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Galls Provide Us with Good Information for Ecological Studies - Methods of Practical Field Survey and Data Analysis

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ABSTRACT

Galls provide us with various ecological data in a convenient way. To facilitate ecological studies of gall midges benefiting from this convenience, methods of field survey to obtain ecological data regarding adult activities, host plant phenology, life history patterns, and life tables are explained based on practical data of my colleagues and myself. In addition to field surveys, the importance of occasional gall dissection is emphasized. To analyze the life table data, the method of key-factor/key-stage analysis is recommended, instead of the conventional key-factor analysis, to detect density-dependent and independent forces operating on gall midge populations and to assess the relative strength of top-down and bottom-up effects.

Key words: Cecidomyiidae, life table, key-factor/key-stage analysis, adult behavior, mortality factor

Introduction

Galls provide life table data in a convenient way (e.g. Redfern and Cameron, 1978). The number of galls can be easily counted continuously from early to final stage of the gall and the galler's development because of their outstanding features and immobility (e.g., Yukawa, 2000). Therefore, population dynamics of gall-inducing cecidomyiids has been relatively intensively studied (e.g., Redfern and Cameron, 1978, 1993; Keller and Schweizer, 1994; Redfern and Hunter, 2005). In recent years, the method of key-factor/key-stage analysis (Yamamura, 1999) has been used (Yamamura, 2012; Yukawa *et al*., 2016b), instead of the conventional key-factor analysis (e.g., Morris, 1959; Varley and Gradwell, 1960, 1968), to detect density-dependent and independent forces operating on gall midge populations at different developmental stages and to assess the relative strength of top-down and bottom-up effects. At the same time of field surveys of gall midges, we can obtain phenological data of host plants, such as seasons of bud burst, shoot-extension, leaf- and flower-openings. These data enable us to analyze the degree of synchronization between gall midge emergence and host plant phenology. Synchronization is a critical event for such short-lived insects as gall midges. Galls also show us patterns of life history strategies of gall midges, including long-term diapause. I provide

some known examples for these topics to facilitate further ecological studies of gall midges and their host plants.

The purposes of this paper are (1) to provide methods of practical field survey, particularly for leaf gall-inducing cecidomyiids on evergreen broad-leaved trees and (2) to show analytical methods of field data. These methods have been practiced and shown in recent papers by my colleagues and myself (Yukawa and Akimoto, 2006; Yukawa *et al*., 2006, 2013, 2016b, c, 2018).

This paper will be useful for students who plan to begin field surveys for ecological studies of gall midges in evergreen broad-leaved forests in subtropical and tropical regions. Taiwan is one of the most appropriate places for such field surveys because of extremely rich gall midge fauna in the forests (Tung and Yang, 2018).

Field survey

Adult gall midge activities

Prior to field surveys of population dynamics, we need to gather information on adult gall midge activities, such as emergence, mating, swarming, flight ability, oviposition and longevity, together with data of sex ratio and the number of ovarian eggs (Yukawa *et al*., 1976; 2013). Information on these behavioral traits can be obtained mostly by direct observation in the field and partly by laboratory experiments. For direct observation, we need to select 10 to 20 census host trees in the field.

Gall midges are roughly divided into two groups in terms of daily activities (Barnes, 1930). Diurnal gall midges emerge early or late in the morning. Sometimes males emerge several hours before females (Yukawa *et al*., 2013). Males usually swarm around host plants from which they emerged and then mating takes place during the morning on the host leaves where emerged females are located (e.g., Yukawa *et al*., 1976; 2013). Females leave the host plant to lay their eggs in or on newly emerged host organs.

Nocturnal gall midges, such as many species of *Asphondylia*, emerge in the evening or after dark. Swarming and mating take place during the nighttime. Frequently, some individuals emerge during the daytime under cloudy conditions.

Hourly emergence pattern can be determined for diurnal gall midges by direct observation in the field and for nocturnal species by rearing experiments under natural lighting conditions in outdoor cages. Field surveys of daily emergence pattern are essential in referring to synchronization between gall midge emergence and host plant phenology. Because the daily emergence patterns fit well to the gamma distribution pattern (Yukawa and Akimoto, 2006), the number of adults emerged can be surveyed at intervals of three to four days, instead of everyday observation, to determine the daily emergence pattern. The emergence of gall midges is distinguishable from that of parasitoids by the exit holes that are larger in gall midges and frequently retain pupal exuviae.

Swarming and mating should be observed in the field (e.g., Mishima *et al*., 2014). The number of females that were not detected by swarming males can be evaluated. Because the observable number of females that are waiting for males on the host leaves is limited for one person in a day, several co-observers are needed to obtain ample data of the rate of females mated. Flight ability can be measured with a strain gage transducer (Kanmiya, 1994). Estimation of flight ability is needed to evaluate dispersal of gall midges, particularly those that exhibit host alternation. If possible, we need to record the daily number of females that visit the host organs for oviposition. With this data we can estimate the rate of females that survive factors operating on adults although identification of the factors is rather difficult.

Sometimes the sex ratio is female-biased and daily emergence patterns are different between sexes. Therefore, at least 50 individuals are needed to determine sex ratio. Because ovarian eggs are matured at the time of emergence, the eggs are countable under a binocular microscope by dissection of female abdomens. Yukawa *et al*. (1976) found 59 eggs on average left in the ovaries of females of *Pseudasphondylia neolitseae* Yukawa. The females fastened on the host buds in the evening and stopped ovipositing after fulfilling their duty. Thus, they calculated that 189 (76%)

of 248 initial ovarian eggs were laid during oviposition on average per female. The number of adults emerged, their sex ratio, and the average number of ovarian eggs are used to estimate the initial number of eggs of the following generation.

Host plant phenology

First we need to count the number of host organs, such as extending shoots or the current year leaves on the census trees selected. Then, we determine, by observing ovipositing females, the condition of host organs that are suitable for oviposition. In the case when shoots are targets of oviposition, we measure the length of shoots with a scale and regard shoots within the range of suitable length as those available for oviposition. Because shoots extension curve available for oviposition fits well to the normal distribution pattern (Yukawa and Akimoto, 2006), the shoot length can be measured in the field at intervals of three to five days. When eggs are laid on the half opened leaves, we count un-, half- and full-expanded leaves. Usually asynchrony between gall midge emergence and host plant phenology is expressed as time lag in days between 50% emergence date and 50% date in the number of host organs that are available for oviposition. In addition to the time lag, Yukawa and Akimoto (2006) took the amount of resources into the degree of synchrony, with which density-dependent effects was evaluated.

Life history patterns

Life-history patterns of gall midges are closely linked to their host plant phenology (Yukawa and Rohfritsch, 2005). In temperate areas, most trees produce new shoots and flowers once a year, and therefore tree-inhabiting gall midges are inevitably univoltine. In contrast, gall midges on herbaceous plants that produce new shoots repeatedly can be multivoltine. Even on trees, gall midges can become multivoltine particularly in the subtropics and tropics, where new shoots are available in seasons other than spring. Some species have two or more generations a year by alternating between different organs of the same host or different species of host plants.

To determine voltinism, periodical dissection of galls is necessary. The galls should be collected from sampling trees that were selected differently from census trees. If necessary, we need to determine the lower development threshold temperatures (LDT) of gall midges and the thermal constants from egg or first instar to adult, with which the number of generations per year can be estimated for multivoltine gall midges (Yukawa *et al*., 2016).

Bergant and Trdan (2006) argued that LDT and thermal constants based on laboratory experiments commonly suffer from a great amount of uncertainty. They emphasized that a sufficient temperature range should be covered in the experiment to approach the borders of the linear response as closely as possible. My colleagues and I reared gall midge larvae under various temperature conditions that could decrease the uncertainty in determining LDT around the borders (see Ohtani *et al*. 1983; Okuda and Yukawa 2000; Yukawa *et al*. 2013, 2016a; Kim *et al*. 2015 for details of the rearing experiments).

Life table data

Life table enumeration provides is one of the most appropriate data sets to detect density-dependent and independent forces operating on gall midge populations at different developmental stages and to assess the relative strength of top-down and bottom-up effects. For this purpose, we select some census trees for field surveys or some lower branches in cases when trees are too tall. In addition, some sampling trees should be selected to collect galls for dissection.

Usually life tables start from the egg stage of the first generation (Table 1). The number of eggs can be directly counted when eggs are laid on the host leaf surface or scars of the oviposited position can be recognized after larval hatching. For example, we can find such scars made on the under surface of *Machilus* leaves by larvae of *Daphnephila* that hatched and sank into the leaf tissue. When eggs are laid inside plant tissue, we have to estimate the number of eggs of the first generation (E_n) by the following equation:

Table 1. A simplified example of life table data for an imaginary gall midge

Developmental stage	Season	Mortality factor	*Lx*	*Dx*	*% Lx*
Eggs on the leaf surface	April		1200		100.00
		Predation by ants		700	
1st instars in young galls	July		500		41.67
		Galls fed by moth larvae		100	
1st and 2nd instars in young galls	September		400		33.33
		Fall of galled leaves by typhoon		350	
3rd instars and pupae in mature galls	March		50		4.17
		Parasitism by *Bracon* sp.		40	
Adults emerged	April		10		0.83
		(Sex ratio: 0.6)			
Females emerged	April		6		
Potential number of eggs for the following generation	April	(6 × 150 ovarian eggs/female)	900		0.75*

Lx: the number of living individuals, *Dx*: the number of dead individuals caused by each mortality factor, *%Lx*: survival rate from the initial number of *Lx* (1200).

* Reproductive rate in comparison with the number of eggs in the previous generation (1800/1200).

$E_n = A_{n-1}$ x S x O

A_{n-1}: The number of adults emerged from the census trees in the preceding generation

S: Sex ratio (the ratio of females among adults emerged)

O: The number of ovarian eggs before oviposition. If possible, the number of realized eggs after oviposition is more appropriate.

Immediately after galls become conspicuous, we need to record the initial number of early-stage galls that contain first instars. At the same time, we record the number of galled and ungalled host organs such as leaves or young fruit. On the way to the maturity of galls, we need to monitor galls that survived optional feeding by cecidophagous lepidopteran larvae and fall of galled organs caused by physical reasons such as strong wind, by biological reasons related to gall density or to lepidopteran leaf feeders. In addition, we count galls that could not mature because of larval death in the galls by unknown factors. Later in the season (in October-November or in the following March in the case of univoltine gall midges), we need to count mature galls remaining on the census trees. During the growth of galls, we should collect some galls from the sampling trees occasionally (once or twice) and dissect them under a binocular microscope to know: (1) the developmental stadium of larvae, (2) presence or absence of ectoparasitoid larvae and their developmental stadium, and (3) presence or absence of inquilines and their developmental stadium. About two weeks before emergence, we dissect mature galls again to record the aforementioned items. At this time, we can find endoparasitoids, if any, which pupate in the host larval skin. At every time of gall sampling, we record the number of host organs remaining on the census trees. Thus, we can obtain data regarding rates of the eggs, larvae and pupae that survived age specific mortality factors.

In the survey of fruit gall midges, special attention should be paid to the following facts (Yukawa *et al*., 2016): (1) The first instars inhabiting flower buds or flowers drop to the ground and die because of flower abortion, which is tremendous in rate. However, the abortion of flowers is a normal event and is not caused by larval infestation. (2) Frequently galled fruit cannot be distinguished from normal fruit in size and color until late autumn. In the light of the aforementioned two facts, occasional dissection of flower buds, flowers and young fruit is more important than direct field observation.

Table 2. Stage specific mortality factors and the calculation of survival rates

The life-table of an imaginary gall midge is divided into 4 developmental stages. N_t is the potential number of eggs on the host leaf surface at the tth year. S_t is the rate of generation change of N_t, that is, N_{t+1}/N_t. Then, S_t can be given by the multiplication of the survival rate (or rate of change) at each developmental stage:

$S_t = s_{1t}s_{2t}s_{3t}s_{4t}s_{5t}$, (1)

900/1200 = 500/1200 x 400/500 x 50/400 x 10/50 x 900/10 (actual data)

where s_{it} is the survival rate in the population entering the ith life stage at the tth year. The survival rate at each life stage is defined as follows:

s_{1t}: proportion of eggs that survived the predation by ants,

s_{2t}: proportion of first instars that survived lepidopteran larval feeding on galls,

s_{3t}: proportion of first and second instars that survived the fall of leaves by typhoon,

s_{4t}: proportion of third instars and pupae that survived parasitism by *Bracon* sp.

s_{5t} is regarded as the rate of change caused by the oviposition of one female, which is calculated from the sex ratio and the mean number of ovarian eggs per female.

Equation 1 is expressed in common logarithms as follows:

$\log_e(S_t) = \sum_{i=1}^{5} \log_e(s_{it})$ (2)

(6.80-7.09) = (6.21−7.09) + (5.99−6.21) + (3.91−5.99) + (2.30−3.91) + (6.80−2.30)

Life tables covering all developmental stages from egg to adult are ideal for analysis, but partial life tables covering a part of the life history (only larval stage, for example) are available for analysis when there are some difficulties in the field survey. The partial life table data during the larval stage tell us about density-dependent and independent forces operating on the gall midge larvae at different developmental stadia. It is much better than nothing.

Data analysis

Yamamura (1999) proposed a key-factor/ key-stage analysis by integrating the conventional key-factor analyses (e.g., Morris, 1959; Varley and Gradwell, 1960, 1968) and ANOVA, emphasizing the importance of discriminating between the key-factor and the key-stage. This analysis can identify the key-factor, the key-stage, and the combination of factor and stage that is most influential in determining the fluctuation of total mortality. By discriminating factors and stages, we can avoid all problems raised by Royama (1996) about the conventional analyses (Yukawa *et al*., 2016b). The effectiveness of the key-factor/key-stage analysis is demonstrated in Yamamura (1999, 2012), Yukawa *et al.* (2016b). Thus, key-factor/key-stage analysis is most recommended to detect density-dependent and independent forces operating on gall midge populations at different developmental stages and to assess the relative strength of top-down and bottom-up effects.

For this analysis, we need to provide multiple life table data sets. The number of data sets should be more than the number of parameters (= factors going to be evaluated), such as the initial number of eggs, temperature, the amount of precipitation, intensity of typhoons and other biotic or abiotic factors, otherwise key-factor/key-stage analysis (and ANOVA) does not work (e.g., Yukawa *et al*., 2018). Multiple data sets can be obtained by long-term surveys in one census field or short-term surveys in several different census fields.

In the analysis, we need to define developmental stages on which stage specific mortality factors operate. Table 2 shows the definition of stages and the calculation method for the survival rates based on the life table data for an imaginary gall midge (Table 1). Then, we define biotic and abiotic factors. Actual equations are indicated in Yukawa *et al*. (2016b) and an '*R*' function to perform key-factor/key-stage analyses is available from the following

web site (Yamamura, 2015, personal information):

http://cse.niaes.affrc.go.jp/yamamura/Key-factor_analysis_program.html

Yukawa *et al*. (2018) used key-factor/key-stage analysis for the first time to analyze long-term data on leaf longevity of *Neolitsea sericea* (Blume) Koidzumi (Lauraceae), a host plant of *P. neolitseae*. The term "stage" was used for "leaf age", as has been used in life table studies of other insects. In this analysis, Yamamura (2018, personal information) recommended to use $\log_e$ instead of $\log_{10}$ when actual survival rates are converted into logarithms. An *"R"* function to perform key-factor/key-stage analyses is available from the following web site:

http://cse.naro.affrc.go.jp/yamamura/Key-factor_analysis_program.html

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References

Barnes HF. 1930. On some factors governing the emergence of gall midges (Cecidomyiidae: Diptera). Proc Zool Soc Lond 1930: 381-393.

Bergant K, Trdan S. 2006. How reliable are thermal constants for insect development when estimated from laboratory experiments? Entomol Exp Appl 120: 251-256.

Kanmiya K. 1994. Flight analysis system using strain-gauge transducers to estimate power and pattern of flying insects. Jpn J Appl Entomol Zool 38: 261-273 (In Japanese with English abstract).

Keller S, Schweizer C. 1994. Populationsdynamische Untersuchungen an der Erbsengallmücke *Contarinia pisi* Winn. (Dipt., Cecidomyiidae) und ihrer Parasitoide. J Appl Entomol 118: 281-299.

Kim W, Matsunaga K, Gyoutoku N, Matsuo K, Minami T, Yukawa J. 2015. Life history strategy and adult and larval behavior of *Macrodiplosis selenis* (Diptera: Cecidomyiidae), a species that induces leaf-margin fold galls on deciduous *Quercus* (Fagaceae). Entomol Sci 18: 470-478.

Mishima M, Sato S, Tsuda, K, Yukawa J. 2014. Sexual isolation between two known intraspecific populations of *Hartigiola* (Diptera: Cecidomyiidae) that induce leaf galls on upper and lower surfaces of *Fagus crenata* (Fagales: Fagaceae), indicating possible diversification into sibling species. Ann Entomol Soc Am 107: 789-798.

Morris RF. 1959. Single factor analysis in population dynamics. Ecology 40: 580-588.

Ohtani T, Yazawa Y, Yukawa J. 1983. Developmental zero point of the ligustrum fruit midge, *Asphondylia sphaera* Monzen and the aucuba fruit midge, *Asphondylia* sp. (Diptera: Cecidomyiidae). Proc Assoc Pl Prot Kyushu 29: 118-121. (In Japanese with English summary)

Okuda S, Yukawa J. 2000. Life history strategy of *Tokiwadiplosis matecola* (Diptera: Cecidomyiidae) relying upon the lammas shoots of *Lithocarpus edulis* (Fagaceae). Entomol Sci 3: 47-56.

Redfern M, Cameron RAD. 1978. Population dynamics of the yew gall midge *Taxomyia taxi* (Inchbald) (Diptera: Cecidomyiidae). Ecol Entomol 3: 251-263.

Redfern M, Cameron RAD. 1993. Population dynamics of the yew gall midge *Taxomyia taxi* and its chalcid parasitoids: a 24-year study. Ecol Entomol 18: 365-378.

Redfern M, Hunter M. 2005. Time tells: long-term patterns in the population dynamics of the yew gall midge, *Taxomyia taxi* (Cecidomyiidae), over 35 years. Ecol Entomol 30: 86-95.

Royama T. 1996. A fundamental problem in key factor analysis. Ecology 77: 87-93.

Tung GS, Yang MM. 2018. Let's gall Taiwan – A guidebook of insect galls. Council of Agriculture Executive Yuan, Forest Bureau, Taipei. 248 pp.

Varley GC, Gradwell GR. 1960. Key factors in population studies. J Anim Ecol 29: 399-401.

Varley GC, Gradwell GR. 1968. Population models for the winter moth. pp 132-142. In: Southwood TRE (ed.). Insect Abundance. Blackwell, Oxford.

Yamamura, K. 1999. Key-factor/key-stage analysis for life table data. Ecology 80: 533-537.

Yamamura K. 2012. Extended key-factor/key-stage analysis for longitudinal data. J Biopharm Statics 22: 1-15.

Yukawa J. 2000. Synchronization of gallers with host plant phenology. Popul Ecol 42: 105-113.

Yukawa J, Rohfritsch O. 2005. Biology and ecology of gall-inducing Cecidomyiidae (Diptera). pp. 273-304. In: Raman A, Schaefer CW, Withers TM (eds.) Biology, Ecology, and Evolution of Gall-inducing Arthropods. Science Publishers, Inc. Enfield, New Hampshire, USA and Plymouth, UK.

Yukawa J, Akimoto K. 2006. Influence of synchronization between adult emergence and host plant phenology on the population density of *Pseudasphondylia neolitseae* (Diptera: Cecidomyiidae) inducing leaf galls on *Neolitseae sericea* (Lauraceae). Popul Ecol 48: 13-21.

Yukawa J, Takahashi K, Ohsaki N. 1976. Population behaviour of the neolitsea leaf gall midge, *Pseudasphondylia neolitseae* Yukawa (Diptera, Cecidomyiidae). Kontyû 44: 358-365.

Yukawa J, Haitsuka S, Miyaji K, Kamikado T. 2006. Influence of the population dynamics of a gall-inducing cecidomyiid and its parasitoids on the abundance of a successor, *Lasioptera yadokariae* (Diptera: Cecidomyiidae). pp 241-249. In: Ozaki K, Yukawa J, Ohgushi T, Price PW (eds.) Galling Arthropods and Their Associates: Ecology and Evolution. Springer-Verlag, Tokyo.

Yukawa J, Nakagawa K, Saigou T, Awa T, Fukuda T, Higashi M. 2013. Adult behavior of an ambrosia gall midge, *Illiciomyia yukawai* (Diptera: Cecidomyiidae) and synchronization between its emergence and host plant phenology. Entomol Sci 16: 400-412.

Yukawa J, Ichinose M, Wanggyu K, Uechi N, Gyotoku N, Fujii T. 2016a. Lower development threshold temperatures and thermal constants for four species of *Asphondylia* (Diptera: Cecidomyiidae) in Japan and their larval developmental delay caused by heat stress. Appl Entomol Zool 57: 71-80.

Yukawa J, Miyamoto K, Yamaguchi T, Takesaki K, Uechi N, Matsuo K. 2016b. Key factor/key stage analysis of long-term life table data for *Asphondylia sphaera* (Diptera: Cecidomyiidae) that induces fruit galls on *Ligustrum japonicum* (Oleaceae). Ecol Entomol 41: 516-526.

Yukawa J, Nakagawa K, Kohno A, Tokuda M, Kiritani K, Matsuo K, Mitsui H, Fujii T. 2016c. Geographical and annual variations in the proportion of extended diapausing individuals of *Illiciomyia yukawai* (Diptera: Cecidomyiidae), with reference to an adaptive significance of its bimodal emergence patterns. Entomol Sci 19: 275–289.

Yukawa J, Yamamura K, Fujimoto K, Tokuda M, Muroya S, Maki T, Sadoshima T, Fukuda T. 2018. Key-factors and key-stages that determine the leaf longevity of an evergreen broad-leaved tree, *Neolitsea sericea* (Lauraceae) at different growing sites in southern Japan. Ecol Res 33: 175-190.

蟲癭為生態學研究提供了良好的資訊-田野調查及數據分析方法

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摘　　要

蟲癭以易取得的方式提供我們多元的生態資訊，為了使蟲癭生態研究的便利性更加優化，在此我與其他研究同仁以實際的數據為基石，闡明獲得相關生態資訊的田野調查方法，包括成蟲活動、寄主植物的物候、生活史模式以及生命表。除了田野調查之外，隨機蟲癭解剖調查也十分重要。分析蟲癭研究的生命表數據，建議使用主導因子/主導時期（key-factor/key-stage）的分析方式來取代慣用的主導因子分析，並檢測作用於癭蚋族群的密度應變或非密度應變因子的作用力，進而去評估生態系中由上而下（top-down）或由下而上（bottom-up）的相對影響強度。

關鍵詞：癭蚋科、生命表、主導因子/主導時期分析、成蟲行為、致死因子