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RESEARCH ARTICLE

Factors affecting the number of leaves included in the shelters of the leaf-folding caterpillar, *Vanessa indica* (Lepidoptera: Nymphalidae)

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ABSTRACT. Larvae of the Indian red admiral butterfly, *Vanessa indica* (Herbst, 1794), fold the leaves of the host plants along the midvein to construct shelters. Usually, one leaf is used for every shelter, although more than one leaf are sometimes used. To determine the conditions under which larvae use more than one leaf to construct a shelter, a field investigation of the larval shelters was conducted. Early instar larvae used multiple leaves in a shelter when a larger leaf served as the main leaf. In comparison, in later instars used multiple leaves when a smaller leaf served as the main leaf. When an early instar larva uses a large, tough leaf, it may not be able to fold it sufficiently and there may be gaps in the surface of the shelter. In such a case, a larva in the early instar may add nearby leaves to cover the gaps, so that the shelter has more than one leaf. Because later instar larvae can fold a leaf tightly, their shelters have no gaps. When later instar larvae use a small leaf, multiple leaves will be needed to expand the shelters within which they hide and feed.

KEY WORDS. Indian red admiral butterfly, leaf size, multiple-leaf shelter, shelter construction, trenching behavior.

INTRODUCTION

Caterpillars from at least 26 families of Lepidoptera construct shelters using host leaf material and their own silk (Baer 2017). There are several shelter shapes, including leaf rolls, leaf webs, leaf ties, leaf folds, and leaf tents (Greeney and Jones 2003, Lill and Marquis 2007, Greeney 2009, Baer 2017). Intraspecific variation in larval shelter architecture is found in some species, but not many studies have been conducted about the reasons for or processes underlying the emergence of such architectural variation (Loeffler 1996, Bodlah et al. 2019), except for ontogenetic changes (Ruehlmann et al. 1988, Lind et al. 2001, Weiss et al. 2003, Abarca et al. 2014).

The Indian red admiral butterfly, *Vanessa indica* (Herbst, 1794), ranges widely from India to China and Japan, and its larvae feed mainly on plants in the nettle family Urticaceae (Fukuda et al. 1983). Larvae of all instars construct shelters by folding leaves of host plants along the midvein with silk, and then feed inside them (Fig. 1). They move to larger leaves and construct new shelters as they grow (Ide 2004). When they use leaves of China ramie, *Boehmeria nipononivea* Koidz. (Urticaceae), one leaf is usually employed to make a shelter, although more than one is sometimes used (Ide 2009). Later instar larvae tend to use more than one leaf to build a shelter when the leaves are small, and adding surrounding leaves enable them to make a larger shelter (Ide 2009). Early instar larvae also sometimes use more than one leaf for a shelter, although they do not need large shelters due to their small bodies. The conditions under which early instar larvae use more than one leaf to construct a shelter are not known.

To construct shelters, *V. indica* larvae cut trenches at the base of the leaves (Ide 2004). Trenching is generally thought to deactivate the chemical defences of host plants through reducing the influx of toxic compounds to the leaf (Dussourd 1993). Trenching also reduces influx of water into the leaf, but it rarely makes the shelter of *V. indica* shrivel up. In addition, the trenching behavior of *V. indica* facilitates leaf-folding; as they trench, larvae gnaw notches

Figure 1. Leaf shelters of *Vanessa indica* larvae: (A) a one-leaf shelter of a later instar; (B) a two-leaf shelter of a later instar, a gap in the shelter surface was covered with a part of another leaf; (C) a two-leaf shelter of an early instar; (D) a one-leaf shelter of an early instar, the shelter was not folded fully, and a gap was covered with silk threads. Scale bars are 20 mm.

into the three main leaf veins, which can then be broken easily at the notches, thereby decreasing the force required to fold the leaf (Ide 2004). Early instar larvae rarely engage in trenching behavior (Ide 2004), probably because they have smaller heads with less mandibular muscle, which likely leads to lower bite force than in later instars (Hochuli 2001, Zalucki et al. 2002). Because the force required for folding correlates with leaf size (Ide 2004), early instar larvae usually use expanding, small and soft leaves, but use larger and larger leaves as instars progress (Ide 2009). However, it is not always possible to find a leaf that is just the right size. When an early instar larva has no choice but to use a large leaf, it may not be able to fold the leaf sufficiently. If a leaf is not folded tightly, there may be gaps in the shelter surface. In such cases, an early instar larva may add nearby leaves to cover any gaps. In short, the reason why early instar larvae use multiple leaves for a shelter may be to cover gaps in the shelter surface, rather than to make the shelter larger.

If this is the case, early instar larvae would tend to use more than one leaf for shelter construction when the leaves are large. In this study, I reanalyzed the data of Ide (2009) and examined whether early and later instar larvae use multiple leaves for different reasons.

MATERIAL AND METHODS

Field investigation

The study was conducted in the area around Kumamoto University in Kumamoto, Japan (130°44'E, 32°49'N; 20 m a.s.l.), from April to November, 2003. China ramie, the main host plant of *V. indica*, forms abundant, dense, monospecific stands at the study site. These stands are mown periodically, so that the plants are usually < 1.5 m in height throughout the season. The larvae of *V. indica* sometimes make a trench after folding a leaf, so a shelter without a trench may be in the process of being constructed (Ide 2004). Therefore, when a larva started eating the shelter, it was considered that the construction of the shelter was completed, and completed shelters were investigated. I searched for larval shelters on the ramie; when a shelter with feeding marks was found, I recorded the number of leaves in the shelter, as well as the leaf length, larval instar, and presence or absence of a trench. If a shelter was constructed from more than one leaf, I recorded the length of the leaf that had been fed on most, i.e., the leaf that had made the main part of the shelter. Leaves whose tips were eaten were excluded from the analysis of leaf size.

Statistical analyses

The statistical analyses were performed using R software (version 3.6.3; R Core Team 2020). To determine whether multiple leaves were used when the main leaf of a shelter was small or large, the relationship between the length of the main leaf and number of leaves included in the shelter was analysed. A multi-leaf shelter usually consisted of a combination of a main leaf and additional leaves. A main leaf provided most of its leaf area as shelter construction, but only a portion of an additional leaf was used for the shelter (Fig. 1B–C). Therefore, it is inappropriate to treat both a main and an additional leaf as the same "one leaf". Then, considering that leaves are added to a shelter one by one, the number of leaves in a shelter was treated as an ordinal variable. An ordinal logistic regression model was used with the length of the main leaf and the larval instar as the independent variable and the number of leaves in a shelter as the dependent variable, using the clm function of the "ordinal" package (Christensen 2019). In addition, a likelihood ratio test was performed to determine overall effects of each variable. First, the effect of the interaction between leaf size and larval instar was calculated by comparing the likelihood ratio of the full model and that of the model without the interaction. Then, the effect of leaf size (or instar) was calculated by comparing the likelihood ratio of the model without the interaction and that of the leaf size (or instar)-only model. The ratio of shelters with trenches to those without trenches was extremely different depending on the instar, and especially, all the shelters of first instars were without trenches (Fig. 1). Therefore, shelters with trenches and those without trenches were analyzed separately.

To determine whether the number of leaves in a shelter varied depending on trenching, the number of leaves was compared between trenched and untrenched shelters with Mann-Whitney U test.

RESULTS

In total, 806 *V. indica* larvae shelters were found, 639 of which used only one leaf of the host plant; the other shelters were made of multiple leaves. The number of leaves in a shelter varied depending on larval instar and the length of the main leaf. In untrenched shelters, larval instar and the interaction of instar and main leaf length, among the fixed effects, affected significantly the number of leaves in a shelter (Table 1). Although the number of leaves in a shelter

Table 1. Summary of ordinal regression for variables predicting the number of leaves in an untrenched shelter. The base category for variable "instar" was first instar.

Parameters	Estimate	S.E.	z	P
Leaf length	0.064	0.027	2.341	0.019
Instar (second)	2.171	1.307	1.661	0.097
Instar (third)	3.067	1.225	2.505	0.012
Instar (fourth)	6.069	1.714	3.541	0.0004
Instar (fifth)	12.454	2.603	4.785	< 0.0001
Instar (sixth)	14.786	3.359	4.402	< 0.0001
Leaf length x Instar (second)	-0.038	0.033	-1.129	0.259
Leaf length x Instar (third)	-0.044	0.030	-1.490	0.136
Leaf length x Instar (fourth)	-0.079	0.035	-2.262	0.024
Leaf length x Instar (fifth)	-0.154	0.044	-3.463	0.0005
Leaf length x Instar (sixth)	-0.168	0.052	-3.220	0.001

Likelihood ratio test of fixed effects

Factor	d.f.	LR stat.	P
Leaf length	1	0.900	0.343
Instar	5	46.617	< 0.0001
Leaf length x Instar	5	23.261	0.0003

of first to third larval instar increased with main leaf size, that of fourth to sixth instar decreased (Fig. 2). In trenched shelters, larval instar and the main leaf length affected significantly the number of leaves in a shelter (Table 2). The number of leaves in a shelter increased with larval instar, and decreased with the length of the main leaf (Fig. 2). The number of leaves in a shelter differed between trenched and untrenched shelters (Table 3). Third to sixth instar larvae used significantly more leaves for untrenched than trenched shelters (Fig. 3).

Table 2. Summary of ordinal regression for variables predicting the number of leaves in a trenched shelter. The base category for variable "instar" was second instar.

Parameters	Estimate	S.E.	z	P
Leaf length	-0.019	0.086	-0.224	0.823
Instar (third)	0.274	4.996	0.055	0.956
Instar (fourth)	2.035	4.905	0.415	0.678
Instar (fifth)	0.805	4.830	0.167	0.868
Instar (sixth)	3.401	4.883	0.696	0.486
Leaf length x Instar (third)	-0.009	0.089	-0.102	0.919
Leaf length x Instar (fourth)	-0.014	0.087	-0.157	0.875
Leaf length x Instar (fifth)	0.003	0.086	0.036	0.971
Leaf length x Instar (sixth)	-0.012	0.086	-0.145	0.885

Likelihood ratio test of fixed effects

Factor	d.f.	LR stat.	P
Leaf length	1	20.145	< 0.0001
Instar	4	26.475	< 0.0001
Leaf length x Instar	4	1.722	0.787

Table 3. Summary of Mann-Whitney U test to evaluate differences in the number of leaves of a *Vanessa indica* shelter by trenching.

Instar	W	P
First (n_u = 103, n_t = 0)	–	–
Second (n_u = 142, n_t = 9)	683	0.6021
Third (n_u = 110, n_t = 71)	5006.5	< 0.0001
Fourth (n_u = 23, n_t = 77)	1270	< 0.0001
Fifth (n_u = 14, n_t = 151)	1763.5	< 0.0001
Sixth (n_u = 6, n_t = 100)	445.5	0.0135

n_u Number of untrenched shelters; n_t Number of trenched shelters.

DISCUSSION

The relationships between the number of leaves included in a *V. indica* larvae shelter and main leaf size varied by larval developmental stage. This suggests that the reason for using multiple leaves for a shelter changed as the larvae grew. In untrenched shelters, first to third instar larvae used multiple leaves in a shelter when they used a larger leaf as the main leaf (Fig. 2, Table 1). This pattern is expected when gaps in the surface of a shelter created by the leaves not folding sufficiently are covered with nearby leaves. Small body sizes impose limitations on the ability to process leaves mechanically (especially by biting and folding) of early instars (Reavey 1993, Hochuli 2001). Therefore, early instars of *V. indica* are affected by leaf toughness. In a skipper butterfly, leaf toughness affects the architecture of larval shelters only at the early stages (Greeney et al. 2010). This pattern was not observed in the trenched shelters of same instars. With trenches, even larger leaves could be folded sufficiently, and there were probably few gaps in the shelters.

Fourth and later instar larvae used multiple leaves in a shelter when a smaller leaf served as the main leaf (Fig. 2, Tables 1, 2). This pattern is expected when surrounding leaves are added to make a larger shelter. As larvae grow large, they need large shelters within which to hide and feed (Ruehlmann et al. 1988, Weiss et al. 2003). Therefore, if the leaves chosen by later instar larvae are small, they will need to add more leaves.

Multiple leaves were used more often in shelters without trenches (Fig. 3). For fourth and later instar larvae that did not cut trenches, cutting trenches was likely to be possible, except just before moulting (Ide 2004). If they were just before moulting, they may not have taken enough time to select leaves, and thus may have used small, unsuitable leaves. In fact, the main leaves of untrenched shelters are significantly smaller than those of trenched shelters (Ide 2004). Therefore, multiple leaves were probably often used for untrenched shelters because surrounding leaves were gathered together to subsequently enlarge the shelters. Note that leaves that are close to each other are easy to use by a larva to construct a shelter with multiple leaves (Marquis et al. 2002). On a ramie shoot, the distance between new expanding leaves near the tip of the shoot is small, i.e., the distance between small leaves is small. Therefore, when using small leaves, it may be easier to add leaves to a shelter because adjacent leaves are close by.

In this study, the effect of leaf size on the number of leaves included in a *V. indica* shelter apparently differed between early and later instars. However, leaf toughness at the early stage, and shelter size at the later stage, were the true factors affecting the number of leaves in a shelter. In other words, because two different factors that correlated with leaf size affected the shelter architecture at each larval stage, leaf size appeared to have different effects on early and later instars.

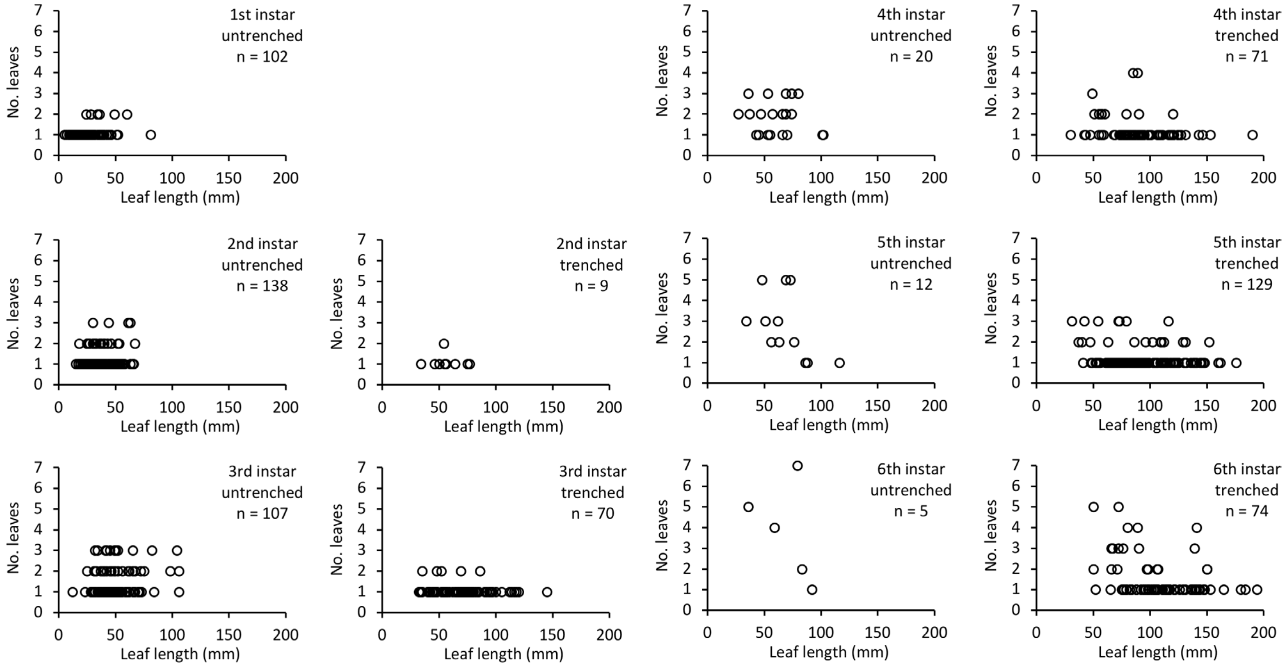

Figure 2. Relationship between the number of leaves in a *Vanessa indica* shelter and the length of the main leaf thereof.

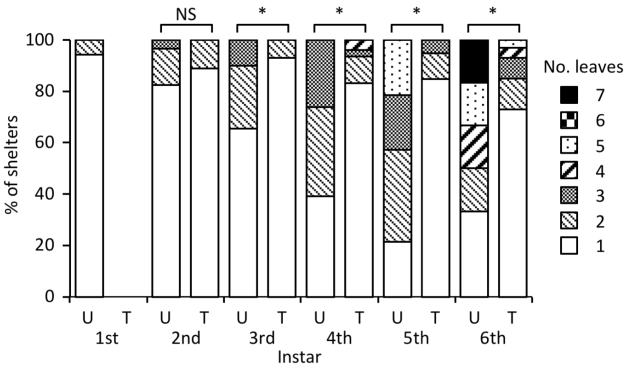

Figure 3. The number of leaves used was compared between untrenched (U) and trenched (T) shelters with Mann-Whitney U test (*p < 0.05; NS, not significant). Sample sizes are shown in Table 3. No shelters of first instar larvae had trenches.

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Competing Interests

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