato, sp. nov., which has been described as new to science in the Appendix), an evergreen oak (*Quercus gilva* Blume), and sika deer (*Cervus nippon* Temminck) in Nara Park, central Japan, where many sika deer have been protected for ≈1,200 yr for religious reasons. *E. cerviparadisicola* is a specialist that feeds exclusively on *Q. gilva.* Mines begin to change from serpentine to blotch-shaped in February, and mature larvae emerge from mines to

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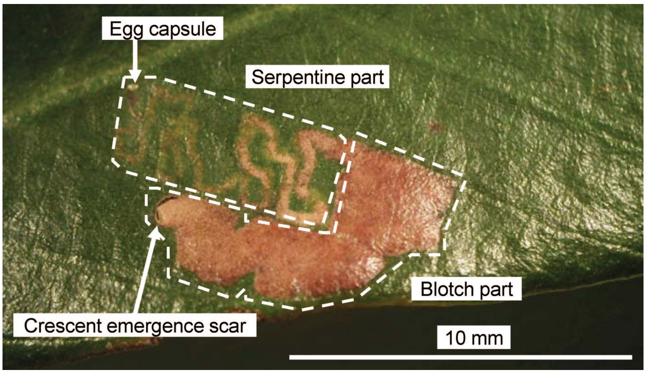

Fig. 1. Mine of *E. cerviparadisicola.* (Online figure in color.)

spin cocoons for pupation on the ground in March. Yamazaki and Sugiura found that 1) leaves that were mined by *E. cerviparadiscola* abscised earlier than unmined leaves, 2) leafmining larvae were able to complete their growth within the mine of abscised leaves, and 3) sika deer consumed many abscised leaves without discriminating between mined leaves and unmined leaves. Thereby, they suggested that early leaf abscission considerably increased the mortality of leafminers not from leaf desiccation or senescence but from deer predation, emphasizing the novelty of the indirect interaction between the leafminer and the ungulate mediated by the host plant's response to the leafminer. Here, one might address a question of the degree to which the ungulate contributes to mortality of the leafminer population. Unfortunately, they did not investigate the life history of the leafminer or survey the leaf-fall pattern through the season, so that they failed to estimate the mortality caused by deer predation.

The objectives of the current study were to 1) reveal the seasonal leaf-fall pattern of *Q. gilva,* 2) confirm whether leaves mined by *E. cerviparadisicola* are apt to abscise earlier than unmined leaves, 3) estimate the larval mortality of *E. cerviparadisicola* caused by leaf abscission, 4) test the Yamazaki and Sugiura's hypothesis that leafminers suffer a considerable mortality because of deer predation via their consumption of abscised leaves, and 5) identify the life history of *E. cerviparadisicola.* For this purpose, we regularly collected abscised leaves and nonabscised leaves of *Q. gilva* in Nara Park, similar to Yamazaki and Sugiura (2008), and examined mines and larvae of *E. cerviparadisicola* in those leaves.

Materials and Methods

Study Site and Organisms. This study was carried out in Nara Park (34° 41′ N, 135° 51′ E, 110 m a.s.l.), Nara Prefecture, central Japan, similar to Yamasaki and Sugiura (2008). This park is adjacent to the town of Nara City, with an area of 6.6 km^2, containing open grasslands and evergreen woods. In the park, more than several hundred sika deer have been protected for ≈1,200 yr for religious reasons (Torii and Tatsuzawa 2009). In 2008, 1,128 sika deer inhabited the park (Foundation for the Protection of Deer in Nara Park 2008).

The study leafminer *Ectoedemia cerviparadisicola* Sato (Lepidoptera, Nepticulidae), which was referred to as *Stigmella* sp. in Yamazaki and Sugiura (2008), has been described as new to science in the Appendix. The following information summarizes the known biology of this leafminer species (Yamazaki and Sugiura 2008; H.S., unpublished data): mines are found exclusively on an evergreen oak, *Q. gilva* (Fagaceae), although such evergreen oaks as *Q. glauca, Q. myrsinaefolia, Q. salicina,* and *Q. phillyraeoides* are common in and around Nara Park; serpentine mines rapidly become angular, blotch-shaped (Fig. 1), and conspicuous in February; mature larvae emerge from mines to spin cocoons for pupation on the ground in March; adults emerge in May and June; because *Q. gilva* sheds almost old leaves in April and May and produces new leaves in late April and May, females oviposit necessarily on new leaves.

Leaf-Fall Pattern. We chose five *Q. gilva* trees ≈10 m in height at or near the edge of the evergreen forest in Nara Park and located a quadrat of 1 × 1 m on the ground under each of the crowns. All abscised leaves within them were collected at intervals of 3–6 d from 29 February to 29 April 2008.

Wind Speed. Because wind speed probably affects leaf-fall pattern, we got data on wind speed which were recorded from February to April 2008 at the Nara Meteorological Station, 2 km north–west of the study site. Three measures of wind speed for each day were used: average wind speed, maximum sustained wind speed (i.e., maximum value of 10-min average wind speed), and maximum instantaneous wind speed (i.e., maximum value of 3-s average wind speed).

Mines of *E. cerviparadisicola* in Abscised Leaves and Nonabscised Leaves. Each of abscised leaves collected from the quadrats was examined for the presence or absence of *E. cerviparadisicola* mines, and mines were classified as active and inactive. Active mines refer to those in which larvae are present and alive, while inactive mines refer to those in which larvae are dead or missing. To examine the relationship between head capsule width and larval instars, living larvae were removed from active mines and head capsule width was measured (see below for detailed method). Inactive mines were examined for the fate of the larvae inside and were categorized as successful development (crescent emergence scar visible on mine surface; Fig. 1), parasitism (parasitoid larva or parasitoid pupa in mine, or emergence hole of parasitoid adult visible on mine surface), and death by other causes (blackish or parched larval body in mine, or larva missing from mine ripped open).

To examine when mature larvae emerge from mines in leaves that retain on trees, we sampled a total of 100 mined leaves randomly within arm's reach from shoots of the above-mentioned trees and their adjacent trees at 3–6 d intervals from 22 February to 3 April 2008, until most larvae had emerged from mines. After recording mines from which larvae had already emerged, we put leaves with active mines individually in a plastic case in which a sheet of moisten filter paper was laid. They were kept in incubators at a photoperiod of 10:14 (L:D) h with temperature simulating outdoor conditions. We checked daily whether mature larvae emerged from the mines. Larvae outside mines were moved onto gardening soil in unglazed ceramic flowerpots. After the manner of Puplesis (1994, fig. 4), the pots were covered with tinfoil and a glass tube was stuck into the cover so that adult moths gathered in the tube. We located them in the shade outdoors, checked daily whether there were adults in the tube, and collected the adults. When all larvae emerged from the active mines in a leaf, the inactive mines in the leaf were dissected to determine the source of larval mortality.

Life History of *E. cerviparadisicola*. To examine the occurrence of larval instars of *E. cerviparadisicola* through the season, we randomly sampled 10–50 mined leaves once or twice a month from July 2008 to February 2009 and from July 2009 to March 2010, except October and November in 2009. Living larvae were collected from the mines, and head capsule width was measured to an accuracy of 1×10^{-4} mm using digital photographs taken with a microscope of 50× magnifications. A histogram of head capsule width was then made to reveal the number of larval instars and identify the instar for each individual. In addition, to examine the relationship between mine shape and instar and whether larvae feed and grow during winter, we randomly selected ≈10 mines on each sampling date from July 2009 to March 2010, scanning them at 300 dpi with an image scanner Canoscan D1250U2 F (Canon, Japan). For each mine, length of serpentine part and area of angular blotch-shaped part (Fig. 1) were measured with image analysis software LIA32 for Windows 95 version 0.376ß1 (Yamamoto 1997).

Statistical Analyses. All statistical analyses such as Mann–Whitney *U* test, *t*-test, and Kendall's ranked correlation were performed with SPSS for Windows version 15.0J (SPSS 2006).

Results

Leaf-Fall Pattern and Wind Speed. In total, 6,611 of abscised leaves was collected from five quadrats during the study period. Because leaf-fall patterns were similar among the quadrats, data from them were compiled (Fig. 2a). Leaves began to fall in mid-March, and peak leaf fall began in mid-April. A massive leaf fall occurred during 6–9 April before the beginning of peak leaf fall. This was probably caused by gale winds (near gale according to Beaufort scale, 13.9–17.1 m/s) that blew on 7 April (Fig. 2b) while abscission layer was forming. However, any other gale winds in February and March did not lead to a massive leaf fall, and no wind blew at gale force during peak leaf fall. Thus, the leaf-fall pattern was unlikely to be affected by winds except during several days before peak leaf fall.

Mines of *E. cerviparadisicola* in Abscised Leaves and Nonabscised Leaves. Of 6,661 abscised leaves, 6,169 (=93.3%) were mined by *E. cerviparadisicola.* The majority of mined leaves (>90%) abscised in April when the massive leaf fall caused by gale winds and peak leaf fall occurred (Fig. 2a). In total, 28,150 mines were obtained from the abscised leaves, and the mean number of mines per abscised leaf (±SD) was 4.3 ± 2.81. Reflecting the leaf-fall pattern (Fig. 2a), the number of mines in abscised leaves remained low until early April, afterwards increasing rapidly (Fig. 2c).

The proportion of mined leaves in abscised leaves kept around 95–100% until early April, when peak leaf fall had not begun yet, afterwards decreasing gradually to 90% at the end of April (Fig. 2d, data from February to early March are not presented because of small sample size). The proportion, overall, decreased with the progress of the season (Kendall's $\tau = -0.781$; $P < 0.001$). The mean number of mines per mined leaf was always significantly higher in abscised leaves than in nonabscised leaves from mid-March to early April during which active mines were found in both abscised and nonabscised leaves (*t*-test, $P < 0.001$; Fig. 2e).

More than half of 28,150 mines obtained from abscised leaves were successful-development mines (16,298/28,150 = 57.9%), followed by death-by-other-causes mines (9,634/28,150 = 34.2%) and parasitized mines (1,684/28,150 = 6.0%; Fig. 2f). Active mines made up only 1.8% (519/28,150). A large part of death-by-other-causes mines did not reach the full size, or most of the larvae in these mines were first to fourth instars (see below for instars in detail). When leaves began to fall in mid-March, the proportion of active mines in abscised leaves was ≈50%, afterwards reducing rapidly and reaching ≈0% in early April before the beginning of peak leaf fall. With a reduction in active mines, the proportion of successful-development mines increased until late March, afterwards reducing gradually. The proportion of death-by-other causes mines increased slowly from late March. Parasitized mines were mainly found in April.

Mature larvae in leaves that retained on trees had already begun to emerge from mines in late February (Fig. 2g). At that time, the proportion of active mines

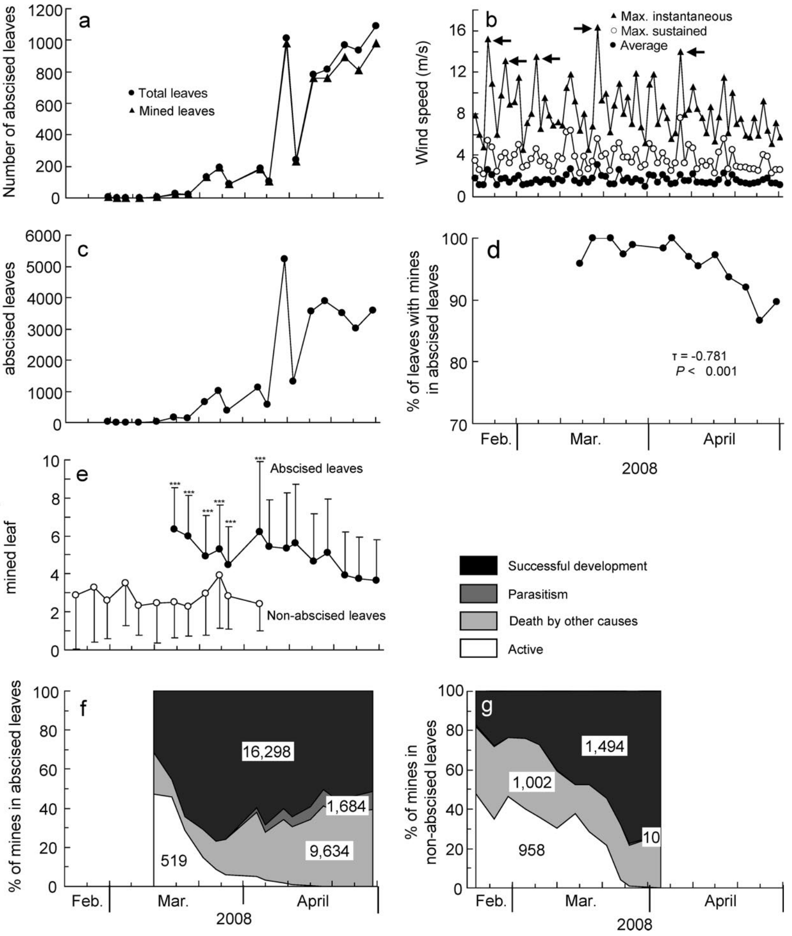

Fig. 2. (a) Leaf-fall pattern of *Q. gilva* on the basis of abscised leaves from five quadrats. (b) Wind speed expressed by maximum instantaneous, maximum sustained, and average. (c) The number of mines in abscised leaves collected from five quadrats. (d) The percentage of mined leaves in abscised leaves collected from five quadrats. (e) The numbers of mines per mined leaf (±SD) in abscised leaves collected from five quadrats and in nonabscised leaves collected from trees by the quadrats. (f) The percentage of mine categories in abscised leaves collected from five quadrats. (g) The percentage of mine categories in nonabscised leaves collected from trees by the quadrats. In panel b, arrows represent gale winds (near gale according to Beaufort scale, 13.9–17.1 m/s). In panel e, *** represents a significant difference between abscised leaves and nonabscised leaves for each date ($t_{cal} > 6.872$; df >118; $P < 0.001$ with Dunn-Šidák's critical probability). In panels f and g, numbers represent the total number of mines in each category.

was ≈50%. All larvae had emerged from mines by early April, before the beginning of peak leaf fall. Parasitized mines were not observed until early April.

Life History of *E. cerviparadisicola*. Frequency distribution of larval head capsule width exhibited five peaks (Fig. 3). Thus, the number of larval instars was estimated to be five. In both study years, first instars appeared in July and molted four times into fifth instars in late December to January (Fig. 4). Larvae made serpentine mines during first to fourth stadia (Fig. 5). When developing into fifth instars, they form angular, blotch-shaped mines. In early February, fifth instars began to emerge from mines to spin cocoons on the ground (Fig. 2g). Because mines extended continuously from November to February, larvae seemed to continue feeding on leaf tissue without dormancy during winter. By early April, almost all larvae had

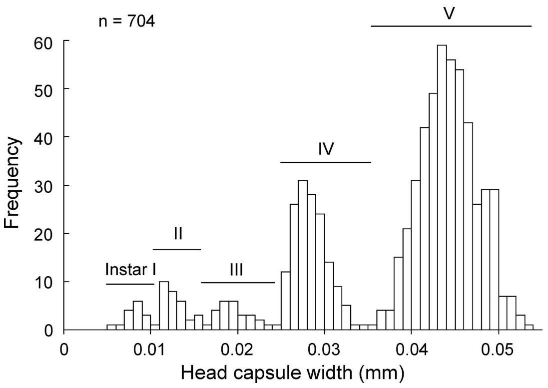

Fig. 3. Frequency distribution of larval head capsule width of *E. cerviparadisicola*. Roman numerals represent larval instar.

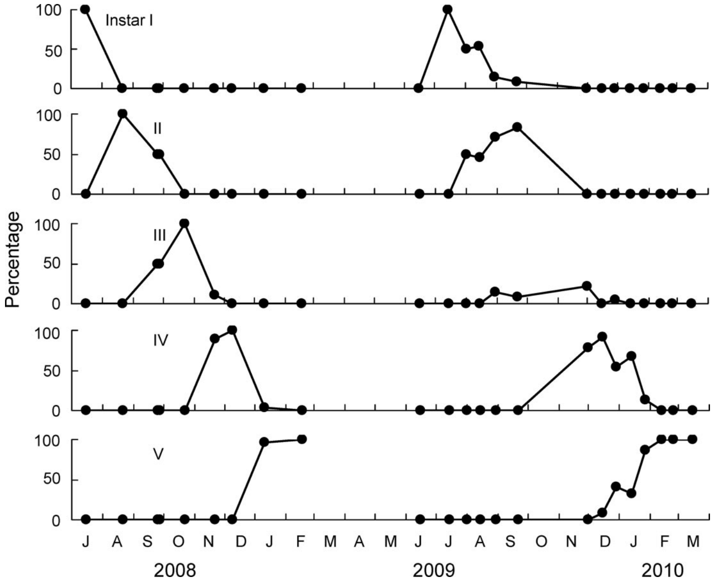

Fig. 4. Seasonal occurrence of larval instars of *E. cerviparadisicola* on the basis of leaf sampling from *Q. gilva* trees.

emerged from mines (Fig. 2f,g). Twelve adults emerged from the soil in flowerpots spontaneously from 7 May to 16 June 2008.

Discussion

In many leafminer-plant systems, mined leaves are apt to abscise earlier than unmined leaves. In some systems, leaf abscission is positively correlated with the number of mines per leaf. This has been confirmed in the *E. cerviparadisicola–Q. gilva* system. The proportion of mined leaves in abscised leaves decreased with the progress of the season (Fig. 2d), and the number of mines per leaf was always significantly higher in abscised leaves than in nonabscised leaves (Fig. 2e). However, the majority of mined leaves abscised at the massive leaf fall caused by gale winds and in the period of peak leaf fall in April (Fig. 1a,c). Thus, the influence of leafminers on leaf abscission would be limited.

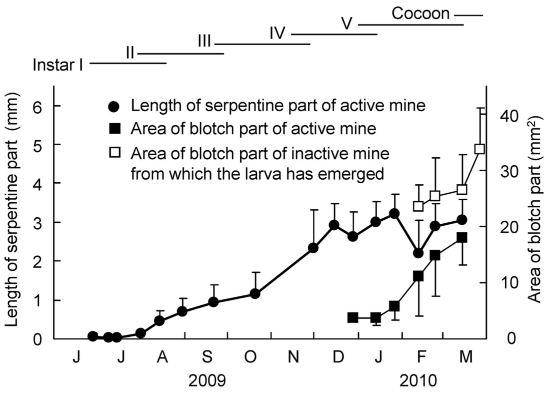

Fig. 5. Seasonal extension of mimes in terms of the length of serpentine part and the area of blotch part (see Fig. 1). Larval stadia represented by horizontal bars are based on Fig. 4.

Although many authors have agreed that mined leaves are apt to abscise earlier than unmined leaves, they have disputed whether leaf abscission causes substantial mortality of leafminers. Some authors estimated larval mortality caused by leaf abscission at >30% (Faeth et al. 1981; Stiling et al. 1987, 1991; Simberloff and Stiling 1987; Auerbach and Simberloff 1989; Preszler and Price 1993; Connor et al. 1994; Waddell et al. 2001), while other authors argued that it was only 2–3% (Prichard and James 1984b), even <1% (Oishi and Sato 2007, Gripenberg and Roslin 2008). In the current study, even when all larvae that are alive in abscised leaves at the time of abscission would die, the larval mortality would be estimated to be 1.8% at most. Moreover, this percentage is overestimated, because when we terminated our leaf sampling at the end of April, peak leaf fall still continued (Fig. 2a), and a large number of leaves with successful-development mines remained on the trees (Fig. 2g). In addition, some living larvae in abscised leaves were about to emerge from mines, and thus the probability that they suffer death from leaf abscission would be fairly low. Death-by-other-causes mines in abscised leaves can be disregarded in estimates of larval mortality caused by leaf abscission, because most larvae in these mines are judged to have already died at the time of leaf abscission from the fact that they were first to fourth instars or their mines did not reach the full size, while all living larvae were fifth instars (Fig. 4) or had emerged from mines to pupate (Fig. 2f). Consequently, we conclude that early leaf abscission has little effect on leafminer mortality in the *E. cerviparadisicola*–Q. *gilva* system.

As mentioned above, larval mortality from leaf abscission in the *E. cerviparadisicola*–*Q. gilva* system is quite low compared with that in other systems. This difference is mainly attributable to the timing of leaf abscission in the leafminer's life cycle. Most larvae of *E. cerviparadisicola* emerge from mines to pupate before the beginning of leaf fall, and thus there are few living larvae in the period of peak leaf fall. Oishi and Sato (2007) have demonstrated that completion of larval development before leaf abscission leads to fairly low levels of larval mortality from leaf abscission in the *Coptotriche japoniella* Puplesis and Diškus–*Eurya japonica* Thunberg system. Furthermore, they suggest that leafminers prevent the hosts from abscising the mined leaves until adults emerge. This hypothesis is based on the fact that mined leaves rarely abscise until adult emergence, although larvae spend a long period of ≈9 mo within the leaves and fully expanded mines often occupy >50% of the leaf area. *E. cerviparadisicola* has a larval period as long as *C. japoniella.* Although mine size relative to the size of host leaves (<10%, unpublished data) is less than that of *C. japoniella,* the number of mines per leaf (4.3 on average) exceeds that of *C. japoniella* (0.17–0.36; Oishi and Sato 2009). Thus, *E. cerviparadisicola* might also inhibit leaf abscission as well as *C. japoniella.* Our data, however, do not allow us to examine this hypothesis more closely.

Yamazaki and Sugiura (2008) suggested that larvae of *E. cerviparadisicola* suffered considerable mortality because of incidental predation by sika deer in Nara Park on the basis of the following observations: 1) mined leaves abscised earlier than unmined leaves, 2) larvae in abscised leaves successfully emerged from mines to pupate in the absence of deer, and 3) deer consumed mined and unmined leaves without discrimination. However, their field survey was conducted only for several days in mid-March, when many larvae had not emerged from mines yet and peak leaf fall did not begin yet. Hence, the proportion of living larvae in abscised leaves seems to have been overestimated. Furthermore, Yamazaki and Sugiura (2008) emphasized the novelty of the indirect interaction between the leafminer and the ungulate mediated by the host plant's response (i.e., early leaf abscission) to the leafminer. However, our results from regular sampling of abscised leaves through the leaf fall season estimated that larval mortality from deer predation was 1.8% at most, which was very low even compared with that from parasitism, 6.0%. This estimation indicates that the findings of Yamazaki and Sugiura (2008) are interesting but irrelevant for the leafminer at the level of population.

The effect of early leaf abscission on leafminer survival depends upon the timing of leaf abscission in the leafminer's life cycle (Bultman and Faeth 1986, Stiling and Simberloff 1989, Kahn and Cornell 1989). Thus, to evaluate the effect precisely, we should examine both life history of the leafminer and seasonal leaf-fall pattern of the host plant. Considering both conditions, we conclude that early leaf abscission has little effect on larval mortality in the *E. cerviparadisicola*–*Q. gilva* system and also that deer hardly contribute to the increase in larval mortality via their consumption of abscised leaves, contrary to Yamazaki and Sugiura's (2008) hypothesis.

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Appendix

Ectoedemia cerviparadisicola Sato, sp. nov.
(Figs. 1 and A1–A4)

Male and Female (Fig. A1a–c). Wingspan including cilia 0.48–0.62 mm (mean ± SD = 0.52 ± 0.036; n = 16) in male; 0.49–0.59 mm (0.53 ± 0.058; n = 4) in female; not significant difference between sexes (Mann–Whitney U test, $U_{\mathrm{cal}} = 28$; $P = 0.750$). Antennal about one-half the length of forewing; upper-surface dark brown; lower surface grayish beige; eyecap whitish beige; segments 46–53 (49.3 ± 2.54; n = 10) in male and 28–31 (29.5 ± 1.29; n = 4) in female, significantly more in male than in female (Mann–Whitney U test, $U_{\mathrm{cal}} = 0$; $P = 0.002$). Frontal tuft soft yellowish orange. Collar light grayish beige, comprising piliform scales. Thorax whitish beige. Forewing whitish beige, speckled with dark brown scales, den-

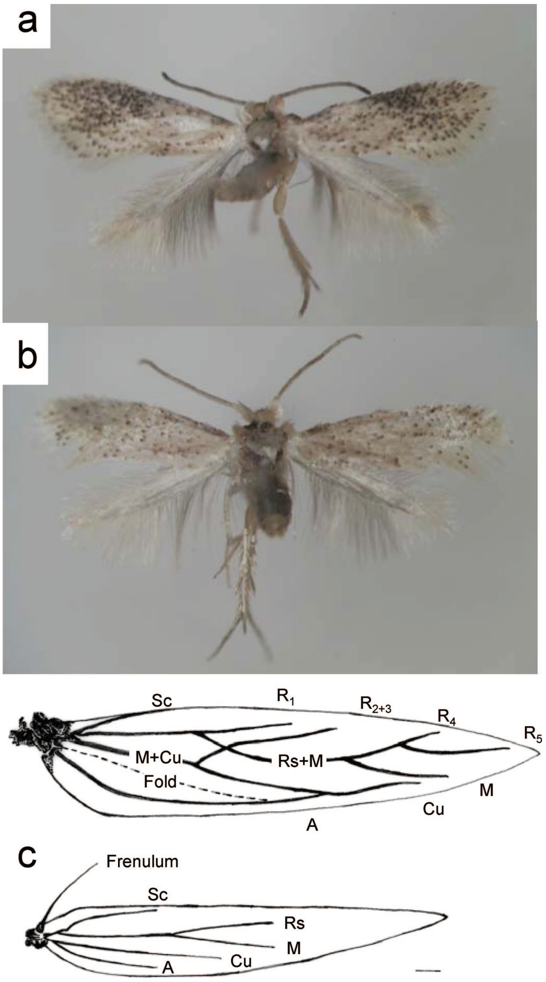

Fig. A1. Adults of *E. cerviparadisicola* sp. nov. (a) Male, forewings speckled densely with brown scales (specimen code YS.08.S3-100). (b) Male, forewings with speckled sparsely with brown scales (02.1-7). (c) Wing venation (slide code 02.1-16). Bar = 0.1 mm. (Online figure in color.)

sity of which highly varying among individuals; fasciae and spots absent; cilia-line distinct; eight veins present; M coalescent with Cu from base, diverging at basal one-third to coalesce with Rs, forming a closed cell; R1, R2 + 3, R4 and R5 present; M unbranched; Cu long, extending almost to anal edge; A thickened, long, sometimes seeming fused with Cu at tips, without anal loop. Hindwing whitish gray; cilia light beige gray; humeral lobe, costal bristles, and hair-pencil absent in male; five veins present; Rs and M coalesced for one-half of length, unbranched; Cu much longer than common stalk of Rs and M; A short, about two-thirds the length of Cu.

Male Genitalia (Fig. A2a–e). Vinculum ring-shaped; ventral plate short, moderately concave anteriorly. Tegumen produced into a widely rounded, distinctly papillate pseuduncus with slender long setae. Uncus absent. Gnathos well sclerotized, simple, V-shaped (Fig. A2a) or W-shaped (Fig. A2b), varying according to method of mounting. Valva rather rectangular, with almost parallel sides; posterior inner corner extending inwardly into pointed process, bearing short setae on ventral surface; posterior outer corner rounded, bearing long setae on both surfaces; outer margins almost straight; middle of inner margin slightly swollen, serrated by prominent sockets of seven to eight long setae; posterior margin concave. Transtilla with ventral arms extending well anteriorly and narrow short lateral arms; transverse bars fused centrally, forming an arch. Aedeagus not constricted, with single pair of carinae ventrally; carina varying single to multifurcate, with up to four horns, not sharply pointed, sometimes with additional spines; vesica with many small triangular cornuti only; cathrema conspicuous.

Female Genitalia (Fig. A3). T8 trapezoid, with bilobed papillae anales bearing slender long setae. Apophysis anterioris strongly arcuate, very broad in basal half, slender in anterior half, with a hole at middle of basal half. Apophysis posterioris straight, slender, swollen basally, approximately same length as apophysis anterioris. Vestibulum with a ring-shaped vaginal sclerite and a spinculate pouch. Corpus bursae without pectinations; signae reticulate, dissimilar; margin of signa narrower than individual cells. Ductus spermathecae with two indistinct convolutions.

Mature Larva (Fifth Instar) (Fig. A4a–c). Body translucent yellow; ventral ganglia brown, visible; a pair of ventral ambulatory calli present on T2-T3; a pair of ventral ambulatory calli present on A1–7. Head longer than broad; frontoclypeus stirrup-shaped, longer than wide, outlined by heavily sclerotized internal ridges; anterior tentorial arm longer than posterior; cranial setae confined to anterior part, one pair located on dorsal side (A1), two pairs on ventral side (SS2 and SS3); five pairs and two pairs of setal pores located dorsally and ventrally, respectively. One pair of stemmata. Labrum with a pair of medial setae and a pair of lateral setae. Mandible with a seta laterally. Maxilla with a long seta on stipes; palpifer consisting of two segments, each of which bearing a short seta ventrally on distal edge. Labial palpus two-segmented, with a long apical seta; distal segment much longer than basal. Spinneret with a pair of setae. Antenna consisting of one segment only, with two basiconic sensilla, two chaetic sensilla, and two minute spines; two types of sensilla arranged not cross-wise, unlike *Stigmella* (Johansson 1990; fig. 50). T1 with a pair of dorsal sclerites and a broad ventral sclerite, covered with microtrichia laterally; T2 with a narrow ventral sclerite, heavily covered with microtrichia; T3 and A1–8 heavily covered with microtrichia; A9 heavily covered with microtrichia ventrally, sparsely dorsally; A10 with a well-defined ventral sclerite and a pair of internal sclerotized rods (=anal rods); anal rod widened posteriorly, with an additional internal sclerite.

Chaetotaxy of Fifth Instar (Fig. A4c). T1 with 13 pairs of setae; L group trisetose; D1 and XD1 closely approximate, in appearance on same pinaculum. T2 with 12 pairs of setae; L group trisetose. T3 with 10 pairs of setae; D2 absent; L group bisetose. A1–8 with

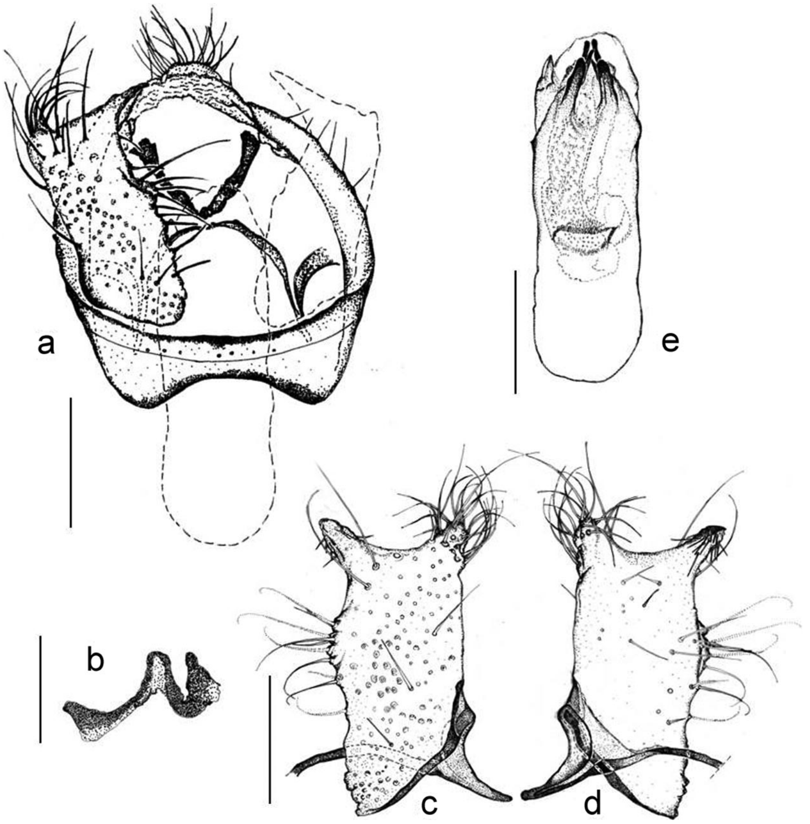

Fig. A2. Male genitalia of *E. cerviparadisicola* sp. nov. (a) Whole genitalia except right valva and aedeagus (slide code HS-G133). (b) W-shaped gnathos (HS-G139). (c) Right valva, ventral view (HS-G139). (d) Right valva, dorsal view (HS-G139). (e) Aedeagus (HS-G133). Bars = 0.1 mm.

six pairs of setae. A9 with two pairs of setae, one dorsal (D1) and one ventral (SV1). A10 with four pairs of setae, two dorsal and two ventral.

Type Material. HOLOTYPE. ♂, Nara City, Nara Pref., 26-II-2002, on *Quercus gilva*, emergence 28-IV-2002, H. SATO (specimen code 02.1-10, genitalia slide code HS–G132). PARATYPES. 14 ♂, 1 ♀, the same data as holotype except on emergence date: 1 ♂, em. 16-IV-2002 (specimen code 02.1-1, genitalia slide code HS-G130); 1 ♂, em. 20-IV-2002 (02.1-2, wing slide code HS–W34); 1 ♂, em. 22-IV-2002 (02.1-4, wing slide code HS–W31); 1 ♂, em. 24-IV-2002 (02.1-5); 1 ♂, em. 27-IV-2002 (02.1-7, genitalia slide code HS–G129); 2 ♂, em. 28-IV-2002 (02.1-9, genitalia slide code HS–G139, wing conditions not good; 02.1-11, HS–G133, wing conditions not good); 1 ♂, em. 30-IV-2002 (02.1-12, genitalia slide code HS–G134, wing conditions not good); 1 ♂, em. 1-V-2002 (02.1-15, wing slide code HS–W15); 2 ♂, em. 4-V-2002 (02.1-16, wing slide code HS–W35; 02.1-19); 2 ♂, em. 6-V-2002 (02.1-18, wing slide code HS–W33; 02.1-20, genitalia slide code HS–G135, wing conditions not good); 1 ♀, em. 26-IV-2002 (02.1-6, genitalia slide code HS–G136, wing conditions not good); 1 ♀, em. 30-IV-2002 (02.1-13, genitalia slide code HS–G140, wing conditions not good). 2 ♂, 3 ♀, the same locality as holotype, Y. Shinozaki: 1 ♂, 1-III-2008, em. 10-VI-2008 (specimen code YS.08.S3-100, genitalia slide code HS–G131); 1 ♂, 4-III-2008, em. 19-V-2008 (YS.08.S4-70); 1 ♀, 4-III-2008, em. 21-V-2008 (YS.08.S4-43); 1 ♀, 4-III-2008, em. 8-V-2008 (YS.08.S4-60, genitalia slide code HS–G138); 1 ♀, 15-III-2008, em. 5-V-2008 (YS.08.S7-92, genitalia slide code HS–G137). All type specimens are deposited in Entomological Laboratory, Osaka Prefecture University, Japan.

Larval Specimens Examined. Thirteen mature larvae (fifth instars), all mounted on slides, the same locality as adult type specimens, Y. Shinozaki: 3 ex, 21-II-2008 (larval slide code HS–L26); 3 ex, 21-II-2008 (HS–L27); 7 ex, 21-II-2008 (HS–L28, HS–L29, HS–L30, HS–L31, HS–L32, HS–L33, HS–L34).

Mine (Fig. 1). Serpentine during first to fourth stadia, becoming angular, blotch-shaped in fifth stadium.

Distribution. Japan (Nara City, Honshu Island).

Host Plant. *Q. gilva* Blume (Fagaceae).

Etymology. *Cerviparadisicola* a noun in nominative case; cervus (L.) = deer; paradisus (L.) = paradise; colere (L.) = to dwell; after Nara Park, which is a paradise for deer because deer have been protected for ≈1,200 yr for religious reasons, and in which the present ecological study was conducted.

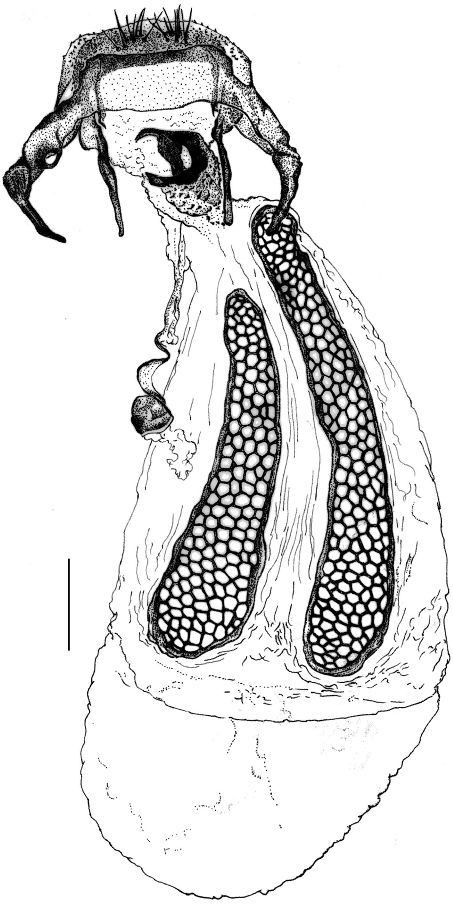

Fig. A3. Female genitalia *E. cerviparadisicola* sp. nov. (slide code HS-G136). Bar = 0.1 mm.

Remarks. In total, 47 species of Nepticulidae has been described from Japan (Kemperman and Wilkinson 1985, Kuroko 1999). Of them, six are members of the genus *Ectoedemia.* Although there is a rich nepticulid fauna in Japan, taxonomic studies have not been well done, except the genus *Stigmella.*

E. cerviparadisicola is the first nepticulid species that is described from *Q. gilva,* belonging to the *subbimaculella* group of the subgenus *Ectoedemia.* This group consists of 23 species (Puplesis and Diškus 2003, Nieukerken et al. 2010), characterized by the following: host plants *Quercus* spp.; pseuduncus triangular or widely rounded; valva with strongly papillate inner margin (but not all species); aedeagus with single pair of carinae; vestibulum with ring-shaped vaginal sclerite and a spiculate pouch; signa dissimilar; and corpus bursae without pectinations (Niieukerken et al. 1985, Puplesis 1994). The last character is considered to be an apomorphy that supports the monophyly of the *subbimaculella* group (van Nieukerken 1985). *E. cerviparadisicola* shares these characters, resembling *E. ermolaevi, E. aligera,* and *E. scoblei* of the species group in that its forewings have no fasciae or spots and are speckled with brownish scales. This new species is easily distinguished from other known members of the group by characteristics of the valvae: rather rectangular; posterior outer corner rounded with long setae; and posterior margin concave. However, I found no distinctive characteristics of female.

Nieukerken et al. (2012) have made a phylogenetic study of the subgenus *Ectoedemia* on the basis of sequences of cytochrome c oxidase I and eclongation factor 1-α. They treated *E. cerviparadisicola* tentatively as *E. Quercus gilva,* suggesting that *E. cerviparadisicola* was related the most closely to *E. albifasciella.*

Larval chaetotaxy of the *subbimaculella* group has been examined in *E. quinquella, E. albifasciella, E. subbimaculella,* and *E. heringi* (Johanson et al. 1990). These species have the L group on T2 bisetose, whereas in *E. cerviparadisicola* it is trisetose.

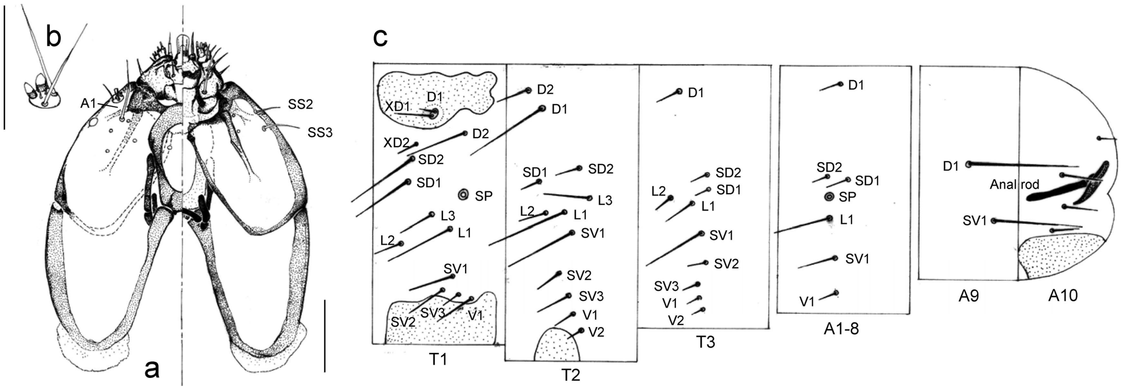

Fig. A4. Mature larva (fifth instar) of *E. cerviparadisicola* sp. nov. (a) Head capsule (slide code HS-L28); left, dorsal view; right, ventral view. (b) Antenna (HS-L28). (c) Setal map. Bars = 0.1 mm.