Population dynamics of *Armigeres subalbatus* (Diptera: Culicidae) across a temperate altitudinal gradient

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Abstract

Understanding the impacts of weather fluctuations, and environmental gradients, on the abundance of vectors is fundamental to grasp the dynamic nature of the entomological risk for disease transmission. The mosquito *Armigeres subalbatus* (Coquillet) is a common vector of filariasis. Nevertheless, its population dynamics have been relatively poorly studied. Here, we present results from a season long study where we studied spatio-temporal abundance patterns of *Ar. subalbatus* across the altitudinal gradient of Mt. Konpira in Nagasaki, Japan. Spatially, we found that abundance of adult *Ar. subalbatus* decreased with altitude and increased in areas where the ground was rich in leaf litter. Similarly, adult activity was observed only when relative humidity was over 65%. Temporally, we found that peaks in abundance followed large rainfall events. Nevertheless, this mosquito was under significant density dependence regulation. Our results suggest that *Ar. subalbatus* population peaks following large rainfall events could reflect the recruitment of individuals that were dormant as dry eggs. We did not find a clear signal of temperature on abundance changes of this mosquito, but only on its phenology. Since ground cover seemed more critical than temperature to its spatial distribution, we propose that this mosquito might have some degree of autonomy to changes in temperature.

Keywords: Schmalhausen's law, Ricker model, filariasis, density-dependence, forcing

Introduction

Mosquitoes have been widely studied because of their role as vectors of several pathogens affecting humans and animals (Silver, 2008). Mosquito population dynamics are of particular interest because mosquito abundance shapes the entomological risk of vector-borne disease transmission (Dye, 1990). Specifically, vectorial capacity, the ability of a pathogen to be propagated by the action of insect vectors, linearly increases with vector-abundance (Garrett-Jones, 1964). Thus, the risk for disease transmission can be both spatially (Kitron, 1998) and temporally

(Chaves *et al.*, 2011) linked to vector abundance. Nevertheless, a detailed knowledge of factors regulating the abundance of mosquitoes, such as density-dependence and the impact of weather changes over a heterogenous landscape, have been relatively poorly studied for species other than dominant vectors (Chaves & Koenraadt, 2010), with few exceptions (Yang *et al.*, 2008a; Hoshi *et al.*, 2014a). Studies of mosquitoes over altitudinal gradients, in the context of climate change, allow to simultaneously evaluate mosquito abundance over the gradient of temperatures associated with elevation (Eisen *et al.*, 2008), where, in general, temperature decreases with altitude. Similarly, information about mosquitoes with minor, or without any, medical importance is fundamental to understand possible evolutionary constraints on the response of mosquitoes, and the diseases they transmit, to climate change (Chaves & Koenraadt, 2010).

The mosquito *Armigeres subalbatus* is a vector of filarial worms with medical importance, *Brugia pahangi* (Muslim

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et al., 2013) and with veterinary importance, *Dirofilaria repens* (Lee *et al.*, 2007). *Ar. subalbatus* has also been found infected with Japanese Encephalitis Virus (JEV) (Tanaka *et al.*, 1979) and has been incriminated as a JEV vector in settings without rice fields (Chen *et al.*, 2000). Regarding bloodfeeding, it has been reported that this mosquito was able to experimentally feed on humans, mice, chickens and reptiles, but not amphibians (Miyagi, 1972), thus reinforcing the view that this mosquito can transmit zoonotic pathogens (Chaves *et al.*, 2010).

The mosquito *Ar. subalbatus* is widely distributed in Asia (Tanaka *et al.*, 1979; Amerasinghe & Munasingha, 1988*a*, *b*) and has been observed across altitudinal gradients (Zea Iriarte *et al.*, 1991), yet no detailed study has assessed its population regulation. Studies on the natural history of *Ar. subalbatus* have shown that its larvae are common in nutrient enriched water (Senior-White, 1925; Barr & Chellappah, 1964), including septic tanks (Moriya *et al.*, 1967), especially with high ammonia concentration (Rajavel, 1992*b*), and the nutrient enriched water can be clear or turbid (Amerasinghe & Munasingha, 1988*b*). This mosquito also colonizes bamboo stumps (Kurashige, 1963), artificial containers (Zea Iriarte *et al.*, 1991) and treeholes (Tsuda *et al.*, 1994). Overwintering occurs in the larval stage (Mogi, 1996), and larvae seem to enter diapause because of a synergistic interaction between low temperature and short day length (Oda *et al.*, 1978). It has also been suggested, based on laboratory observations, that dry conditions may lead to egg diapause (Barr & Chellappah, 1964). The phenology of adults also seems to be strongly seasonal, with latitudinal variation. For example, in Taiwan adult *Ar. subalbatus* were absent from mosquito traps during January and February (Sun, 1964), at the higher latitude of Shandong province, China from November to early May (Zhang *et al.*, 1992). At the even higher latitude of Kyoto, Japan adult mosquitoes have been only observed from August to October (Nakata & Ito, 1955). Rainfall has been suggested as an important factor for *Ar. subalbatus* population changes, with significant adult abundance increase during and after high rainfall (Amerasinghe & Munasingha, 1988*a*). Here, we present results from a season long study of *Ar. subalbatus* abundance patterns in Mt. Konpira in Nagasaki, Japan. We asked what factors of the landscape were correlated with *Ar. subalbatus* abundance across the altitudinal gradient of Mt. Konpira, and whether its population dynamics were under density dependence regulation while accounting for potential impacts of weather changes on its abundance.

Materials and methods

Study site

Larval and adult mosquitoes were biweekly sampled across 27 locations in Mt. Konpira (fig. 1). Mt. Konpira is located in Nagasaki city, northwest Kyushu, western Japan (fig. 1). Nagasaki has a temperate seasonal climate, with four well defined seasons. Temperature in the winter (from December to February) rarely drops below 0°C and snowfall is minimal, often absent. By contrast, the summer (mid July–mid September) has both high relative humidity, above 65%, and temperature, above 27°C (Isida, 1969). Our sampling locations in Mt. Konpira followed those of a previous study in the same area, ranging from 109 to 330 m in altitude (Zea Iriarte *et al.*, 1991). The sampling setting consisted of three radial transects, across an altitudinal gradient, joined at a middle point (fig. 1). Our sampling locations were mainly located

across a secondary forest, with diverse types of dominant vegetation according to the Japanese Ministry of the Environment (available at http://www.vegetation.biodic.go.jp/), but there were some urban sites in the periphery of all the sampling locations (fig. 1). Exact coordinates for each sampling location were measured with a Garmin Oregon 650 GPS (Garmin Ltd, KS, USA), which was also used to measure the site altitude, in metre. The altitude measures were compared with those from an ASTER digital elevation model (DEM), for Mt. Konpira (available at http://gdem.ersdac.jspacesystems.or.jp/), and given the low disagreement between the two measurements, we employed the DEM data in our analysis. At each sampling location we characterized the canopy cover in 25 May 2014 by following a standard methodology (Frazer *et al.*, 2001) where four photographs were taken with a fisheye ball lens located at ground level within a 1.5 m radius of a focal tree, i.e., a tree where an ovitrap was located. For the photos we used a EOS 40D camera (Canon Co., Tokyo, Japan) that had attached a normal lens (EF-S17-85 mm F4-5.6 IS USM; Canon Co., Tokyo, Japan) and a 180° fisheye lens (Nordward optronics 0.25X Super fisheye lens 180° G2; Revel Royal Inc, Aichi, Japan), which allowed to take hemispherical photos. The images were subsequently analyzed with the gap light analysis mobile application for android tablets (http://gap-light-analysis-mobile-app.android.informer.com/) to determine the average and standard deviation (SD) of canopy cover for each sampling location. We also quantified the ground cover by sampling twelve 2.5 m long transects radiating from each focal tree. Each transect had an approximate angular separation of 30°. We categorized each transect as: grass, leaf litter, bush, trees, concrete and terrace, based on the dominant group type among those six categories. A matrix with the percent of transects in each category at each sampling point was then analyzed with principal components analysis (Chaves *et al.*, 2011), and the first principal component (online Table S1) was then used as a ground cover index.

Mosquito sampling

To sample immature mosquito stages we made 'ovitraps' (Zea Iriarte *et al.*, 1991) by using 350 ml Coca-Cola® cans painted black with an acrylic spray paint (Kanpe Hapio Co., Ltd., Osaka, Japan) inside and outside. We made one 5 mm opening to hold both the can to a tree, using a cord, and to drain excess water above 280 ml. All traps were uniformly set at 1.2 m above the ground level. We selected this height given that previous reports indicated that *Ar. subalbatus* preferentially oviposits in containers around this height (Amerasinghe & Alagoda, 1984). Ovitraps were filled with rain water collected during April 2014 and were set in 18 May, and then biweekly surveyed from 1 June until 29 November of 2014. During each survey we determined the presence of first to third instar mosquito larvae of any species, and we counted the number fourth instar larvae of *Tripteroides bambusa* Yamada and *Ar. subalbatus*, due to their easy identification in the field (Tanaka *et al.*, 1979), and removed all other fourth instar larvae and all pupae for laboratory rearing and identification of emerged adults (Hoshi *et al.*, 2014*a*).

Adult mosquitoes were sampled using a sweep net (36 cm diam; Model 61-1B; Shiga Insect Co., Tokyo, Japan), a common tool to sample adult mosquitoes (Hoshi *et al.*, 2014*b*). Sampling started at sunrise. We selected this sampling time given previous reports that indicated this mosquito is active at sunrise (Berlin *et al.*, 1975). Also sweep nets are appropriate to sample
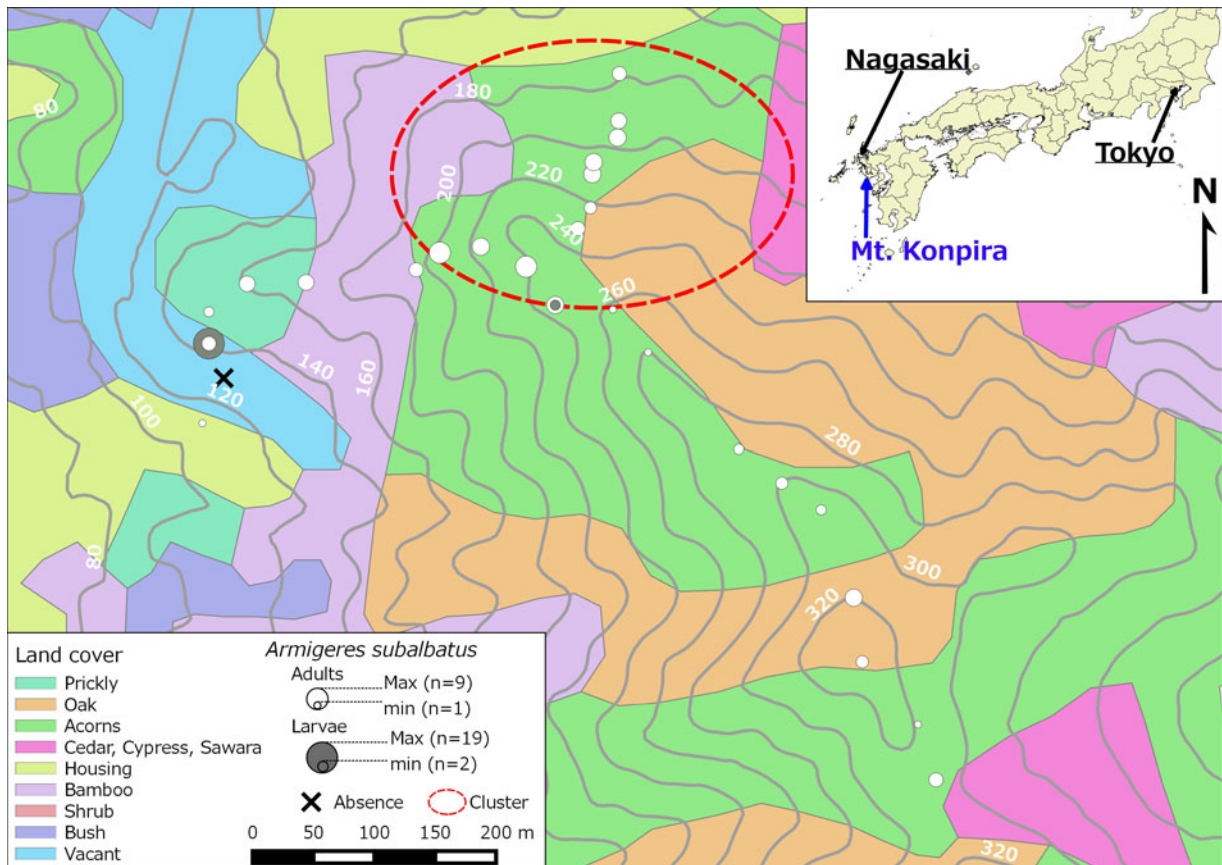
Fig. 1. Map of sampling locations. The inset figure shows the location of Mt. Konpira in Nagasaki, Japan. Contour lines indicate the elevation. The inset legend indicates the land use types and vegetation cover, and there is also a guide to the total abundance of *Armigeres subalbatus* adults and fourth instar larvae sampled during May–November 2014. A dashed red line indicates a significant spatio-temporal cluster.

this mosquito, which is predominant at ground level (Tsuda *et al.*, 2003). The use of sweep nets was standardized by staying at a fixed point within a 2.5 m radius from the focal tree where the ovitraps were set up. After arriving to a sampling location the net was swept around the body of the person sampling for 2 min, after this time period the person sampling stopped for 1 min and then swept the net for another 2 min. During each sampling session the order of the sampling locations was determined randomly in order to avoid any possible bias due to sampling the same locations always at the same time. Adult mosquitoes were biweekly sampled between 4 May and 15 November of 2014. We selected a biweekly sampling to have a sampling interval that coincided with the pre-adult developmental time of *Ar. subalbatus* (Weathersby, 1962). Similarly, we started to collect mosquitoes in May, given that in Shandong Province, China, at the same latitude of Nagasaki, adult mosquitoes have only been caught after 10 May (Zhang *et al.*, 1992).

Weather variables

At each sampling session we recorded the air temperature and relative humidity using a portable O-230 termohygrometer (Dretec Co., Saitama, Japan). The water temperature in the ovitraps was measured using an AD-5617WP infrared thermometer (A&D Co., Tokyo, Japan). Data on daily records for rainfall from 1 May to 1 December 2014 were obtained for Nagasaki city from the Japanese Meteorological Agency Website (http://www.jma.go.jp/jma/index.html). For the analyses we employed the cumulative amount of rainfall on the 14 days prior to the mosquito sampling. The weather station is located within a 5 km radius from our study site.

Mosquito identification

Fourth instar larvae and adult mosquitoes were identified using the taxonomic key of Tanaka *et al.* (1979). Voucher specimens are available in the Entomological Collection in the Institute of Tropical Medicine of Nagasaki University, Japan and in the Mosquito Collection in the Walter Reed Biosystematics Unit – Smithsonian Institute, Washington DC, USA.

Statistical analysis

Larvae data of *Ar. subalbatus* were not analyzed given that we found them at two localities, once at one site, inside a vacant lot bordering a prickly forest, and two times at another other site, inside an area whose vegetation was dominated by acorns (fig. 1).

To detect spatio-temporal clusters, i.e., hotspots, of *Ar. subalbatus* adults, we employed an elliptical cluster detection mode in a SCAN spatio temporal Poisson model (Kulldorff & Nagarwalla, 1995; Kulldorff *et al.*, 2005). We constrained the maximum cluster size up to 50% of the samples while scanning the whole study site. We chose the elliptical shape over a circular one given that our sampling followed transects (Kulldorff *et al.*, 2006). For statistical inference we employed 999 Monte Carlo simulations. We then proceeded with a spatial analysis of *Ar. subalbatus* abundance in Mt. Konpira, Nagasaki. We fitted Poisson generalized linear models (Faraway, 2006) to the total abundance per site. The total abundance per site was obtained by adding the abundance through the 14 biweeks of sampling at each sampling location (fig. 1). We started by building a model that included elevation (m), mean and SD canopy openness (%), mean, SD and kurtosis of temperature (°C) and relative humidity (%), and the ground cover index. This model was simplified by a combined backward-elimination and forward-addition algorithm (Kuhn & Johnson, 2013) that minimized the Akaike Information Criterion (AIC). Briefly, AIC is a model selection criterion that weighs the trade-off between the goodness of fit in a model and the number of parameters, and the best model is chosen by minimizing the difference between a function of a model log-likelihood and the number of parameters (Kuhn & Johnson, 2013). Moran's I test (Venables & Ripley, 2002) was employed to test the spatial independence of residuals in the model selected as best, in order to ensure that inferences from the best Poisson generalized linear model were sound (Chaves, 2010).

For the temporal analysis of *Ar. subalbatus* abundance we generated a 14 observations adult abundance time series, given the absence of adult mosquitoes during 4 May 2014. The time series was generated by adding the biweekly records across the 27 sampled sites per sampling session. We used this time series for some preliminary time series statistical analysis. We estimated the autocorrelation- (ACF) and partial autocorrelation (PACF)-functions to describe the patterns of temporal autocorrelation in *Ar. subalbatus* abundance (Chaves *et al.*, 2012, 2013). The ACF presents the correlation of a time series with itself through different time lags, while PACF shows a similar profile that only considers the correlation between consecutive time lags (Shumway & Stoffer, 2011). We also estimated the cross-correlation functions (CCFs), i.e., the temporal correlation function between two time series for different time lags (Shumway & Stoffer, 2011), between the *Ar. subalbatus* abundance time series and rainfall, temperature and relative humidity. We estimated the 95% confidence limits (CL), of the ACFs, PACFs and CCFs to test if the correlations departed from what would be expected by random, with only correlations outside the 95% CL being considered statistically significant (Shumway & Stoffer, 2011). Then, we proceeded to study the density-dependence regulation of *Ar. subalbatus* adults. We started by plotting the per-capita growth rate (*r*) of this mosquito as function of its total adult abundance (N_t). The per-capita growth rate is defined by (Turchin, 2003):

$$r = \ln(N_t) - \ln(N_{t-1}) \tag{1}$$

Based on the results from this preliminary analysis, we fitted the Ricker model to the *Ar. subalbatus* time series data. The Ricker model has been widely used to study mosquito populations (Yang *et al.*, 2008*a*, *b*; Hoshi *et al.*, 2014*a*) and is defined by the following equation:

$$N_t = \lambda_0 N_{t-1} \exp(-bN_{t-1}) \tag{2}$$

Where λ_0 is the intrinsic rate of population growth and *b* is a density-dependence coefficient (Turchin, 2003). When $-b < 0$, it can be affirmed that a population undergoes density-dependent regulation (Turchin, 2003). Further details about this model are presented elsewhere, including its derivation (Turchin, 2003; Mangel, 2006) and several stochastic versions (Melbourne & Hastings, 2008).

To fit the models to our data we assumed biweekly adult counts had a negative binomial (NegBin) distribution, given our observations had a variance larger than their mean, i.e., they were over-dispersed (Bolker, 2008). This assumption for the model presented in (2), when also assuming the model has an observation error, i.e., without feedback on future states (Bolker, 2008), coincides with the assumption that mosquito abundance follows a negative binomial environmental stochastic Ricker model (Melbourne & Hastings, 2008):

$$N_t \sim NegBin(\text{mean} = \lambda_0 N_{t-1} \exp(-bN_{t-1}), \ \text{overdispersion} = k) \tag{3}$$

which implies stochasticity affects individuals, in this case *Ar. subalbatus* mosquitoes, independently of their density. We also fitted the model assuming stochasticity was demographic, i.e., a function of mosquito density (Melbourne & Hastings, 2008):

$$N_t \sim NegBin(\text{mean} = \lambda_0 N_{t-1} \exp(-bN_{t-1}), \\ \text{overdispersion} = kN_{t-1}) \tag{4}$$

We expanded the model presented in (2) to account for the potential impacts of weather variables (WV) on the dynamics, as follows:

$$N_t = \lambda_0 N_{t-1} \exp(-bN_{t-1} + \gamma WV_{t-z}) \tag{5}$$

Where γ is a coefficient for the impact of a WV at any time lag *z*. The lag *z* for the WVs was estimated with the CCFs previously described. To incorporate the forcing of equation (5) into equations (3) and (4), we simply changed the mean of the negative binomial to the following:

$$\text{mean} = \lambda_0 N_{t-1} \exp(-bN_{t-1} + \gamma WV_{t-z}) \tag{6}$$

To fit the model presented in (3) and its forced version (6), we employed a negative binomial generalized linear model (Faraway, 2006), using the natural logarithm of N_{t-1} as an offset, and N_{t-1} and WV_{t-z} as covariates. Nevertheless, to fit the model presented in (4) and its forced version (6), we wrote an R function that is available as a supplementary online material (online Appendix S1). We computed the AIC of the models for comparison.

All statistical analysis was implemented with the statistical software R version 3.2.0, with the exception of the SCAN cluster analysis that was performed with SaTScan version 9.3.1.

Results

We collected a total of 114 adults and 21 fourth instar larvae of *Ar. subalbatus* during the duration of our study (fig. 1). Adults of *Ar. subalbatus* were present through all the land cover uses and vegetation types that we sampled, and were only absent from one of the points that we sampled (fig. 1). The average (±SD) number of adults per sampling location was 4.22 ± 2.35, ranging from 0, i.e., a place where *Ar. subalbatus* was never detected, to 9 individuals (fig. 1). Figure 1 also shows that adults of *Ar. subalbatus* were clustered across the

Table 1. Parameter estimates for the best spatial Poisson Generalized Linear model explaining *Armigeres subalbatus* abundance across an altitudinal gradient. This model was selected by a combined backward-elimination and forward-addition algorithm, from a model that included elevation (m), mean and SD canopy openness, mean, SD and kurtosis of temperature (°C) and relative humidity (%), and a ground cover index. The Akaike Information Criterion (AIC) of the starting model was 124.05, and the AIC for the model presented here is 111.25.

Parameter	Estimate	SE	z	P
Intercept	2.46	0.45	5.496	<0.0001*
Elevation (m)	−0.0051	0.0020	−2.521	<0.01*
Ground Cover	0.124	0.032	3.881	<0.0001*
Moran's I	−0.0652	–		0.574

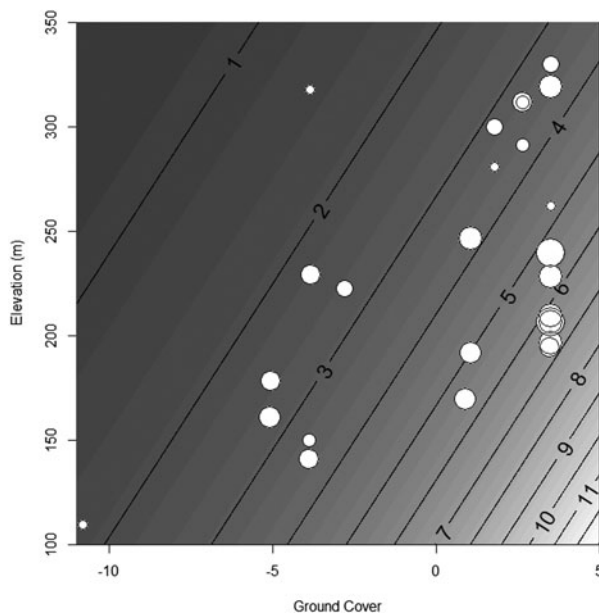*Statistically significant ($P < 0.05$).

Fig. 2. Spatial patterns of *Armigeres subalbatus* abundance. Dot size is proportional to abundance of *Ar. subalbatus*, ranging from 1 to 9, as function of ground cover and altitude. The grey gradient indicates the abundance estimated with the model presented in Table 1.

northernmost sampling locations, which were all inside an area of the forest dominated by acorns.

The best spatial model showed that elevation and ground cover (table 1) were the best covariates explaining the spatial patterns of *Ar. subalbatus* abundance. The lack of significance for the Moran's I index (table 1) indicates spatial independence in the residuals, thus fulfilling the assumptions for a sound statistical inference (Chaves, 2010). Figure 2 clearly shows how the number of adult *Ar. subalbatus* decreased with elevation and increased with the ground cover index, where positive values indicate a dominance of leaf litter over bushes and/or concrete (Table S1 online only), while negative values indicate an abundance of bushes and/or concrete over leaf litter.

Temporal patterns of *Ar. subalbatus* (fig. 3) showed that its abundance peaked during the 5th, 7th and 9th sampling

biweek, i.e., 12 July, 9 August and 6 September, respectively, reaching a maximum of 34 individuals (fig. 3a). From the 13th biweek of sampling we did not catch any *Ar. subalbatus*. The average (±SD) number of adults per sampling biweek was 8.14 ± 10.23. In general, *Ar. subalbatus* abundance time series had temporally independent observations, as indicated by the lack of significant lags in its ACF (fig. 3b) and PACF (fig. 3c). Regarding fluctuations in the weather variables, it can be seen that peaks in mosquito abundance (fig. 3a) overlapped with those of rainfall (fig. 3d), but not those of temperature (fig. 3e) nor relative humidity (fig. 3f), nevertheless, these two last factors seem to be related with the presence of adult mosquitoes, which were only captured when their values were high. CCF analysis showed that *Ar. subalbatus* abundance was significantly associated with rainfall without a temporal lag (fig. 3g), but not with temperature (fig. 3h), nor with relative humidity (fig. 3i).

As expected in a population undergoing density-dependent regulation (fig. 4) we found that the per capita growth rate of *Ar. subalbatus* decreased with its population size.

Estimates of the environmental negative binomial Ricker model (table 2) outperformed those of the demographic one (Table S2, online only). Table 2 shows the fit considering the forcing by rainfall was better, i.e., with a lower AIC, than an autonomous model. The effect of rainfall on the density dependent regulation of *Ar. subalbatus* (fig. 5) was to increase the number of mosquitoes, nevertheless, in all cases the abundance of *Ar. subalbatus* was under a strong density dependence, with the per capita growth rate diminishing with adult abundance in all instances.

Discussion

We found that temporal changes in adults of *Ar. subalbatus* were autonomous from changes in temperature and relative humidity. Nevertheless, these two factors may be important for the phenology of *Ar. subalbatus* adult activity, since we only found the species when temperatures were above 14°C and relative humidity above 65%. In fact, we found that phenology of adults of *Ar. subalbatus* at our study site was similar to observations at a similar latitude in China, where adult mosquitoes were only observed from May to October (Zhang *et al.*, 1992). Moreover, our spatial model indicated that abundance of *Ar. subalbatus* adults decreased with altitude, further supporting that temperature might be important to the phenology and distribution of this mosquito, as suggested by observations across different latitudes (Nakata & Ito, 1955; Sun, 1964; Zhang *et al.*, 1992) and studies on its overwintering (Oda *et al.*, 1978). However, spatially we also found that adults of *Ar. subalbatus* were more common on grounds with abundant leaf litter, suggesting that organic matter necessary for enriching potential larval habitats (Senior-White, 1925; Rajavel, 1992b) is a factor that might modulate habitat use by adults. This ground cover also resembles the habitats where this mosquito has been found transmitting filarial worms (Muslim *et al.*, 2013). Therefore, ground cover might be important to understand landscape heterogeneities in pathogen transmission risk by *Ar. subalbatus*.

The positive impact of rainfall on adult *Ar. subalbatus* abundance can be related to two aspects of its biology: (i) under dry conditions, eggs can enter diapause (Barr & Chellappah, 1964) and (ii) the low variability in oviposition above the water surface, where more than 75% of the eggs were within a 'threshold'
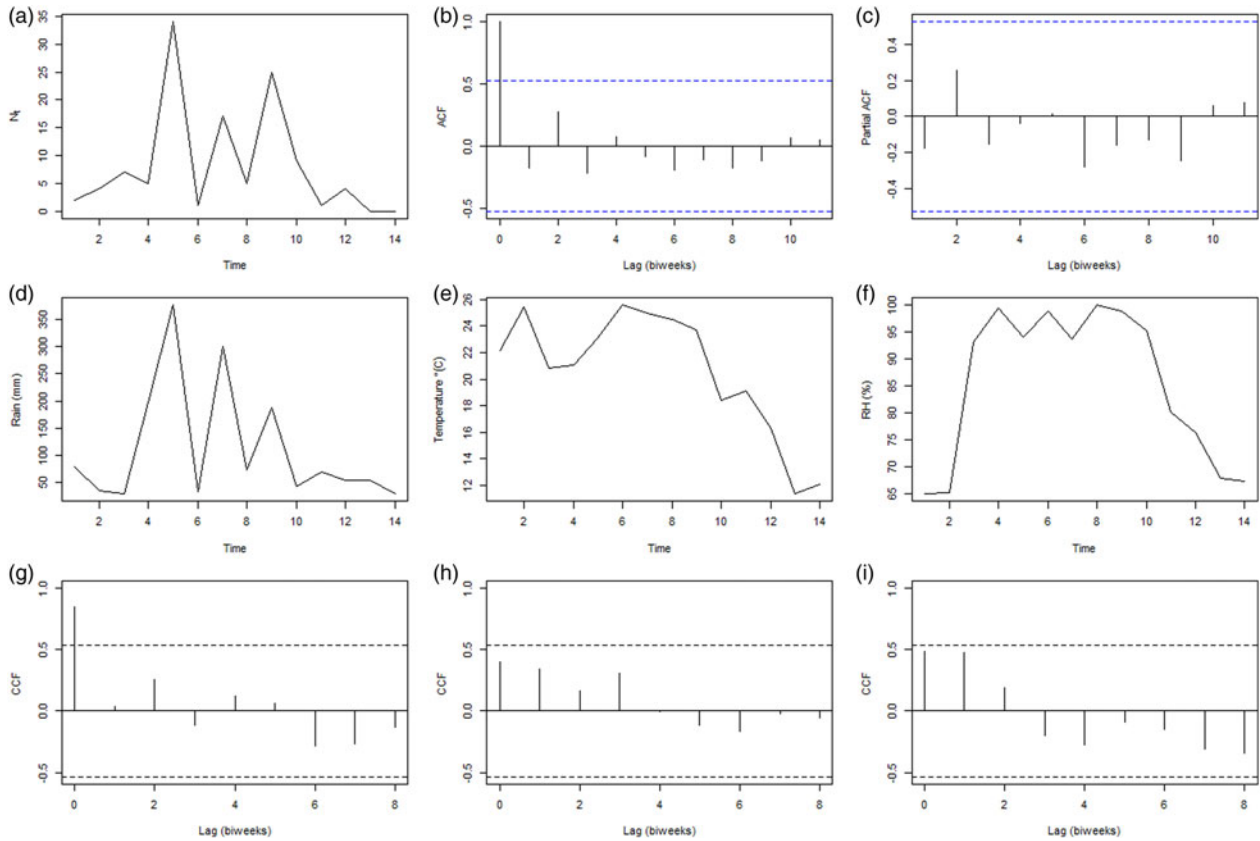
Fig. 3. Temporal patterns of *Armigeres subalbatus* abundance. (a) Time series of *Ar. subalbatus* (TSAS), biweekly abundance from 18 May to 15 November 2014. (b) Auto-correlation function (ACF), of TSAS, (c) partial ACF, PACF, of TSAS. Time series of weather variables: (d) rainfall, (e) temperature and (f) relative humidity (RH). Cross-correlation functions between TSAS and: (g) rainfall, (h) temperature and (I) RH.
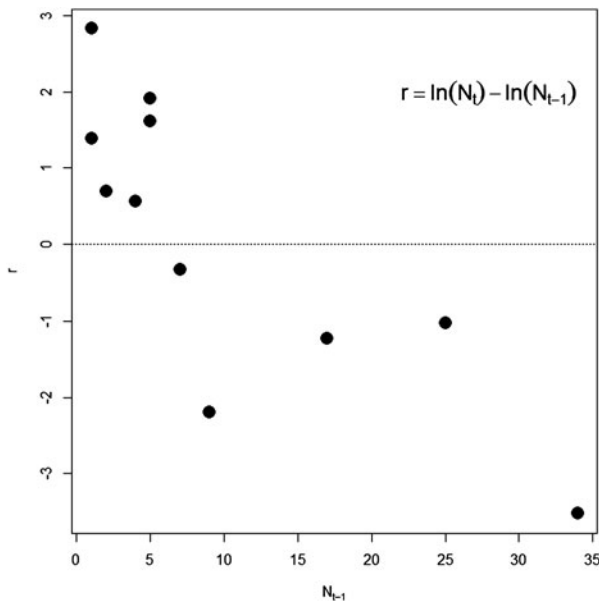

Fig. 4. Per capita population growth rate (*r*) of *Armigeres subalbatus* as function of its density, (N_{t-1}), in Mt. Konpira, Nagasaki, Japan.

of 16 mm above the waterline (Amerasinghe & Alagoda, 1984), suggest that any rainfall above the oviposition 'threshold' has the potential to synchronously trigger egg hatching in *Ar. subalbatus* larval habitats, which can lead to an eventual 'outbreak' or sudden large change in mosquito abundance (Chaves *et al.*, 2014), as we observed in our study site, a pattern also observed in Sri Lanka (Amerasinghe & Munasingha, 1988*a*). Unfortunately, the scarcity of larval samples did not allow us to connect any potential changes in larval density with adult abundance. However, a previous study (Rajavel, 1992*a*) suggests that at high larval density *Ar. subalbatus* keeps its numbers in check by cannibalizing larvae, especially if *Ar. subalbatus* does not co-occur with other mosquito species, and in that regard we can mention that we found fourth instar larvae of *Ar. subalbatus* only co-existing with larvae of *Tp. bambusa*, the most common and abundant mosquito in our ovitraps, following a similar pattern also observed by Zea Iriarte *et al.* (1991) in our study area. However, we were unable to asses any potential interaction between these two species given the scarce *Ar. subalbatus* larval data that we collected.

Our results clearly indicate that there is density dependence regulation in *Ar. subalbatus*. The density dependence parameter *b* in the Ricker model was negative as expected under density dependence regulation (Turchin, 2003), and in congruence with patterns observed in several mosquitoes (Makiya, 1973, 1974, Yang *et al.*, 2008*a*, *b*, Chaves *et al.*, 2012; Hoshi *et al.*, 2014*a*). However, although rainfall can transiently

Table 2. Parameter estimates for the negative binomial environmental stochastic Ricker model fitted to the time series of *Armigeres subalbatus* biweekly abundance. Models were fitted with (Forced) and without (Autonomous) rainfall as covariate. AIC stands for the Akaike Information Criterion.

Parameter	Forced				Autonomous			
	Estimate	SE	z	P	Estimate	SE	z	P
Intrinsic rate of population growth ($\hat{\lambda}$)	2.17	1.08	2.01	*	7.37	2.75	2.68	*
Density dependence coefficient ($\hat{b}$)	−0.12	0.03	−4.50	**	−0.15	0.03	−5.38	**
Rainfall coefficient ($\hat{\gamma}$)	0.0054	0.0020	2.70	**	–	–	–	–
Negative binomial overdispersion ($\hat{k}$)	2.68	1.53	–		1.43	0.66		
AIC	74.44				78.00			

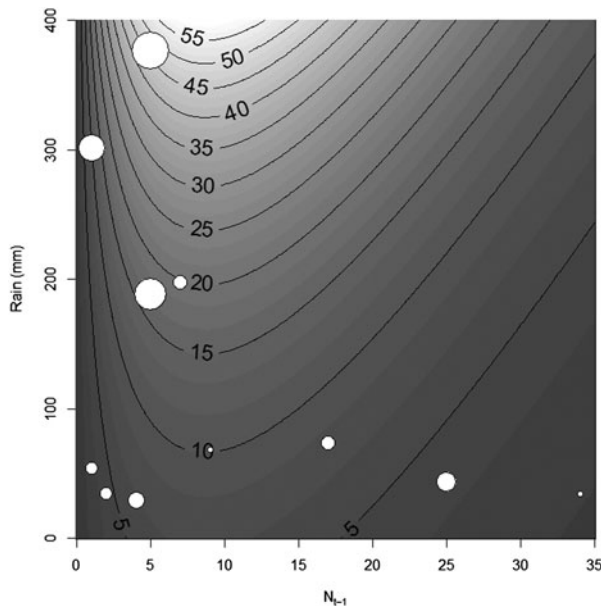NS, not significant ($P > 0.05$), *Statistically significant ($P < 0.05$), **Statistically significant ($P < 0.001$).

Fig. 5. Temporal abundance of *Armigeres subalbatus* (N_t) as function of its previous abundance (N_{t-1}) and rainfall. In the plot dots are proportional to the biweekly abundance of *Ar. subalbatus* (N_t), which ranged from 1 to 34. The grey gradient indicates the expected number of *Ar. subalbatus* according to the Ricker model forced by rainfall (for parameters see Table 2).

increase the number of adults, population growth still decreases with population abundance, highlighting the importance of density dependence regulation. It is also interesting to note that for the autonomous environmental stochastic Ricker model, the natural logarithm of the intrinsic rate of population growth parameter, i.e., $\ln(\lambda_0) = 1.98$, was close to the threshold value for weather independent oscillations, a.k.a., bifurcations, which is $\ln(\lambda_0) \geq 2$ (Mangel, 2006). Nonetheless, when considering rainfall it became clear that any potential periodicities likely reflected rainfall patterns. Finally, we can conclude that population dynamics of adult *Ar. subalbatus* follows Schmalhausen's law, the prediction that systems are more sensitive to environmental variables more unpredictable around their mean (Chaves & Koenraadt, 2010; Chaves *et al.*, 2012). Rainfall, the only weather variable associated with abundance changes in *Ar. subalbatus* adults, undergoes less extreme changes in its mean behaviour than temperature or relative

humidity at our study site, which although more extreme, have a more predictable pattern of change and seem to impact *Ar. subalbatus* phenology, but not its abundance dynamics.

Supplementary Material

The supplementary material for this article can be found at http://www.journals.cambridge.org/BER

Acknowledgements

References

Amerasinghe, F.P. & Alagoda, T.S.B. (1984) Mosquito oviposition in bamboo traps with special reference to *Aedes albopictus*, *Aedes novalbopictus* and *Armigeres subalbatus*. *Insect Science Applications* 5, 493–500.

Amerasinghe, F.P. & Munasingha, N.B. (1988a) A predevelopment mosquito survey in the Mahaweli development project area, Sri Lanka: adults. *Journal of Medical Entomology* 25, 276–285.

Amerasinghe, F.P. & Munasingha, N.B. (1988b) a predevelopment mosquito survey in the Mahaweli development project area, Sri Lanka: Larvae. *Journal of Medical Entomology* 25, 286–294.

Barr, A.R. & Chellappah, W.T. (1964) The colonization and laboratory maintenance of *Armigeres subalbatus* (Coquillett). *Bulletin of the World Health Organization* 31, 439

Berlin, O.G.W., Dwarakanath, S.K. & Pandian, R.S. (1975) Relation between diel activity and respiration in *Armigeres subalbatus* (Coquillet) (Diptera: Culicidae). *Journal of Medical Entomology* 12, 479–480.

Bolker, B.M. (2008) *Ecological Models and Data in R*. Princeton, Princeton University Press.

Chaves, L.F. (2010) An entomologist guide to demystify pseudoreplication: data analysis of field Studies with design constraints. *Journal of Medical Entomology* 47, 291–298.

Chaves, L.F. & Koenraadt, C.J.M. (2010) Climate change and highland Malaria: fresh air for a hot debate. *Quarterly Review of Biology* 85, 27–55.

Chaves, L.F., Harrington, L.C., Keogh, C.L., Nguyen, A.M. & Kitron, U.D. (2010) Blood feeding patterns of mosquitoes: random or structured? *Frontiers in Zoology* **7**, 3.

Chaves, L.F., Hamer, G.L., Walker, E.D., Brown, W.M., Ruiz, M. O. & Kitron, U.D. (2011) Climatic variability and landscape heterogeneity impact urban mosquito diversity and vector abundance and infection. *Ecosphere* **2**, art70.

Chaves, L.F., Morrison, A.C., Kitron, U.D. & Scott, T.W. (2012) Nonlinear impacts of climatic variability on the density-dependent regulation of an insect vector of disease. *Global Change Biology* **18**, 457–468.

Chaves, L.F., Higa, Y., Lee, S.H., Jeong, J.Y., Heo, S.T., Kim, M., Minakawa, N. & Lee, K.H. (2013) Environmental forcing shapes regional house mosquito synchrony in a warming temperate Island. *Environmental Entomology* **42**, 605–613.

Chaves, L.F., Scott, T.W., Morrison, A.C. & Takada, T. (2014) Hot temperatures can force delayed mosquito outbreaks via sequential changes in *Aedes aegypti* demographic parameters in autocorrelated environments. *Acta Tropica* **129**, 15–24.

Chen, W.-J., Dong, C.-F., Chiou, L.-Y. & Chuang, W.-L. (2000) Potential role of *Armigeres subalbatus* (Diptera: Culicidae) in the transmission of Japanese encephalitis virus in the absence of rice culture on Liu-Chiu Islet, Taiwan. *Journal of Medical Entomology* **37**, 108–113.

Dye, C. (1990) Epidemiological significance of vector-parasite interactions. *Parasitology* **101**(Pt 3), 409–415.

Eisen, L., Bolling, B.G., Blair, C.D., Beaty, B.J. & Moore, C.G. (2008) Mosquito species richness, composition, and abundance along habitat-climate-elevation gradients in the northern colorado front range. *Journal of Medical Entomology* **45**, 800–811.

Faraway, J.J. (2006) *Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models*. Boca Raton, CRC Press.

Frazer, G.W., Fournier, R.A., Trofymow, J. & Hall, R.J. (2001) A comparison of digital and film fisheye photography for analysis of forest canopy structure and gap light transmission. *Agricultural and forest meteorology* **109**, 249–263.

Garrett-Jones, C. (1964) Prognosis for interruption of Malaria transmission through assessment of mosquitos vectorial capacity. *Nature* **204**, 1173.

Hoshi, T., Higa, Y. & Chaves, L.F. (2014*a*). *Uranotaenia novobscura ryukyuana* (Diptera: Culicidae) population dynamics are denso-dependent and autonomous from weather fluctuations. *Annals of the Entomological Society of America* **107**, 136–142.

Hoshi, T., Imanishi, N., Higa, Y. & Chaves, L.F. (2014*b*). Mosquito biodiversity patterns around urban environments in South-Central Okinawa Island, Japan. *Journal of the American Mosquito Control Association* **30**, 260–267.

Isida, R. (1969) *Geography of Japan*. Tokyo, Kokusai Bunka Shinkokai.

Kitron, U. (1998) Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *Journal of Medical Entomology* **35**, 435–445.

Kuhn, M. & Johnson, K. (2013) *Applied Predictive Modeling*. New York, Springer.

Kulldorff, M. & Nagarwalla, N. (1995) Spatial disease clusters: detection and Inference. *Statistics in Medicine* **14**, 799–810.

Kulldorff, M., Heffernan, R., Hartman, J., Assunção, R. & Mostashari, F. (2005) A space–time permutation scan statistic for disease outbreak detection. *PLoS Med* **2**, e59.

Kulldorff, M., Huang, L., Pickle, L. & Duczmal, L. (2006) An elliptic spatial scan statistic. *Statistics in Medicine* **25**, 3929–3943.

Kurashige, Y. (1963) Ecological studies on mosquitoes in the bamboo groves of Tochigi Prefecture : V. On the mosquito larvae in bamboo groves during winter season. *Japanese Journal of Sanitary Zoology* **14**, 213–215.

Lee, S.-E., Kim, H.-C., Chong, S.-T., Klein, T.A. & Lee, W.-J. (2007) Molecular survey of *Dirofilaria immitis* and *Dirofilaria repens* by direct PCR for wild caught mosquitoes in the Republic of Korea. *Veterinary Parasitology* **148**, 149–155.

Makiya, K. (1973) Population dynamics of mosquitoes in Nagoya district V. Seasonal change of composition rate of *Culex pipiens molestus* and *Culex pipiens pallens* observed in a midtown building. *Japanese Journal of Sanitary Zoology* **24**, 89–96.

Makiya, K. (1974) Population dynamics of mosquitoes in Nagoya district B. Larval and imaginal populations of *Aedes albopictus* (Skuse) in a cemetery of Nagoya City. *Japanese Journal of Sanitary Zoology* **25**, 41–49.

Mangel, M. (2006) *The Theoretical Biologist's Toolbox: Quantitative Methods for Ecology and Evolutionary Biology*. Cambridge, Cambridge University Press.

Melbourne, B.A. & Hastings, A. (2008) Extinction risk depends strongly on factors contributing to stochasticity. *Nature* **454**, 100–103.

Miyagi, I. (1972) Feeding habits of some Japanese mosquitoes on cold-blooded animals in laboratory. *Tropical Medicine* **14**, 203–217.

Mogi, M. (1996) Overwintering strategies of mosquitoes (Diptera: Culicidae) on warmer islands may predict impact of global warming on Kyushu, Japan. *Journal of Medical Entomology* **33**, 438–444.

Moriya, K., Harada, F. & Yabe, T. (1967) Some observations on mosquitoes occurring in the septic tanks in Kanagawa Prefecture II. *Medical Entomology and Zoology* **18**, 247–255.

Muslim, A., Fong, M.-Y., Mahmud, R., Lau, Y.-L. & Sivanandam, S. (2013) *Armigeres subalbatus* incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia. *Parasites and Vectors* **6**, 219.

Nakata, G. & Ito, S. (1955) Ecological studies on mosquitoes about Kyoto City (2): on the annual succession of the mosquitoes captured by the light trap. *Japanese Journal of Sanitary Zoology* **6**, 82–93.

Oda, T., Wada, Y., Kurokawa, K., Ueda, M. & Ito, T. (1978) Studies on the overwintering of the mosquito *Armigeres subalbatus* in Nagasaki area. *Tropical Medicine* **20**, 157–166.

Rajavel, A.R. (1992*a*). Cannibalistic behavior in *Armigeres subalbatus* (Diptera: Culicidae). *South East Asian Journal of Tropical Medicine and Public Health* **23**, 453–457.

Rajavel, A.R. (1992*b*). Larval habitat of *Armigeres subalbatus* (Coq) and its characteristics in Pondicherry. *South East Asian Journal of Tropical Medicine and Public Health* **23**, 470–473.

Senior-White, R. (1925) Physical factors in mosquito ecology. *Bulletin of Entomological Research* **16**, 187–248.

Shumway, R.H. & Stoffer, D.S. (2011) *Time Series Analysis and its Applications*. 3rd edn. New York, Springer.

Silver, J.B. (2008) *Mosquito Ecology: Field Sampling Methods*. 3rd edn. New York, Springer.

Sun, W.K.C. (1964) The seasonal succession of mosquitoes in Taiwan. *Journal of Medical Entomology* **1**, 277–284.

Tanaka, K., Mizusawa, K. & Saugstad, E.S. (1979) A revision of the adult and larval mosquitoes of Japan (including the Ryukyu Archipelago and the Ogasawara Islands) and Korea (Diptera: Culicidae). *Contributions of the American Entomological Institute* **16**, 1–987.

Tsuda, Y., Takagi, M. & Wada, Y. (1994) Ecological study on mosquito communities in tree holes in Nagasaki, Japan, with

special reference to *Aedes albopictus* (Diptera; Culicidae). *Japanese Journal of Sanitary Zoology* **45**, 103–111.

Tsuda, Y., Maekawa, Y., Saita, S., Hasegawa, M. & Takagi, M. (2003) Dry ice-trap collection of mosquitoes flying near a tree canopy in Nagasaki, Japan, with special reference to *Aedes albopictus* (Skuse) and *Culex pipiens pallens* Coquillett (Diptera : Culicidae). *Medical Entomology and Zoology* **54**, 325–330.

Turchin, P. (2003) *Complex Population Dynamics*. Princeton, Princeton University Press.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. New York, Springer.

Weathersby, A. (1962) Colonization of six species of mosquitoes in Japan. *Mosquito News* **22**, 31–34.

Yang, G.-J., Bradshaw, C., Whelan, P. & Brook, B. (2008*a*). Importance of endogenous feedback controlling the long-term abundance of tropical mosquito species. *Population Ecology* **50**, 293–305.

Yang, G.-J., Brook, B.W., Whelan, P.I., Cleland, S. & Bradshaw, C.J.A. (2008*b*). Endogenous and exogenous factors controlling temporal abundance patterns of tropical mosquitoes. *Ecological Applications* **18**, 2028–2040.

Zea Iriarte, W.L., Tsuda, Y., Wada, Y. & Takagi, M. (1991) Distribution of mosquitoes on a hill of Nagasaki city, with emphasis to the distance from human dwellings. *Tropical Medicine* **33**, 55–60.

Zhang, X.Z., Huang, K.J., Wang, L.K., Peng, X.W., Fu, X.Z. & Liu, H.L. (1992) A study on the ecological habit of *Armigeres subalbatus* in Dawa area of the Mengshan Mountain in Shandong Province. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*. **10**, 48–51.