Production of winter eggs in *Schizotetranychus brevisetosus* (Acari: Tetranychidae) inhabiting evergreen Japanese blue oak

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Abstract

The overwintering pattern of parasitic herbivorous arthropods is closely related to host phenology, because defoliation imposes strong selection pressures on various developmental stages. This relationship has been well studied in populations of spider mites (Acari: Tetranychidae) on deciduous hosts, but is little studied in populations on evergreen hosts, probably because their leaves are always available. However, spring defoliation may also influence the life cycle. We studied the overwintering pattern of *Schizotetranychus brevisetosus* (Acari: Tetranychidae), a specialist on evergreen oak, *Quercus glauca*, in Kochi, Japan. Only adult females and their eggs (winter eggs) survived the coldest months. We also observed a conspicuous seasonal change in egg colour and size: December winter eggs were $1.7 \times$ larger than September summer eggs, suggesting the winter eggs are diapausing. Adult females produced summer eggs until November and winter eggs from late November until they disappeared in March. The winter eggs hatched in early March. The immature stages developed in mid- to late March, when 39% of old leaves fell, some of which carried hundreds of immature individuals. Therefore, even in evergreen hosts, defoliation places potentially strong selection pressure on the immature stages. Despite this, *S. brevisetosus* appears not to change its egg hatching season to avoid the defoliation season, so it may have evolved a behavioural adaptation to escape falling leaves. The existence of several species with similar lifestyles suggests the importance of host phenology and predation pressure for evolution of the overwintering pattern.

Introduction

Terrestrial herbivorous arthropods that are parasitic to certain host plants have developed two types of adaptation to temporally escape unfavourable conditions: abiotic ones to deal with low temperatures and desiccation, and biotic ones to

deal with a lack of food resources and high predation pressure (Tauber et al. 1986). In particular, temporal variation in host availability imposes strong selection pressure on the seasonal timing of development and reproduction. Diapause, which is usually induced at one specific life stage, plays an important role in controlling such seasonal timing, as is suggested by the fact that diapause intensity, which governs the seasons of diapause induction and termination (Cheng et al. 2019), varies considerably among species and populations with differing host availability (Pratt and Ballmer 1993; Kurota and Shimada 2002).

In Sapporo, Japan, the hatching date of the overwintering eggs of the nonmigratory galling aphid, *Kaltenbachiella japonica* (Matsumura), has become genetically differentiated among populations occurring on different individual trees, and correlates with the timing of budburst in those trees (Komatsu and Akimoto 1995). Of three sawfly species in Kyoto, all of which feed on cruciferous plants, only *Athalia japonica* enters summer diapause. This is because its larvae feed chiefly on the new leaves of wild cardamine plants, which sprout only in spring and autumn. The other two species feed mainly on the leaves of cultivated plants that are available year-round (Nagasaka 1992). Thus, summer diapause in *A. japonica* may be an adaptation to the unavailability of their primary host.

The relationships between host phenology and the seasonal timing of development and reproduction in parasitic herbivorous arthropods have been studied chiefly in species inhabiting deciduous hosts, because seasonal changes in host availability can be easily recognised based on defoliation and leaf extension. In contrast, such changes are less conspicuous for many evergreen plants, on which some of the leaves that have grown in the previous years (hereafter *old leaves*) fall in the spring while new leaves are opening, so there is no obvious gap in host availability. This leaf replacement may have little effect on the lifecycle of ~~herbivorous~~ arthropods with a high dispersal ability, but may have a large effect on that of species with limited ability to escape from falling leaves.

Spider mites (Acari: Tetranychidae) are suitable subjects for clarifying the association between the seasonal activity of herbivorous arthropods and host phenology. All stages feed on host leaves and have limited dispersal ability, so their lifestyles depend on the availability of host leaves. Individuals can overwinter, though not necessarily in diapause, in either the egg or the adult female stage (Veerman 1985). In most species it is either one or the other, but in a few both stages can overwinter (Gotoh 1986b; Yanagida 1999). These overwintering stages appear to be loosely constrained by phylogeny. The *Panonychus*, *Oligonychus* and *Schizotetranychus* species overwinter as eggs, whereas *Tetranychus*, *Eotetranychus*,

Oligonychus and *Stigmaeopsis* species overwinter as adult females (Saito 2010). The overwintering stage of each genus is purportedly associated with host phenology (Gutierrez and Helle 1985; Saito 2010), ~~but~~though several genera have been shown to be polyphyletic (Sakagami 2002; Matsuda et al. 2014, 2018). In addition, overwintering stages vary within the same genus (Takafuji et al. 1991; Takafuji and Morishita 2001), suggesting that this proposition requires scrutiny.

Adaptation to evergreen hosts seems to relax the constraint of having a single overwintering stage. Overwintering as both eggs and adult females occurs in *Yezonychus sapporensis* Ehara (Yanagida 1999; Saito 2010) and *Sasanychus akitanus* (Ehara) (Gotoh 1986a, b) on evergreen *Sasa* bamboos. Such an overwintering style is considered to be a transient evolutionary state from egg to adult diapause or vice versa (Gotoh 1986b). In addition, *Schizotetranychus shii* Ehara, which occurs on Japanese chinquapin *Castanopsis sieboldii* in temperate areas, is considered to have secondarily lost its diapause capacity, since various stages survive through the winter (Ehara and Gotoh 2009). However, the impact of vernal defoliation and leaf extension on the lifecycle has not been evaluated in any of these species, and the relationship between evergreen hosts and the relaxation of spider-mite lifecycles is yet to be investigated.

In the present study, we focused on *Schizotetranychus brevisetosus* Ehara (Acari: Tetranychidae). This species, which is thought to display communal sociality (Y. Saito, pers. comm.), is found in Japan and is specialised on the evergreen oak, *Quercus glauca* L. (Fagaceae; Ehara 1989; Ehara et al. 2009). In this species, some of the old leaves fall in the spring while the new leaves are growing (Yukawa and Tsuda 1986). All stages of the mite live gregariously in silk-web nests constructed in the depressions near leaf veins, inside which they feed and reproduce. Female development time at 25 °C under a 16L:8D photoperiod is rather long (mean $\pm$ SD $= 22.6 \pm 3.1$ days, $n = 22$), but their fecundity is low (13.7 ± 5.9, $n = 37$), resulting in a very low intrinsic rate of natural increase (0.060; Tamura and Ito 2017). With respect to behaviour, adult males display harsh lethal aggression towards other males during copulation with unmated females, and sometimes create a harem (Masuda et al. 2015), so there are far fewer males than females.

All stages are present from spring to autumn, during which time the adult females lay white or yellow eggs (hereafter summer eggs). In winter they only produce bright orange eggs (winter eggs), and only these eggs and the adult females are present in this season (Saito 2010; Ito and Hamada 2018). However, details regarding stage structure throughout the year and the relationships between the timing of winter-egg production and *Q. glauca* phenology are unknown. However, these

points are important to know the evolutionary significance of laying the two forms of eggs in different seasons in several species on evergreen hosts.

The present study principally aims to clarify the association between host phenology and seasonal patterns of winter-egg production and immature development (Gotoh 1986a) in *S. brevisetosus*. We predicted that the winter eggs develop to adulthood, which affords a high dispersal ability, before the onset of defoliation. We first determined the morphological differences between summer and winter eggs in this species, and then observed seasonal transitions in the stage structure over two years, to better understand the life cycle of *S. brevisetosus*. We also investigated the abundance of each stage on old and new leaves in the spring, to investigate whether immature individuals avoid the risk of defoliation. Based on these results, we summarise the characteristics of winter egg production in *S. brevisetosus* in terms of host phenology with reference to other mite species that overwinter in multiple stages.

Materials and methods

Morphology of summer and winter eggs

The initial study site was located in the Kochi Prefectural Forestry Technology Research Centre in O'hira, Tosayamada, Kami-city, Kochi Prefecture, Japan (33.633°N, 133.708°E, WGS84, 96 m asl; Geospatial Information Authority of Japan 2018).

Thirty summer eggs were sampled from a *Q. glauca* leaf on 25 September 2017, and the same number of winter eggs were similarly sampled on 6 December (10 eggs) and 20 December (20 eggs) of the same year. Each egg was placed on a glass slide using a fine brush. Because the surface of the eggs is sparsely covered with adhesive threads, the eggs could be fixed on the glass slide without additional materials. The top and side views were photographed (× 200) using a DP20 microscope camera (Olympus, Tokyo, Japan) attached to a differential interference microscope BX50 (Olympus). For calibration during the image analysis, an objective micrometer (1 mm/100 div., Olympus) was also photographed at the same magnification.

Eggs were close to an oblate spheroid in shape, and the top of some rose slightly to form a small stipe. Egg images were analysed using the software Image J v.1.50i (Schneider et al. 2012). First, the measurement scale was calibrated using a

straight line drawn on the micrometer scale. To measure egg height, an ellipse was fitted to the outer surface of the side-view image excluding the egg stipe, and the length of the minor axis was measured. To measure egg width, a circle was fitted to the outer surface of the top-view image of the egg, and the diameter was measured. Assuming that the egg shape is an oblate spheroid, the volume of each egg was approximated as:

$$V = \frac{4\pi}{3}a^2c,$$

where a and c are the equatorial radius (width/2) and the polar radius (height/2), respectively (Weisstein 2019). In addition, the presence or absence of the stipe was also recorded for each egg.

Data for winter eggs collected in December were pooled into a group for analysis. The mean height, width and volume of the two types of eggs were compared using Welch's t tests. The coefficient of variation (the ratio of the standard deviation to the mean) was estimated for each size parameter to quantify and compare their variability. The percentages of eggs with a stipe was compared between months using χ^2 tests with Yates continuity corrections. All statistical tests, including those outlined below, were conducted on R v.3.5.1 (R Core Team 2018).

Stage occurrence

Three to 26 infested leaves, i.e., those carrying at least one individual, were sampled at the study site from 6 October 2014 until 19 December 2016, once per month until February 2016, and twice per month thereafter. The numbers of sampled shoots and plants could not be fixed, because population sizes and distributions varied over sampling dates. These leaves were individually packed in plastic bags (140 × 100 mm, GP E-4; Seisannipponsha, Tokyo, Japan) and placed in a biomedical freezer (− 30 °C, MDF-236; Sanyo, Osaka, Japan) within 24 h of collection, and they were stored there for several days for fixation.

The individuals of each stage on each leaf were counted using a stereomicroscope (Olympus SZX7). Stages were classified into the following categories: eggs, immature stages not including female quiescent deutonymphs, female quiescent

deutonymphs, adult males, and adult females. The quiescent deutonymphs were counted separately to precisely identify the season when immature stages successfully developed to adulthood.

The proportion of individuals at each stage to the total number of individuals on all leaves was calculated for each date. In addition, the number of individuals at each stage was averaged over all leaves, and this value was called the density. Note that as only infested leaves were sampled, the statistical population is the total number of infested leaves on the trees. Because *S. brevisetosus* tends to exhibit a heavily clumped distribution on the leaves, and the population size often decreases dramatically as a result of predation (< 0.001 individuals per leaf; K. Ito, unpubl. data), numerous samples are required to estimate the density across all leaves (Eq. 9 in Iwao and Kuno 1968).

Seasonal changes in the proportion and density of each stage were investigated based on the above estimates for each date, and periods of summer- and winter-egg production were identified. The data for 7 June 2015 were excluded because there were few samples due to bad weather.

Stage density on old and new leaves

In February 2018, the study site was changed to *Q. glauca* shrubs in Sakagawa, Tosayamada, 3.8 km from the original study site (33.611°N, 133.738°E, 90 m asl; Geospatial Information Authority of Japan 2018), because the previous site became unavailable due to construction.

On 23 February 2018, numbered paper tags were tied with thin wires to nine branches with 4–13 old leaves each (80 leaves in total). Each of these branches had at least one leaf that was infested by *S. brevisetosus* individuals. The leaves were surveyed seven times7 ×, from 2 March to 9 May, at an interval of 8–20 days (mean ± SD = 11.3 ± 4.6). On each date, the old and new leaves on each branch were counted, and the individuals of each stage on these leaves were counted on site, using a magnifying glass (14 ×, NKL-14; Winner, Chiba, Japan). The classification of stages was done in the same way as in the previous survey, except that quiescent deutonymphs were pooled with the immature stages because it was difficult to distinguish them from other stages using only a magnifying glass.

The number of intact leaves and the proportion of infested leaves to old or new leaves were investigated for each date. The mean density of each stage on the leaves was calculated for each date. The timing of emigration from old leaves was

inferred from the changes in the density of each stage on the leaves. In addition, the proportion of infested old leaves that had fallen by the next survey, and the density of each stage on these leaves, was investigated to infer the impact of defoliation on the survival of each stage.

Results

Morphology of summer and winter eggs

Egg size and colour are summarised in Table 1. Summer eggs were white or yellow and easy to collapse by rough manipulation with a fine brush, whereas winter eggs were bright orange and hard to break. The winter eggs were significantly larger than the summer eggs in terms of diameter, height, and quotient (Table 1). Winter eggs were also 1.7 × larger than summer eggs in volume. The coefficient of variation was very low for all items (1.9–4.3% for summer eggs and 2.4–6.6% for winter eggs), confirming that the sizes of the two types of egg were distinct. The proportion of winter eggs with an egg stipe was smaller than that of summer eggs ($\chi^2 = 5.056$, df = 1, $P = 0.025$).

Table 1

Differences in colour and size between summer eggs of *Schizotetranychus brevisetosus* collected in September and winter eggs collected in December 2017

Trait	Summer eggs	CV (%)	Winter eggs	CV (%)	*P*
Colour	White or yellow		Bright orange		
Egg surface	Fragile		Robust		
Diameter (μm)	150.6 ± 2.9	1.9	169.9 ± 4.1	2.4	< 0.001[a]

Means ± SD (n = 30) and the coefficient of variation (CV = SD/mean) (%) are shown
[a] Welch's *t* test
[b] χ^2 test with Yates continuity correction

Trait	Summer eggs	CV (%)	Winter eggs	CV (%)	P
Height (µm)	104.1 ± 3.2	3.0	138.2 ± 7.4	5.4	< 0.001[a]
Height/diameter	0.69 ± 0.03	3.9	0.81 ± 0.05	6.3	< 0.001[a]
Volume (μm^3; $\times 10^4$)	~~124~~123.5 ± 5.3	4.3	209.0 ± ~~14~~13.7	6.6	< 0.001[a]
Frequency of egg stipe (%)	60.0		33.3		0.025[b]

Means ± SD (n = 30) and the coefficient of variation (CV = SD/mean) (%) are shown

[a] Welch's t test

[b] χ^2 test with Yates continuity correction

Stage occurrence

The proportion and density of each stage is illustrated in Figs. 1 and 2, respectively. As shown below, the switching of egg type occurred in May and November, and both types of eggs seldom occurred in the same period.

On 6 October 2014, the percentage of summer eggs of the total number of individuals was 64% (n = 2140; Fig. 1) and the density was 75.6 ± 118.5 (mean ± SD, 18 leaves; Fig. 2). These values declined steeply to 2% (n = 572) and 1.6 ± 2.5 (eight leaves) on 12 November, and thereafter there were no summer eggs. In contrast, the percentage and density of adult females rose to 52% and 36.9 ± 43.5, respectively. In November, immature stages and quiescent deutonymphs still comprised 38% (26.9 ± 42.1) and 7% (5.0 ± 7.8) of the population, respectively, but the proportion and density of these stages dropped in December.

Winter eggs appeared on early December (52%, n = 1181) at a density of 43.5 ± 42.7 (14 leaves). The total number of eggs and adult females (38%) accounted for 90% of the total number of individuals. The percentage and density of winter eggs increased through the winter and peaked on mid- to late February 2015 (90.9%, n = 4069; 528.6 ± 281.1, seven leaves), when only eggs and adults were found. Adult females were found until 18 March.

The percentage and density of winter eggs decreased to 5% ($n = 1071$) and 14.3 ± 21.4 (four leaves) on 14 April 2015, respectively, and immature stages increased to 94% and 252.8 ± 342.6. Adult females emerged on 10 May 2015 (3%, $n = 3111$; 16.6 ± 9.6, five leaves), and the percentage and density of summer eggs reached 75% and 466.6 ± 519.5. The overall density then decreased towards July (Fig. 2), as a result of predation by species such as rove beetles *Holobus kashmiricus beneficus* and predatory mites *Anystis baccarum* (data not shown). The percentage of summer eggs fluctuated in the 16–59% range during the period from 13 July to 4 October ($n = 147$–1857), and all stages were found during this period. After a small peak in the percentage and density of summer eggs on 4 October 2015 (39%, $n = 563$; 36.2 ± 36.9, six leaves), they declined towards December. However, the percentage and density of summer eggs was lower on 4 November (0.3%, $n = 649$; 0.40 ± 0.89, five leaves) though adult females survived (Figs. 1, 2). This finding suggests that the majority of eggs found in October had already hatched, and adult females did not oviposit from October to November. Immature stages (67%) and quiescent deutonymphs (11%) comprised the largest proportion. No summer eggs were found after December.

Similar seasonal trends regarding stage structure was found in the next year (Figs. 1, 2). Winter eggs first appeared on 4 December 2015 (20%, $n = 264$; 8.0 ± 12.3, six leaves), with a high percentage of adult females (71%, 29.2 ± 50.5). The percentage of eggs peaked on 7 March 2016 (97%, $n = 1452$), and their density peaked on 22 March (390.4 ± 370.1, five leaves). The percentage and density of winter eggs dropped to 9% ($n = 676$) and 5.5 ± 8.8 (11 leaves) on 5 April, respectively. By that stage, the immature stages that had developed from the winter eggs comprised 91%, with a density of 56.0 ± 80.6 (11 leaves). The first adult females had emerged by 20 April (70%, $n = 247$; 15.7 ± 14.4, 11 leaves), and summer eggs appeared on 3 May, with a percentage and density of 93% and 280.7 ± 177.3 (11 leaves), respectively. As a result of reduction in the population size via predation after May, the percentage of summer eggs fluctuated within the 10–81% range, and all stages were found from 20 May to 18 October ($n = 35$–4158), except for adult males and quiescent deutonymphs, which were not found on a few of the dates. The percentage of eggs peaked on 18 October (81%, $n = 2188$), and declined towards 6 December (1.4%, $n = 141$). On 6 December, the sum of adult females (64%), immature stages (20%), and quiescent deutonymphs (6%) comprised the largest proportion of the population, and the first winter eggs had appeared (4%). By 19 December, the percentage and density of winter eggs had reached 62% ($n = 81$) and 16.7 ± 9.9 (three leaves), respectively.

Stage density on old and new leaves

Seasonal changes in the proportion of old and new leaves are illustrated in Fig. 3a. All tagged branches except for the lowest one sprouted new leaves. The percentage of old leaves found on 2 March 2018 had decreased to 91% on 22 March, 75% on 1 April, and 63% on 10 April. Defoliation then slowed, and 61% of leaves remained by 9 May. New shoots were elongated during the period from 22 March to 1 April (data not shown). Most of the new leaves had expanded on 10 April, but they were covered with dense hairs, so the mites were unable to settle on them. After the leaf hairs had decreased, the first colonies of mites were found on 18 April. The total number of new leaves did not change thereafter, except for a few leaves that were heavily damaged by herbivorous insects.

Changes in the stage structure and proportion of individuals are shown in Fig. 3b. The density of winter eggs decreased steeply from 108.8 ± 121.6 (mean $\pm$ SD; 13 leaves) on 2 March to 28.3 ± 42.9 (13 leaves) on 22 March, whereas the density of immature stages rose from 0 to 32.2 ± 46.9. The density of immature stages peaked on 1 April. Adults emerged on 10 April (18.5 ± 43.9, 16 leaves), when the egg density dropped to 2.8 ± 8.0 (16 leaves). The first individuals were found on new leaves on 18 April. The percentage of individuals on the old leaves fell to 24% on 2 May ($n = 819$).

Figure 4 shows the change in the density of each stage on old and new leaves separately. On April 18, adult females (3.8 ± 5.3), their eggs (3.3 ± 3.9), and only a few individuals in the immature stages (0.3 ± 0.6) that had dispersed from the old leaves, appeared on 11 new leaves. The densities of each stage on the old and new leaves were similar after 26 April.

During the defoliation period, 4 out of 13 (30.8%) infested leaves surveyed on 22 March had been lost by 1 April, and 3 out of 13 (23.1%) leaves surveyed on 1 April had been lost by 10 April. No other infested leaves were lost during the study period. The number of individuals on the lost leaves was 38.4% of the total number of individuals on 22 March ($n = 790$) and 27.9% of those on 1 April ($n = 803$). Percentages of females, immature stages, and winter eggs on the lost leaves were 5.9, 37.3, and 56.8% ($n = 303$) on 22 March, and 0.4, 94.2, and 5.4% ($n = 224$) on 1 April.

Discussion

Host phenology and seasonal patterns of herbivorous arthropods are considered to be linked, but the evidence of the link is still insufficient. Using *S. brevisetosus* as the study subject, we tried to test the association based on the field surveys of

seasonal transitions in the stage structure, and to clarify the characteristics of winter egg production in terms of host phenology.

We observed an overwintering pattern in *S. brevisetosus* that has not been previously recorded in the Tetranychidae. In most egg-diapausing species, which occur chiefly on deciduous hosts, for example *P. ulmi* on apple trees (*Malus* spp.) and *P. mori* on mulberry trees (*Morus* spp.), adult females deposit diapausing eggs in the autumn, finish oviposition and die immediately before the onset of the winter, and only these eggs survive the winter (Lees 1953; Takafuji and Morimoto 1983). However, the adult females of *S. brevisetosus* remain on the leaves without oviposition during most of November, and only begin to produce winter eggs in late November or early December, under short day-length conditions (approximately 10L:14D, Kochi City, Japan Meteorological Agency 2018; Figs. 1, 2). Females survive the coldest month (January) on host leaves, and probably continue producing winter eggs during the winter, as suggested by the fact that the density of these eggs was highest in February/March, without an obvious increase in the female density (Fig. 2). The adult females may then die before the end of winter, since they had disappeared by March. It is possible that the winter eggs are in diapause, because they are morphologically distinct from those laid in the summer; they are approximately 70% larger than summer eggs in volume, bright orange in colour, tough, and tend to lose the egg stipe. The size of individual eggs was not found to vary within each egg type (low coefficients of variation, Table 1), so these eggs may represent distinct physiological states. Egg morphology may reflect the process of oogenesis and oviposition (Feiertag-Koppen and Pijnacker 1985), and a larger size of winter eggs is likely to be caused by a higher yolk content, but the function of the egg stipe, and the reason why only a fraction of eggs have them (Table 1) remains to be elucidated. In addition, environmental cues for diapause termination should be clarified to know how winter eggs adjust their development to leaf opening.

The lifecycle of *S. brevisetosus* appears to be similar to those of *P. akitanus* (Gotoh 1986a, b) and *Y. sapporensis* (Yanagida 1999), which live on evergreen *Sasa* bamboos in Hokkaido Prefecture, northern Japan, in that two stages survive on host leaves in winter. However, in these species large proportions of both females and eggs survive the cold winter. Particularly in *P. akitanus*, both winter eggs and eggs from the overwintering females develop during the same season, and different generations interbreed in spring (Gotoh 1986a). Therefore, we consider that *S. brevisetosus* is not a species that truly overwinters in two stages like these *Sasa*-inhabiting species, but a novel egg-diapausing species in which females that deposit eggs survive very late into the winter. Lifestyles like those of *S. brevisetosus* could be achieved only on evergreen hosts under benign temperate conditions, which allow winter reproduction. The overwintering patterns of spider mites are

thus variable even among species that use evergreen hosts, and we have to clarify which biotic or abiotic factors affect overwintering stages by empirical and theoretical studies as conducted in a few insects (e.g. bruchid beetles, Kurota 2001; Kurota and Shimada 2002).

Host phenology may potentially impose strong selection pressures on the activity at each stage, but we could not identify a direct association between them. Although winter eggs must hatch at the beginning of March, before the start of defoliation, immature stages develop on old leaves during the period from mid-March to early April, when 39% of leaves fell in Sakagawa (Figs. 3, 4). We could not precisely estimate the emigration rate of immature stages to suitable, but 23–31% of old leaves fell in late March and early April, and these carried 28–38% of the total population. Notably, immobile eggs comprised 57% of the individuals present in March. These results may run counter to intuition, which might predict that the overwintering generation should complete development before the season of heavy defoliation to reduce the risk to stages with low mobility. Perhaps older nymphs have a strong ability to migrate to good leaves, as is suggested by the spring stage movement of *P. akitanus* (Gotoh 1986a). *Schizotetranychus brevisetosus* females frequently leave their eggs behind in their old nests, unlike *Stigmaeopsis* species (Mori and Saito 2005; Yano et al. 2011; Saito and Zhang 2017), and move by themselves—actually, we observed that older nymphs moved as a group to the young leaves near the tip of the branch. Thus, the immature stages may have evolved behavioural adaptations to escape seasonal defoliation. Another, not exclusive, possibility is that adult females deposit winter eggs of different diapause intensity on multiple leaves as a bet-hedging strategy. This may be the case if the females cannot assess the quality of leaves where offspring will grow in future, or if climatic conditions, and thus the phenology of *Q. glauca*, yearly fluctuate. This hypothesis should be tested in future studies.

The selection pressures on stage structure in winter are both biotic and abiotic. For abiotic factors, cold hardiness is a major factor affecting stage survival as numerous studies have revealed, but responses to temperature vary among species or populations (Lees 1953; Veerman 1985). In a Kochi population of *St. longus*, which inhabits evergreen bamboo leaves, eggs and quiescent individuals were highly vulnerable to low temperature (4 °C) but active stages were cold hardy (Ito and Chae 2018). In the present study, immature individuals were rare in winter and this rarity might be attributed to low temperature, but the influence of mild winter conditions on stage survival requires further elucidation. For biotic factors, low predator activity in the winter may be a large factor promoting winter oviposition in these species. In *P. akitanus*, the number of their primary predators, phytoseiid mites, declined with decreases in the number of *P. akitanus* individuals, and

almost disappeared in winter (Gotoh 1986a). In *Y. sapporensis*, females deposit eggs on the tip of host leaf hairs from June to September, and this habit improves the survival rate of eggs against predation by various predators such as the egg-eating *Agistemus summersi* (Stigmaeidae) (Yanagida et al. 2001). However, in the winter, when predator density decreases, females deposit eggs directly on leaf surfaces (Yanagida 1999). For *S. brevisetosus*, predation may also be an important factor affecting oviposition in the winter. The main predator of *S. brevisetosus* is the rove beetle *H. k. beneficus*, which predates numerous eggs (Shimoda et al. 1993; Kishimoto and Adachi 2008) and is active until the beginning of winter, but scarce in January and February (K. Ito in prep.). However, the density of *H. k. beneficus* in *S. brevisetosus* habitats increases after late April. It is likely that the adults of *H. k. beneficus*, which mature in *Tetranychus* spp. habitats on nearby weeds, and immigrate to the *S. brevisetosus* habitat at that time (K. Ito, unpubl. data). Thus, low predation pressure may account for egg production in the winter, but this is a chicken-and-egg situation: the predators may be less abundant because their prey is less abundant, or the spider-mite females may lay eggs then because their predators are less abundant. At present, this problem is speculative, but previous research and the present study findings strongly suggest that both host phenology and predation pressure are involved in interspecific variation in overwintering patterns.

Surviving the coldest months as both adults and eggs represents a missing link between egg diapause and adult diapause (Gotoh 1986b), and such lifestyles are restricted to only a few species that live on evergreen hosts. The life cycle of *S. brevisetosus* observed here suggests that overwintering patterns vary among species and may provide a clue to aid our understanding of the evolution of overwintering stages. Overwintering in multiple stages is rarely reported, even among other arthropods (Tauber et al. 1986), so spider mites may be a useful model organism to clarify the evolutionary pathway of the overwintering stages. Although we have focused on host defoliation, the success of winter egg development and future reproduction may also be reduced by untimely hatching and development (Ito and Chae 2018) or a reduction in the reproductive ability of the females as a result of the consumption of metabolic reserves while overwintering, in other words, the cost of diapause (Ishihara and Shimada 1995; Kroon and Veenendaal 1998; Ito 2004, 2007, 2011; Sadakiyo and Ishihara 2012). The costs and benefits of overwintering as multiple stages are complex, and these factors should be elucidated to understand the process of adaptation on evergreen hosts.

Fig. 1

Change in stage structure of *Schizotetranychus brevisetosus* on the leaves of *Quercus glauca* from 2014 to 2016. Sampling occurred once a month until February 2016 and twice thereafter. The bars above the graph indicate March and April, when old leaves (of previous years) fell and new leaves extended. Note that the majority of summer eggs (indicated in white) emerged after leaf extension
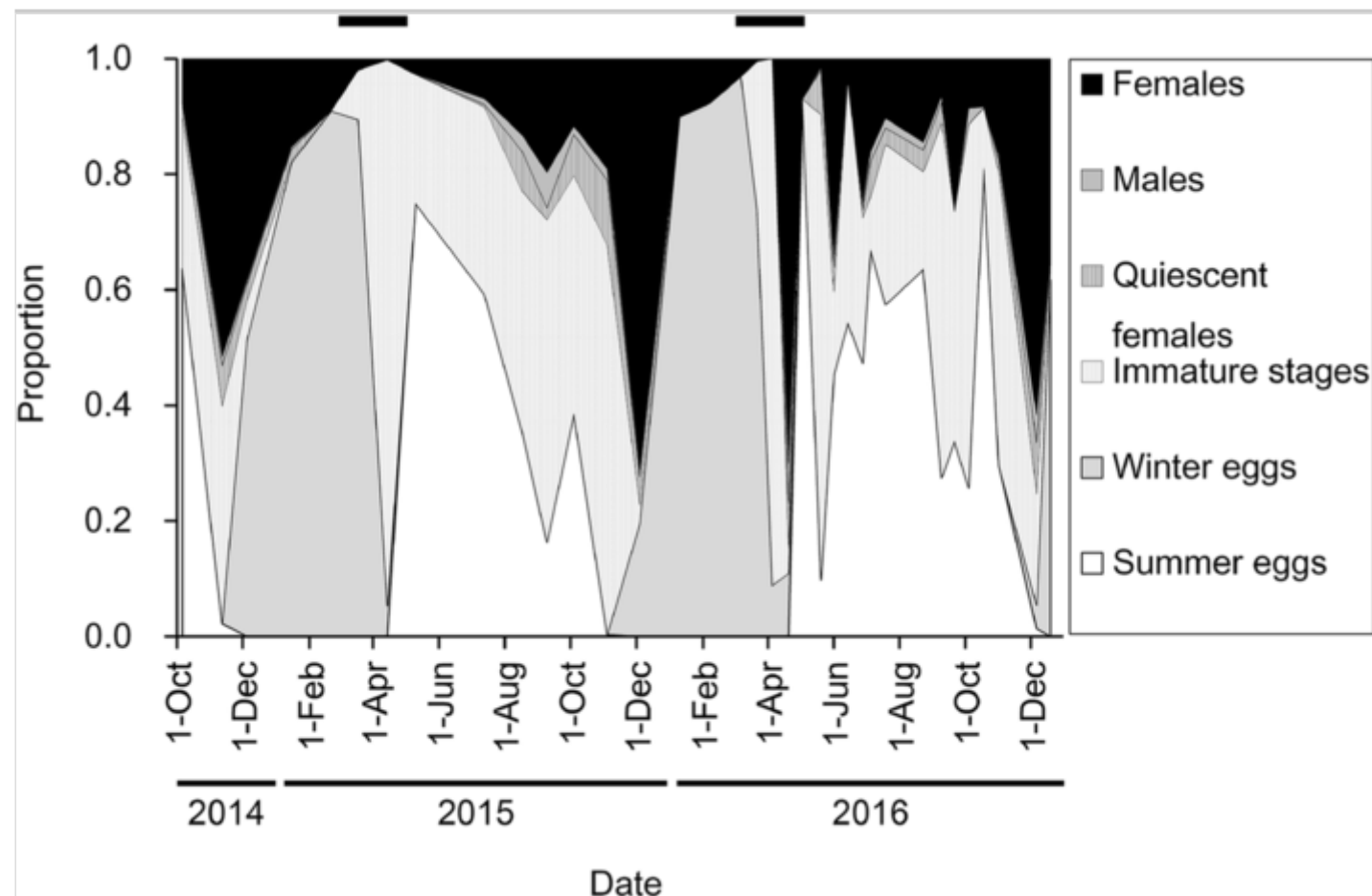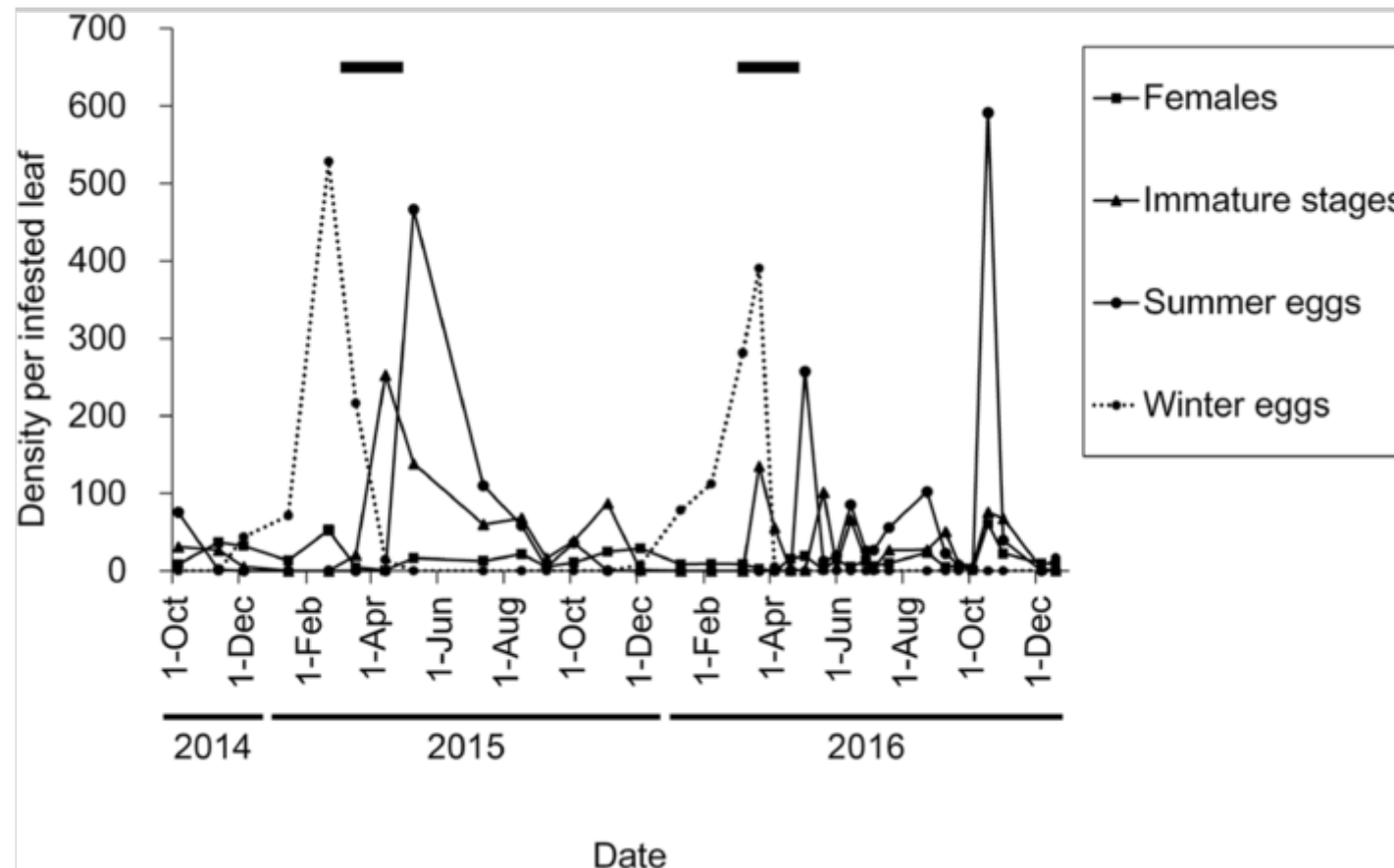

Fig. 2

Change in the density of *Schizotetranychus brevisetosus* individuals of each stage per infested leaf of *Quercus glauca* from 2014 to 2016. Data for adult males and quiescent deutonymphs, which always occurred at low densities, were omitted for simplicity. The bars above the graph indicate March and April, when defoliation and leaf extension occurred
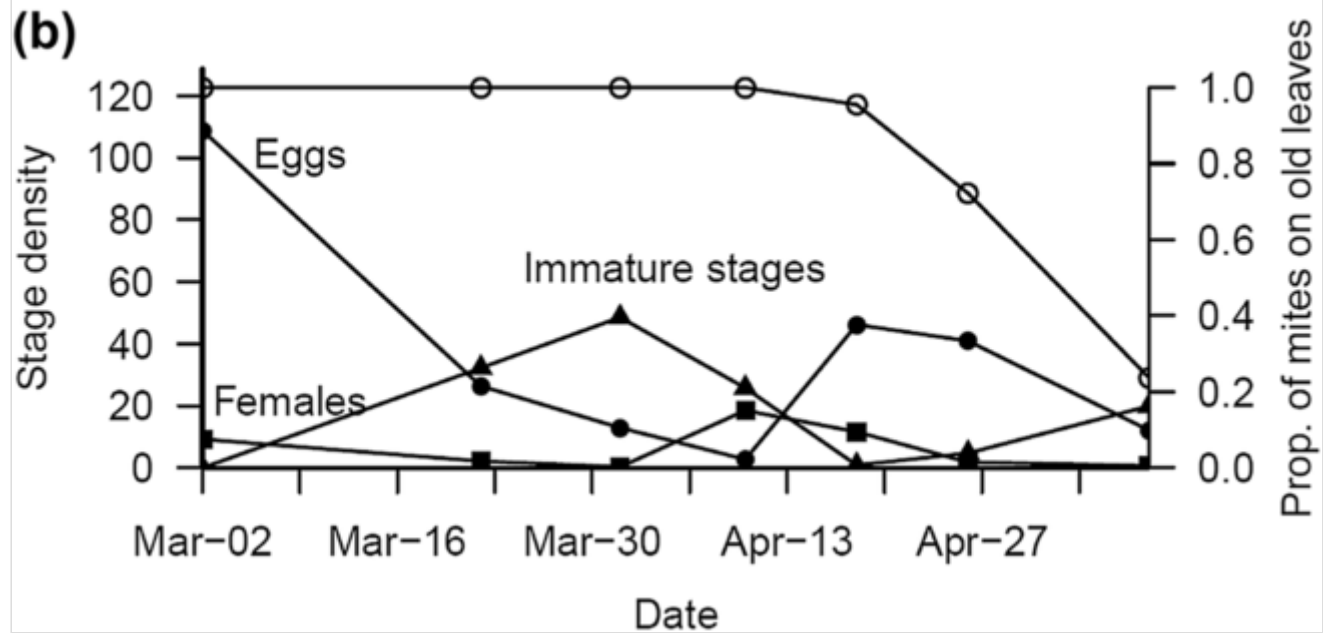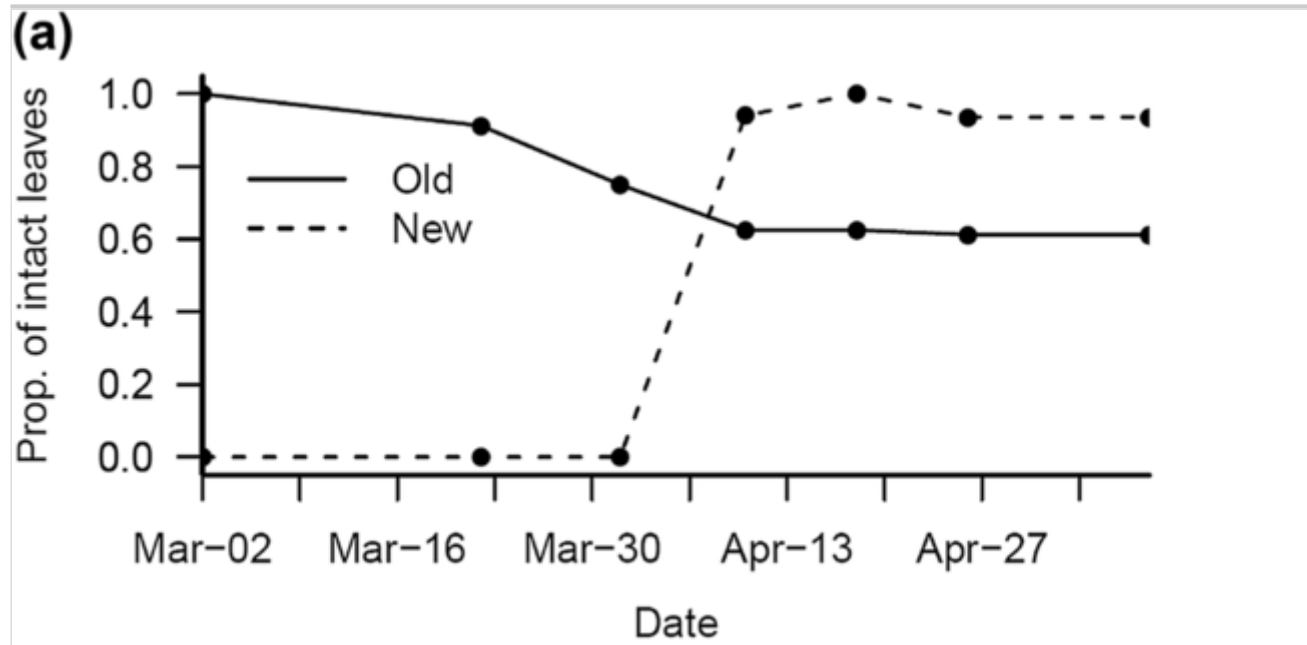

Fig. 3

a Proportions of old and new *Quercus glauca* leaves of the total from March to May 2018 in Sakagawa. The proportion of new leaves did not increase monotonically due to insect herbivory. **b** Average number of *Schizotetranychus brevisetosus*
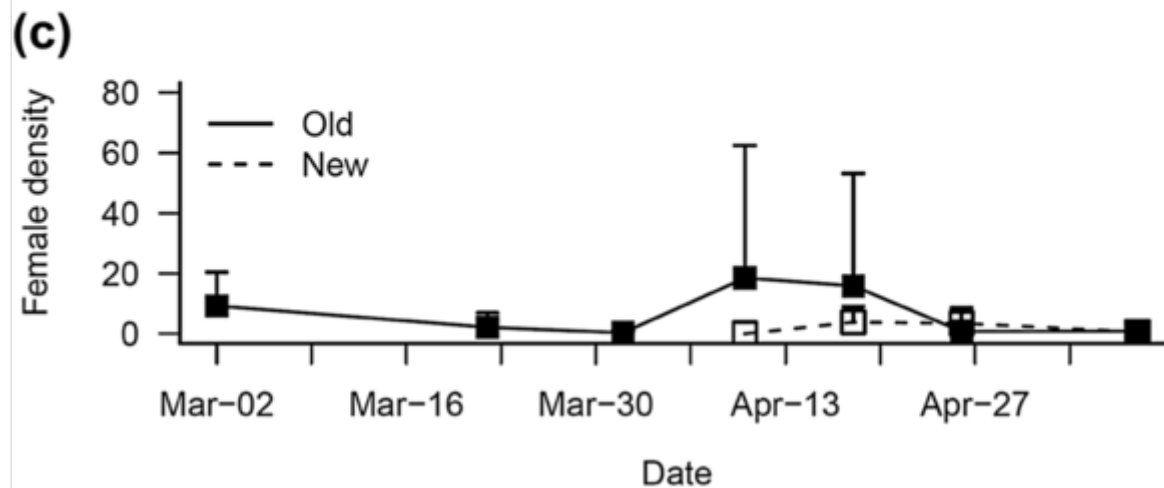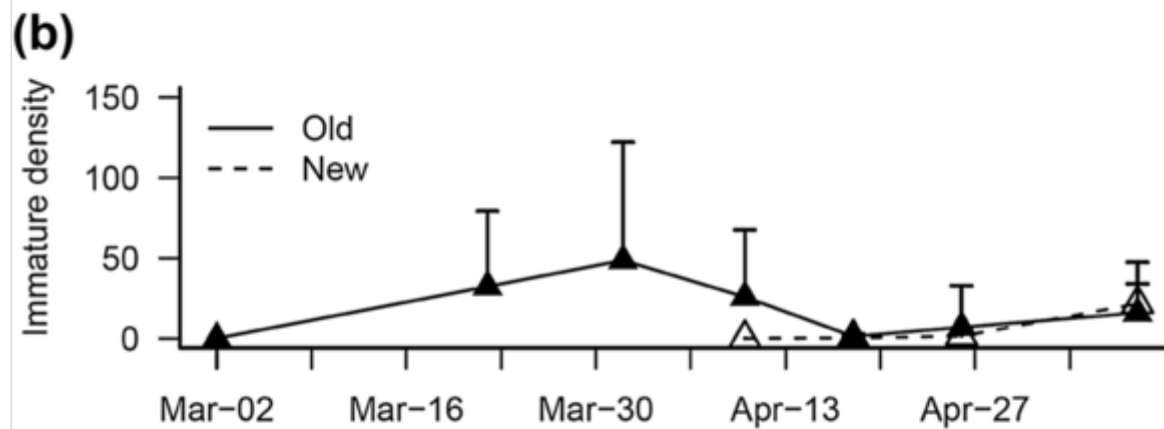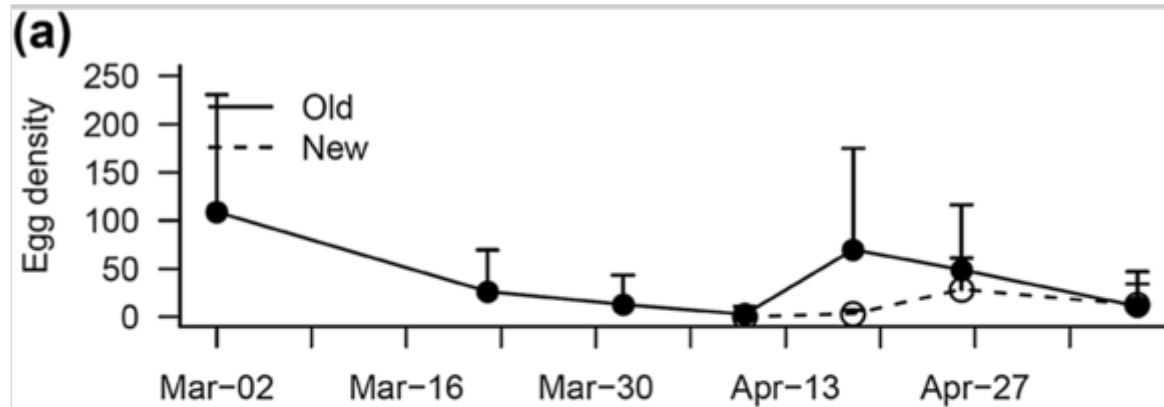
individuals at each stage per infested leaf (mites on old and new leaves are not separated). Data for adult males were omitted due to low density. The right axis indicates the proportion of individuals of all stages staying on old leaves (open circles)

(a)

Y-axis: Prop. of intact leaves (0.0, 0.2, 0.4, 0.6, 0.8, 1.0)

Legend:
— Old
-- New

X-axis: Date (Mar-02, Mar-16, Mar-30, Apr-13, Apr-27)

(b)

Left Y-axis: Stage density (0, 20, 40, 60, 80, 100, 120)

Right Y-axis: Prop. of mites on old leaves (0.0, 0.2, 0.4, 0.6, 0.8, 1.0)

Labels: Eggs, Immature stages, Females

X-axis: Date (Mar-02, Mar-16, Mar-30, Apr-13, Apr-27)

Fig. 4

Mean density of *Schizotetranychus brevisetosus* individuals of each stage on infested *Quercus glauca* leaves in 2018 at Sakagawa. **a** Eggs, **b** larvae and nymphs (including quiescent stages), and **c** adult females. The density on old and new leaves is indicated in filled and open symbols (corresponding to Fig. 3), respectively. Note the differences in the scales of the *y*-axes. Males are not shown since they were only present at very low densities

(a)

Egg density

- Old
- New

Mar–02 · Mar–16 · Mar–30 · Apr–13 · Apr–27

(b)

Immature density

- Old
- New

Mar–02 · Mar–16 · Mar–30 · Apr–13 · Apr–27

(c)

Female density

- Old
- New

Mar–02 · Mar–16 · Mar–30 · Apr–13 · Apr–27

Date

Acknowledgements

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