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Predicting the Start of the *Aedes albopictus* (Diptera: Culicidae) Female Adult Biting Season Using the Spring Temperature in Japan

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Abstract

Aedes albopictus (Skuse) (Diptera: Culicidae) is distributed widely and is common in much of Japan. In Japan, female adults begin to bite in between April and June, except in the southern subtropics where the mosquito has no dormant period. It is difficult to estimate the first *Ae. albopictus* biting day because it varies annually depending on the location. Over several years, we surveyed the mosquitoes at different locations that covered a range of warmer to cooler areas of Japan. We found an association between the timing of first biting day by *Ae. albopictus* and spring temperature. In spring months, the strongest correlation was found with mean April temperatures, followed by March. Based on these data, it may, therefore, be possible to apply a simple formula to predict the timing of the first biting day at various geographical locations in Japan. Forecasting maps were created using a simple prediction formula. We found that the first biting day for *Ae. albopictus* changed depending on early spring temperatures for each year. There was an approximate 20-d difference in first biting day between years with warmer and cooler springs. This prediction model will provide useful insight for planning and practice of *Ae. albopictus* control programs, targeting larvae and adults, in temperate regions globally.

Key words: spring temperature, first female adult biting day, forecasting maps

Aedes albopictus (Skuse) (Diptera: Culicidae), or the Asian tiger mosquito, is one of the most invasive vector mosquito species globally. *Aedes albopictus* bites are both a nuisance and a potential transmitter of arboviruses, such as dengue, Chikungunya, Zika, and dog heartworm (*Dirofilaria immitis*). Local transmission of dengue in Japan was first recorded during the 1940s (Hotta 1998) with no local transmission occurring since 1945. Between 1999 and 2010, 868 imported cases of dengue were reported in Japan with no local transmission (Takasaki 2011). In 2014, Japan experienced its first dengue outbreak after 70 yr in the Tokyo Metropolis, consisting of 160 locally transmitted cases between August and October, transmitted exclusively by *Ae. albopictus* (Kutsuna et al. 2015, Tsuda et al. 2016).

Ae. albopictus was previously limited to areas such as Southeast Asia and the islands of the Western Pacific and the Indian Ocean. Over the past 30–40 yr, the species has spread globally to areas that include North, Central, and South America, parts of Africa, northern Australia, and several countries in Europe (Moore 1999, Benedict et al. 2007, Paupy et al. 2009, Roiz et al. 2011). *Ae. albopictus* is a typical 'container mosquito' and prefers artificial environments (Hawley et al. 1987). In Japan, it can be found in various habitats, including, but not limited to, flower vases and wash basins in shrines, the graveyards of Buddhist temples, used tires, used cans, plastic containers, buckets, and bamboo stumps (Kobayashi et al. 2002). Rainwater drainage pits, which are scattered around public roads, parks, public spaces, and private land, are an important larval habitat in urban areas. These pits maintain a suitable depth of water throughout much of the year, even in the dry season, because they are often nonpermeable (Hata and Kurihara 1982, Ogata 2013).

Similar to most other invasive aedine species, the eggs of *Ae. albopictus* are desiccation resistant. The eggs often overwinter under dry conditions in temperate areas (Makiya 1968). They also can be laid inside water holding cargo that is shipped globally, such as used car tires (Knudsen 1995) or houseplant pots such as 'lucky bamboo (Madon et al. 2003, Deblauwe et al. 2015).' The ability of the eggs to survive for long periods without flooding has facilitated their rapid spread globally (Gratz 2004, Sebesta et al. 2012). Eggs can survive in the field for several months over winter (Ishii et al. 1954, Toma and Miyagi 1990). When exposed to a temperature of −10°C for 24 h, the survival rate of dormant eggs from temperate Asia and the United States was 70% (Hawley et al. 1987); however, the survival rate depends on the conditions during embryonation, as well as chemical and physiological changes to the overwintering eggs. After overwintering in temperate areas, such as Japan, the eggs begin to hatch in late March or early April. The temperature is particularly important for terminating the dormancy of the eggs because *Ae. albopictus* is poikilothermic.

To date, no reports regarding the season in which the eggs hatch after overwintering in the field have been created. *Ae. albopictus* biting activity occurs in Japan from late April to mid-June in areas of infestation with different annual mean temperatures and altitudes. This study was undertaken to predict the date when *Ae. albopictus* female adults start to bite in temperate regions of Japan using high-resolution temperature analysis. Japan was a suitable location for this study for two main reasons: first, *Ae. albopictus* is a native species and is widely distributed throughout Japan, within a large population where most people come into contact with *Ae. albopictus*; second, Japan covers a range of climates. The country extends north to south, ranging from tropical to subarctic zones, with most of the country located within the temperate zone. The seasonal variation of temperature is also very marked. In this study, we used high-quality and high-resolution meteorological data from weather observation stations throughout Japan.

Materials and Methods

Ae. albopictus Field Surveillance

Human bait collection was used to determine the first *Ae. albopictus* biting day. Surveillance began roughly by the second half of April (depending on the collection location) and continued through until the date when the first female adult *Ae. albopictus* was collected using human bait. The start date meant that there were a sufficient number of days before the mosquito season begins in Japan, where mosquitoes were not collected. *Ae. albopictus* individuals were caught during 8 min of sweeping with an insect net (diameter 40 cm). The mosquitoes were caught at the human bait before they landed on the exposed skin of the collector (Kobayashi et al. 2014). Sampling was conducted in vegetated shady areas in urban areas. Data relating to the mean monthly field surveillance temperatures, latitude and longitude, and the survey year are shown in Table 1. The collection locations are plotted in Fig. 2. Surveillance was initiated from mid-April (depending on the collection location), before the completion of larval development. The date on which the first female adult was captured by human bait collection was recorded at each location for each year.

Climatological Data

Meteorological data (daily mean temperature in 1981–2015) was obtained from a public database of the Automated Meteorological Data Acquisition System (AMeDAS), which was developed by the Japan Meteorological Agency. The system collects data from 1,316 stations, which are located throughout Japan. The data for each collection site were acquired from the AMeDAS observation station that was located nearest to the sampling location. The AMeDAS stations, which have a mean separation of approximately 20 km, have monitored the temperature and other weather conditions throughout Japan since 1974.

Growing Degree Day

The growing degree days (GDDs) value was calculated based on the daily mean temperature. Although there were some differences in the developmental zero temperature of *Ae. albopictus* in past studies (Udaka 1959, Teng and Apperson 2000), we assumed the value was 10°C based on past results. The GDD value was estimated by accumulating the daily difference in the temperature from the developmental zero from the first day of March or April to 1 d before the first female mosquito was collected. The GDDs were calculated for each field sampling location.

The Basic Grid Square Climatological Data and the Population Density of Japan

The basic grid square climatological normal values were standardized using the raw data from 1981 through 2010. The data were downloaded from the Japan National Land Numerical Information download service (Ministry of Land, Infrastructure and Transport National and Regional Planning Bureau, Japan). The Japan Meteorological Agency originally collected the climatological data and estimated the distribution of the climatological elements on each basic grid square. These elements included the temperature, precipitation, and insolation conditions. This system divides the whole area of Japan into blocks, with a basic grid square being equivalent to one degree of longitude by two-thirds of one degree of latitude (approximately 1 × 1 km). The basic grid square system is standardized by the Statistics Bureau of Japan. The basic grid square climatological data (Seino 1993) for each year were obtained from the Japan National Institute for Agro-Environmental Sciences. The population density of each grid was obtained from the results of the national census, which was performed by the Japan Ministry of Internal Affairs and 123 Communications.

Mapping the Forecast of the *Ae. albopictus* First Biting Day

The forecast dates of when *Ae. albopictus* first biting day began in residential areas were mapped to a resolution of one basic grid square. The date was forecast based on the results of the correlation analyses of the field surveillance and climatological data.

Computer Software

The R software program (version 3.2.0, http://www.R-project.org/) with the rstan package (version 2.6.0, http://mc-stan.org/) and Microsoft Excel were used for the general statistical analyses. The high-resolution data that were used in mapping were calculated using programs that were written in the Python programming language (version 3.4, https://www.python.org/). The Q-GIS software program (version 2.8, http://qgis.org) was used for mapping the results.

Results

The *Ae. albopictus* field surveillance results are shown in Table 1. The dates at which *Ae. albopictus* adults started to bite in the field ranged from late April (Nagasaki) to the middle of June (Morioka, Ueda). Nagasaki was the most southerly and warmest of the surveillance

Table 1. The date on which the first *Ae. albopictus* adult was captured using human-baited insect net sweeping

Location	Year	Latitude/longitude	Date[a]	Mean monthly temperature				GDDs[b]
				Mar.	April	May	June	
Ebina	2014	35.45/139.39	May 13	9.1	13.9	18.0[c]	22.4	231.6
Kasukabe	2015	35.98/139.75	May 7	9.4	14.0	20.8[c]	22.1	220.7
Morioka	2015	39.70/141.15	June 10	4.4	10.5	16.6	19.3[c]	326.6
Nagasaki	2015	32.75/129.88	April 27	11.1	16.5[c]	19.9	21.7	220.3
	1975		May 7[d]	10.0	15.1	18.8[c]	22.2	234.3
	1974		May 7[ed]	9.8	14.8	19.5[c]	20.9	227.2
Niigata	2015	37.92/139.04	May 18	7.0	12.1	18.6[c]	21.2	211.1
Nishinomiya	2015	34.74/135.34	May 16	10.2	16.0	21.1[c]	22.7	380.7
	2008		May 13	10.6	15.1	19.6[c]	22.9	304.3
Oiso	2014	35.30/139.27	May 9	9.3	14.4	18.5[c]	22.0	186.2
	2013		May 12	11.5	14.1	18.1[c]	21.8	258.4
	2012		May 13	8.6	13.6	18.1[c]	20.7	220.6
	2010		May 23	7.3	12.5	18.0[c]	22.8	245.9
	2011		May 15	9.2	14.0	18.1[c]	22.5	299.8
Toyama	2015	36.70/137.21	May 10	7.0	13.2	19.6[c]	21.4	199.9
	2011		May 19	8.1	11.3	17.0[c]	22.3	184.9
Ueda	2015	36.40/138.25	June 7	5.5	11.3	18.0	19.8[c]	370.3
	2014		June 9	4.4	10.2	16.4	20.6[c]	328.8
	2013		June 9	6.2	9.8	16.2	20.7[c]	325.1
	2012		June 18	3.7	10.3	15.4	19.4[c]	375.0
	2011		June 10	2.5	9.5	15.4	20.8[c]	273.4
	2010		June 5	4.4	8.6	15.5	21.0[c]	226.4
Uwajima[e]	2015	33.22/132.56	May 2	10.6	16.7	20.1[c]	21.8	261.2
Yokohama	2015	35.57/139.52	May 14	10.5	14.6	20.8[c]	22.2	315.6
	2014		May 12	10.1	14.3	19.5[c]	22.6	275.1
	2013		May 11	12.0	14.8	19.1[c]	22.0	295.2
							Mean GDD	269.0
								95% credible interval: 245.8 –293.6

[a] The date on which the first female adults were captured in the year.

[b] The GDDs were accumulated from April 1 using 10°C as the assumed developmental zero temperature.

[c] The month when the first female adult was captured.

[d] The dates were quoted from Mori and Wada (1978).

[e] Yuta Wakayama (a science teacher at Ehime prefectural Uwajima Higashi High School, Japan) informed us of the date.

locations; Morioka is located near the northern limit of the *Ae. albopictus* habitat boundary in Japan (Kobayashi et al. 2002); and Ueda is located inland with an altitude of approximately 500 m. In Nagasaki and Ueda, the monthly mean temperatures from April to June are similar to the mean temperature of March in Morioka. At other locations, the start of the biting season is usually in May.

Although geographically there was a difference of up to 2 mo between the first *Ae. albopictus* biting day, the GDDs were almost the same. The mean GDD value was 269.2 GDDs (95% confidence interval [CI], 245.2 – 292.9). The appearance of the first adults was observed at a daily mean temperature of approximately 20°C. Since the developmental zero temperature of *Ae. albopictus* was 10°C, the difference was 25°C × d/(20 – 10°C) = 2.5 days.

A correlation was found between the field surveillance data and the climatological data (Fig. 1). The most important factor in determining the dates when *Ae. albopictus* adults first started to bite in the field was the temperature during larval development. There were high correlations between the monthly mean temperatures from March to June and the date at which the first mosquitoes were captured. The correlation coefficient was highest in April (Pearson product–moment correlation coefficient: $r = -0.900$), followed by March (−0.8750), May (−0.821), and June (−0.693), respectively.

The dates could be forecasted using a linear regression formula that showed the relationship between the April mean temperature and the number of successive days from April 1. The date was estimated using the following equation: (Days from April 1st (=1 d) to the date) = 123.48 − 5.65 × (April monthly mean temperature). A forecast map created using climatological data is shown in Fig. 2. The climatological data were standardized based on the data over 30 yr (from 1981 through 2010) by the Japan Meteorological Agency. The first forecast map ranges from April to June (Fig. 1). April applies to the part of the southwestern Kyushu districts (i.e., Nagasaki) that face the Pacific Ocean; May 1–15 applies to the southern areas facing the Pacific Ocean (i.e., Kyushu), parts of Chugoku, and 164 Shikoku (Uwajima), which face the inland sea, and Kinki (Nishinomiya), Kanto (Oiso, Ebina, Yokohama, Kasukabe), and the Chubu district; May 15–31 applies to part of Chugoku and Hokuriku district (Toyama, Niigata), which face the Sea of Japan, and Chubu, and the northern Kanto Districts; and June applies to the areas of Tohoku (Morioka) and the inland area of Chubu district (the altitude of Ueda is approximately 500 m). A second map, which was derived from a typical hot spring year (1998) and a third map from a cold spring year (1996) were also created (Fig. 3). In Japan, the spring season occurs from March to May. The Japan Meteorological Agency provided the criteria for hot and cold seasons based on the historical climate data; their definitions were used in the present study. Almost all of the locations found a difference of approximately 20 d in the first biting day (Fig. 3).

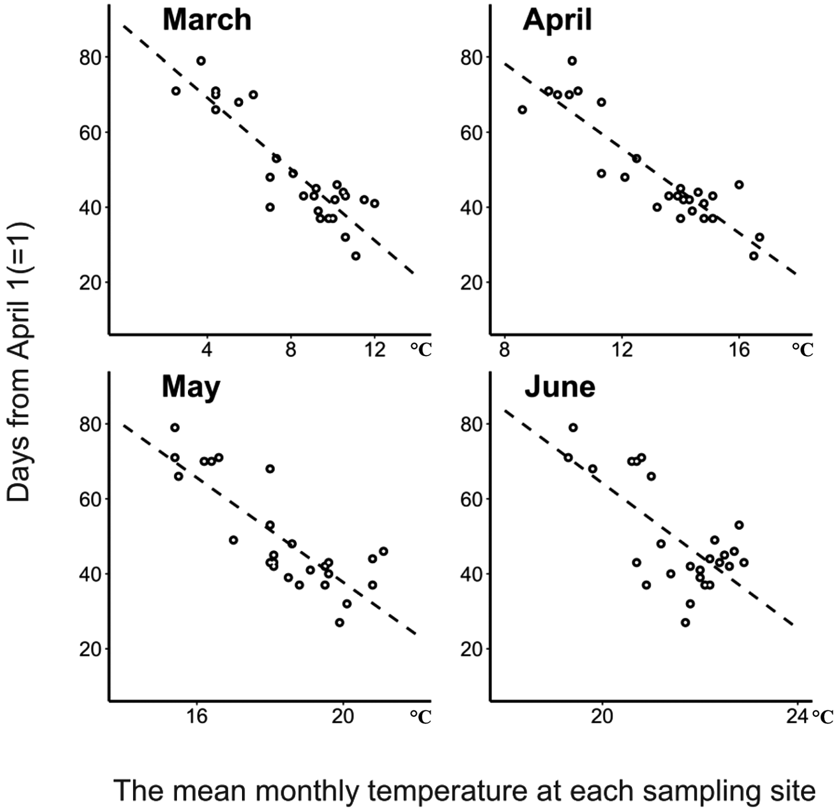

Fig. 1. The correlation between the mean monthly temperature and the days until the first *Ae. albopictus* female adult was captured from April 1 (= 1). Each circle shows the surveillance results for each field and year. The correlation coefficient is highest in April (Pearson product-moment correlation coefficient: $r = -0.900$), followed by March (–0.875), May (–0.821), and June (–0.693), respectively.

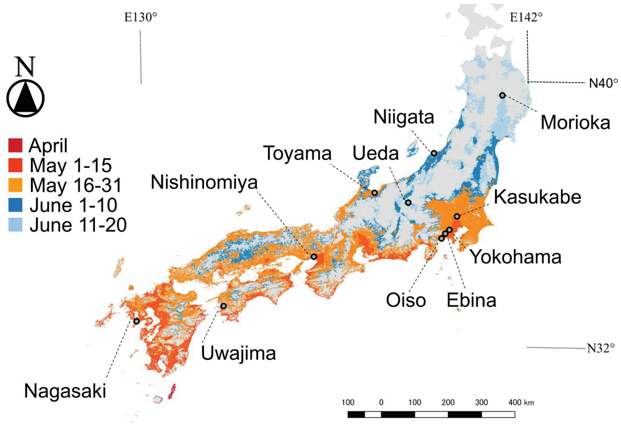

Fig. 2. The forecast of the *Ae. albopictus* first adult biting day. The date was forecast based on the mean April temperature (normal values in 1981–2010; approved by the Japan Meteorological Agency). The base map of Japan and the April mean temperature data were obtained from National Land Numerical Information download service (National Land Information Division, National Spatial Planning and Regional Policy Bureau, MLIT of Japan, http://nlftp.mlit.go.jp/ksj/). The first biting day was predicted from the temperature. Refer to the results section for details of the forecast calculation formula.

Discussion

We discovered that the timing of seasonal initiation of biting by female *Ae. albopictus* could be estimated using the monthly mean temperature (Fig. 1). The April mean temperature showed the highest correlation with date of first captured female adults, whereas the mean March temperature showed a moderate correlation. The difference in the correlation coefficient between April and March seems to be derived from the number of cold days below development zero (10°C) in March. From our experience in the field, it seems that the hatching of dormant eggs and the start of larval development occur in early April rather than early or mid-March. The correlation coefficient between the first biting days from April 1 and the May mean temperature was clearly low because, in contrast to March and April, there were no distinct differences in the daily mean temperature. No contradiction was found between the estimation of the *Ae. albopictus* first biting day and comparisons of the GDDs that theoretically allow for larval development in field or laboratory conditions (Matsuzawa and Kitahara 1966).

There are several reports on egg dormancy and the termination of overwintering of *Ae. albopictus* in laboratory experiments as well as a small amount of observational data from the field. Several complex factors, such as the age of the eggs, desiccation, the duration of colonization in the laboratory, temperature changes, and the oxygen concentration, determine whether or not *Ae. albopictus* eggs will hatch upon flooding by spring rain. There are several reports on the number of days from *Ae. albopictus* ovipositing to hatching, where the duration of embryonation was found to be dependent on the temperature (Hawley 1988). In a strain of *Ae. albopictus* from China, the period from ovipositing to hatching was 2 d at 25°C, whereas a strain from Japan took 6 d at 17°C and 3 days at 24° °C (Sheng and Wu 1951, Udaka 1959). Laboratory studies found that the mean duration (in days) of the larval stage of *Ae. albopictus* varies with temperature: 19 d at 14°C, 7 d at 20°C, 8 d at 25°C (Udaka

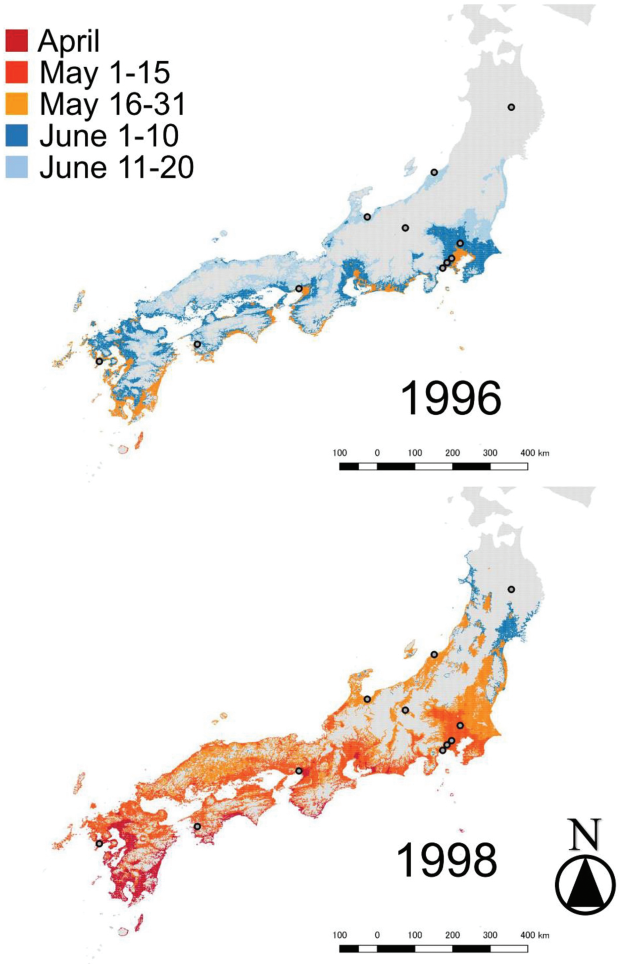

Fig. 3. The difference in the start of the *Ae. albopictus* biting season during a typically cold (1996) and hot (1998) spring. The cold and hot springs were defined based on the mean spring (March to May) temperatures in Japan. These were forecast using the mean April temperature for each year. The base map of Japan was obtained from the National Land Numerical Information download service (National Land Information Division, National Spatial Planning and Regional Policy Bureau, MLIT of Japan, http://nlftp.mlit.go.jp/ksj/). The April mean temperature data in 1996 and 1998 were obtained from Dr. Yasushi Ishigooka (National Institute for Agro-Environmental Sciences, Japan). The first biting day was predicted from the temperature.

1959), 5–6 d at 26–30°C (Livingstone and Krishnamoorthy 1985), and 5–7 days at 30°C (Galliard 1968). Unfortunately, detailed experiments have not been performed using field collected eggs or the early instar larvae of *Ae. albopictus*. It is not clear whether minor meteorological differences influence the termination of egg dormancy or the duration of larval development in spring in countries such as Japan, the United States, European countries, Korea, and China. However, in Japan, the timing depends strongly on the April temperature. A strong correlation was observed between the first *Ae. albopictus* biting day and the April mean temperature in southern to northern Japan (Table 1). This finding was supported by field observations.

Conversely, in some locations, first adult female biting day did not depend on mean April temperature. These locations included the warmer Nansei island regions (around Okinawa), the colder regions of northern Japan (Hokkaido), and mountainous areas (highlands and unpopulated areas). In the warmer regions of Japan, such as the subtropical southern island of Okinawa, *Ae. albopictus* neither deposits overwintering eggs (Hawley 1988) nor has an annual dormant overwintering period (Toma and Miyagi 1990). Furthermore, *Ae. albopictus* is not found in the cold or mountainous parts of Japan as they cannot survive the winter in areas where the annual mean temperature is less than 11°C (Kobayashi et al. 2002). In areas in which the first biting day was predicted to be after July, we hypothesized that *Ae. albopictus* would not survive the winter. This hypothesis was based on two factors: first, there are no reports to indicate that dormant eggs can survive for more than half a year, and therefore, we excluded these locations from this study; second, the northern edge of the area in which the first biting day is predicted to be late June, corresponds with the northern limits of the distribution of *Ae. albopictus,* indicating that the dormant eggs of *Ae. albopictus* fail to survive the winter as the dormancy period under the winter low temperatures may be too long.

We predicted that there would be multiple factors affecting the timing of the initiation of spring biting activity in *Ae. albopictus*. However, in this study, we clearly demonstrated that there is a strong correlation between the April mean temperature and the duration of larval development in the temperate regions of Japan. It is possible that a simple formula can be used to predict the first *Ae. albopictus* biting day at various geographic locations in Japan, as well as the start date in other temperate countries such as the United States and in European. Due to a variation in temperature from year to year, the first *Ae. albopictus* biting day changes, meaning the start date can vary up to approximately 20 d (Fig. 3) in Japan. Thus, it is necessary to make the prediction based on the April temperature for the current year. This prediction will be helpful in the implementation of programs to control the populations of *Ae. albopictus* larvae and adult mosquitoes in potential risk areas such as Japan for the mosquito-borne diseases in temperate regions.

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