

Invasion of *Wolbachia* at the residential block level is associated with local abundance of *Stegomyia aegypti*, yellow fever mosquito, populations and property attributes

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Abstract. *Wolbachia* can suppress dengue and control mosquito populations and this depends on the successful invasion of *Wolbachia*-infected mosquitoes into local populations. Ovitrap data collected during the recent invasion of wMel-infected *Stegomyia aegypti* (Diptera: Culicidae) (Linnaeus) into Gordonvale near Cairns, Australia, were used to identify variables that help predict the success of localized invasion. Based on the variance in *Wolbachia* frequencies across Gordonvale as well as at another release site at Yorkeys Knob in comparison to simulations, it was estimated that on average 2–4 females contributed eggs to an ovitrap. By collating ovitrap data from two collection periods at the start of the release from residential blocks, it was found that uninfected mosquitoes had a patchy distribution across the release site. Residential blocks with relatively high uninfected mosquito numbers were less easily invaded by *Wolbachia* than blocks with low numbers. The numbers of uninfected mosquitoes in ovttraps were negatively correlated with the proportion of brick houses in a residential block, whereas local *Wolbachia* frequencies were correlated positively with this variable as well as negatively with the amount of shading in a yard and availability of breeding sites. These findings point to proxy measures for predicting the ease of localized invasion of *Wolbachia*.

Key words. *Stegomyia aegypti*, *Wolbachia*, density, invasion success, ovitrap, sampling, spatial variation.

Introduction

There is currently growing interest in the release of modified *Stegomyia* mosquitoes into natural populations to decrease disease transmission through the introduction of *Wolbachia* (Hoffmann *et al.*, 2011) or to suppress populations through sterile releases involving *Wolbachia* (O'Connor *et al.*, 2012) or genetically modified mosquitoes (Harris *et al.*, 2011; Wise de Valdez *et al.*, 2011). When determining the number of individuals for

release, accurate estimates of population size across space are required. For instance, introductions of *Wolbachia* infections require the frequency of the infection to exceed an unstable equilibrium point which varies depending on the *Wolbachia* strain (Turelli, 2010; Hancock *et al.*, 2011; Walker *et al.*, 2011). Overcoming this unstable point will depend on the overall size of the mosquito population along with any spatial heterogeneity resulting in local areas that are difficult to invade (Barton & Turelli, 2011; Hancock & Godfray, 2012).

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A variety of techniques are used to routinely sample *Stegomyia aegypti* (Linnaeus) from wild populations for the purposes of surveying population numbers particularly from the perspectives of assessing control methods and determining disease dynamics. Commonly used methods include ovitraps to sample immature stages from ovipositing females (Fay & Eliason, 1966) and 'sticky' ovitraps that collect adults ovipositing in traps (Ritchie *et al.*, 2003; Chadee & Ritchie, 2010). Other approaches include surveying of pupae in containers, aspiration of adult mosquitoes from resting sites (Focks & Chadee, 1997; Barrera, 2009) and sampling of adults using traps such as the Biogents Sentinel Trap (BGS) (Krockel *et al.*, 2006).

Sampling of immature stages from ovitraps represents a relatively simple approach that can give a high level of spatial variation across a release site (Hoffmann *et al.*, 2011). However, this approach may lead to inaccurate estimates of local population size and invasion dynamics if only a few females oviposit on a resource. *Stegomyia aegypti* is thought to use skip oviposition to spread its eggs across breeding sites (Reiter, 2007) but multiple offspring may still be commonly expected from the same site. This issue is important not only for population manipulations but also when sampling for genetic surveys or when estimating the distribution of life stages in populations. To overcome it, genetic markers have been advocated as a way of estimating the number of females ovipositing in a breeding site, and these suggest that many females contribute only a few offspring per site, with several families contributing per container (Apostol *et al.*, 1993, 1994; Colton *et al.*, 2003).

Ovitraps can be set out in large numbers and provide a detailed picture of invasions across an area which may be essential when using adaptive management to locally increase or decrease release rates to counter areas of high mosquito abundance (Hoffmann *et al.*, 2011). Moreover, ovitrap data may also point to other measures that can be used as proxies for estimating local population density across an area (Williams *et al.*, 2006) and developing treatments such as pesticide applications (Chadee, 2009). Potential proxies of local mosquito density in northern Queensland include the availability of flooded containers in yards for breeding (Williams *et al.*, 2013) and the frequency of houses that are unscreened. Unscreened houses are typically older wooden pole homes referred to as 'Queenslanders' and often harbour large numbers of adult *S. aegypti* and a higher incidence of dengue than newer suburbs with screened concrete block houses (Hanna *et al.*, 2006; Ritchie *et al.*, 2011). While these are unlikely to indicate cryptic breeding sites such as subterranean habitats (Kay *et al.*, 2000), they might nevertheless provide an indication of changes in relative mosquito densities across an area because adult females have access to humans, although the relative importance of mosquito sources is expected to vary across sites (Tun-Lin *et al.*, 1995b).

The present study tests whether fine-scale data collected from ovitraps in recent successful introductions of the wMel strain of *Wolbachia* into a North Queensland population of *S. aegypti* could be used to predict local invasion success within the release areas. The distribution of *Wolbachia* frequencies across ovitraps placed in release areas were used to estimate the number of females contributing to each ovitrap in each release, and also compare variance estimates across release blocks derived from ovitraps. The ability of the ovitraps to provide information

on local *Wolbachia* frequencies for adaptive management was considered, by assessing spatial structure across traps and testing whether areas where local invasions were slow coincide with those where ovitraps suggested high numbers. The association between high density/low invasibility areas of mosquitoes and various attributes including housing density, proximity to the boundary of the release site, nature of housing (screening/air conditioning, raised/flat) and property shading was then assessed. Results are discussed within the context of facilitating invasion in future releases.

Materials and methods

Ovitrap data

Data comes from two releases carried out in North Queensland populations in 2011 described in Hoffmann *et al.* (2011). Ovitraps were placed two per property in the period of the release and immediately afterwards (when numbers were reduced to one per property). They consisted of 1200-mL plastic buckets with an oviposition strip consisting of red fabric clipped to the side of the bucket and extending into the water contained in the bucket; a 0.5-g alfalfa (lucerne) pellet was added to create an infusion to attract gravid *S. aegypti* (Ritchie, 2001).

Ovitraps were set up to estimate *Wolbachia* frequencies after release and estimate the distribution of adults prior to release. From each bucket, a maximum of 10 *S. aegypti* larvae were sampled and scored for *Wolbachia* (Hoffmann *et al.*, 2011). The number of uninfected mosquitoes in a property was estimated by subtracting the number of infected mosquitoes from the total number scored in a bucket, with the maximum value being 20 (no infected larvae, samples of 10 mosquitoes retrieved from each of the two buckets placed at a property). These data were combined for larvae collected in the first two ovitraps events with traps at Gordonvale (24 January and 7 February) when only infected larvae derived from adults of the first release contributed to the adult population and the infection frequency was low. Yorkeys Knob was not considered because trapping was disrupted by cyclone activity and pesticide applications in response to a dengue case.

Under an even distribution of uninfected mosquitoes across the release site but patchy distribution of infected males, areas with a locally high infection rate were expected to exhibit a low number of uninfected larvae owing to cytoplasmic incompatibility. However, if there are areas with high mosquito numbers which are attractive (or support high survival) of both infected and uninfected mosquitoes, a positive correlation between uninfected and infected larval numbers was expected. High-density areas might arise from cryptic breeding sites or areas suitable for resting mosquitoes that are highly vegetated. Therefore, the number of uninfected larvae early in the release was considered. In addition, the frequency of infecteds in the later sampling dates before a very high frequency was reached (average of 7 and 21 March) was considered. This period was used to test if areas with an initial high abundance of mosquitoes showed lower frequencies. With a daily survival around 0.9 or less (Ritchie *et al.*, 2013a), adults contributing uninfected larvae early on would not have influenced *Wolbachia* frequencies a month later.

Spatial signature for infected and uninfecteds was detected based on Moran's *I* at distances of 100–300 m (see Results), and we therefore analysed data at the residential block level that encapsulated these types of distances. The blocks were defined in Hoffmann *et al.* (2011) and range from 8500 to 32 800 m² in area (and 60 to 300 m in length). Spatial associations in uninfected and infected larval numbers and *Wolbachia* frequencies were estimated across blocks for the early dates. In later releases, adaptive management (release of larger numbers of *Wolbachia*-infected mosquitoes) was undertaken at the block level to counter areas where infection frequencies remained low. However this would not have influenced larval data in the January–early February sampling period. As blocks contained data from a substantial number of ovitraps at Gordonvale (eight ovitraps/block in the initial period), block level effects at the presence/absence level at this location were also analysed, by considering the proportion of traps that were positive for uninfected or infected mosquitoes across the two sampling dates. These analyses follow on from the estimation of population size based on presence/absence data (Ritchie *et al.*, 2013a), and obviate issues associated with the low number of females that might contribute to ovitraps (see Results). It was then tested if blocks with a relatively high abundance of uninfected mosquitoes at the start of the trial had lower infection frequencies, in this case excluding blocks with adaptive management [see Hoffmann *et al.* (2011)].

Simulations

To estimate the number of females contributing to an ovitrap, the observed variance in *Wolbachia* frequencies across buckets with expectations based on simulated data sets where different numbers of females oviposited into a bucket were compared. In the simulations, the probability of a female being infected or uninfected (and compatible) is assigned randomly depending on the overall frequency of infection in the population (as estimated from larval data). Each female contributing eggs is assumed to have an equal probability of ovipositing in a bucket and maternal transmission of *Wolbachia* to offspring is assumed to be perfect. If X_i is an indicator random variable that is 1 if the *i*th larva is infected and 0 if uninfected (based on the infection status of its mother), values of X_i are summed across the 10 sampled larvae to produce infection frequencies for each container. The predicted variance in infection frequencies (angular transformed) from the buckets based on assessments of larval samples of 10 individuals is then determined. Simulations were based on 1000 replicates run with the PopTools add in for MS Excel. Simulated results were compared with actual variance estimates between buckets obtained from ovitrap collections during the invasion by *wMel* at Gordonvale and Yorkeys Knob which covered a range of *Wolbachia* frequencies (Hoffmann *et al.*, 2011). Only data from ovitraps where 9–10 larvae were sampled were used in computing the empirical variance estimates.

Predicting local mosquito numbers: surveys

Premises surveys. All premises in both the Gordonvale and Yorkeys Knob release sites were surveyed in November 2009 in

both release areas. Buildings were scored as being residential or commercial (shops or small businesses), and houses were scored as being low to the ground or raised off the ground with an open/closed area underneath. Buildings were also scored as being brick, wood or made of other material, and whether windows were screened or unscreened. House yards were scored for the degree of shade typically provided by high vegetation (trees, palms) in the yards. A survey of breeding containers was also undertaken but not used further because of a relatively low incidence of identified containers. The state of yards and potential breeding containers was however used in the surveys. The proportion of premises per block in a particular category was then computed and used in the analyses. Variables computed were the proportion of premises that (a) were classed as domestic rather than commercial (including holiday apartments), (b) had buildings low set to the ground without an open space underneath, (c) had buildings that were constructed from brick rather than another material, (d) had buildings where all windows were screened rather than open, (e) had yards that had some shade, (f) had yards that contained any potential breeding sites such as pot plant bases, bromeliads and other containers, and (g) had yards that contained rubbish, and that often had an overgrown lawn. Indices 5 and 7 are from the Premise Condition Index and scored from 1 to 3 (Tun-Lin *et al.*, 1995a,b). It was anticipated that mosquito numbers might have been higher in blocks where there was a high abundance of houses raised off the ground made from material other than brick and with unscreened windows, where yards were shady and contained breeding sites and rubbish (Tun-Lin *et al.*, 1995a; Vazquez-Prokopec *et al.*, 2010). To ensure that proportions were based on at least seven premises, data from adjacent blocks (in one case) had to be combined based on weighted averages.

Spatial analysis

ArcGIS 9.3, R ('ape' and 'vegan' libraries) and SAM v. 4.0 (Spatial Analysis in Macroecology) (Rangel *et al.*, 2010) was used to investigate spatial structure in the data and to link mosquito numbers and *Wolbachia* frequencies to block attributes. Initially the spatial structure in the original mosquito population was investigated by considering the average number of larvae sampled from the buckets in the early collections that were uninfected (see above), treating each premise where ovitraps were placed as a sampling point and considering both the number of traps that were positive for uninfected larvae and the number of uninfected larvae. Spatial structure was tested using the overall value of Moran's *I* computed in R, and then computed Moran's *I* for different distance classes and used 1000 permutations run in SAM to assess the significance of spatial structure at different scales. The lag of the spatial structure was confirmed by computing a semi-variogram in SAM. Because these analyses suggested spatial structure up to around 100–300 m, block data were used to assess the association between house attributes, the number of uninfected mosquitoes and the frequency of *Wolbachia* infection reflecting the ease of invasion within a block. The density of uninfected mosquitoes across space in the ovitraps was visualized across the release area using ArcGIS after ordinary kriging; this kriging method has been used previously

for *S. aegypti* ovitrap data and is considered appropriate because it assumes an unknown dynamic mean defined by nearby data values (Duncombe *et al.*, 2013). A visual representation of patterns for infected mosquitoes in the initial period of release and the *Wolbachia* frequencies across space was also provided.

It was initially considered whether the number of uninfected mosquitoes at the start of the release could predict the frequency of *Wolbachia* reflecting the ease at which a block became invaded. An ordinary least squares regression using block values was run to test this prediction. The regression residuals were then examined for spatial autocorrelation using Moran's I to test if there was any spatial structure left in the model, with values of 0 indicating a random association and a value of 1 indicating complete autospacial correlation. To test the association between measures on premises at the block level and the relative abundance of uninfected numbers in the ovitraps, multiple regressions with all premises variables included were used initially. The proportions of premises in a block falling into a particular category were arcsine transformed before analysis. Forward selection run in SPSS for Windows v. 19 (SPSS Inc., Chicago, IL, U.S.A.) was also used to identify variables in the multiple regression models contributing most to variation in mosquito numbers according to the forward selection procedure recommended for ecological data (Blanchet *et al.*, 2008). This process was then repeated to assess if premises features could be related to block level *Wolbachia* frequencies. Multicollinearity in these analyses was tested by examining correlations between housing attributes and by computing the variance inflation factor (VIF) in SPSS, which indicates how much the variance of the estimated coefficients are increased over the case of no correlation among variables. VIF values > 5 can indicate issues around multicollinearity (Rogerson, 2001) but in the present analyses they were < 2 and were therefore not deleted. Once predictive models were identified for premises features, residuals for spatial structure were tested using Moran's I. Correlations also computed at the block level between infected and uninfected mosquito numbers were spatially adjusted using the Dutilleul (1993) procedure where appropriate.

Results

Mosquitoes and Wolbachia

There was significant spatial structure for the average number of uninfected larvae caught in ovitraps in Gordonvale (Moran's $I = 0.037$, $P = 0.032$). When spatial patterns were considered at individual distance classes, Moran's I was highly significant by permutation ($P = 0.005$) at around 100 m but fell to 0 at 300 m and was not significant at other distance classes. Similarly, the semi-variogram based on the uninfected data produced a sill pattern at distances greater than 200 m, confirming that spatial structure was only evident over a short distance. By contrast, the number of infected mosquitoes in these early collections did not show evidence of spatial structure (Moran's $I = 0.013$, $P = 0.341$), as might be expected given the uniform release of infected mosquitoes across the site. The pattern produced by ordinary kriging suggested that there were high numbers of uninfected *S. aegypti* in two locations within Gordonvale, at the

south eastern and north eastern areas of the release (Fig. 1). Infected and uninfected numbers of mosquitoes were not correlated with each other, either with or without spatial correction (all P -values > 0.1 regardless of whether trap incidence or larval numbers were considered).

At the block level, the frequency of infected mosquitoes in March was predicted by the average number of uninfected larvae present early in the release ($R^2 = 0.198$, $P = 0.006$). When there were high numbers of uninfecteds in an area, there was a slower rate of invasion by the *Wolbachia* ($b = -0.15 \pm 0.002$) (Fig. 2). No spatial structure in the residuals of this relationship computed using SAM (Moran's I values not significant at any spatial scale) were found. In a geographically weighted regression that takes into account spatial structure, the R^2 value was much higher (0.692) than in the least square regression and the association was highly significant ($P < 0.001$) reflecting the strong spatial overlap between these variables. The spatial association is apparent from the maps produced by ordinary kriging (Fig. 1) which indicate clearly the low frequency of *Wolbachia* in the north eastern and south eastern areas where there was a high incidence of uninfected mosquitoes.

Females contributing to ovitraps

When compared with simulated results (Fig. 3), it was estimated that 2–4 females contributed offspring to each ovitrap across the trapping period as *Wolbachia* frequencies increased to near fixation. These estimates are similar for both release sites and suggest individual females contributing at least 2.5–5 eggs to a container based on the fact that 9–10 larvae were scored per container to obtain the empirical estimates.

Premise features

Data from Gordonvale are collated in Table 1 and presented alongside the same information from Yorkeys Knob. The latter is presented for comparative purposes because mosquito populations at that release site are lower based on counts of adults from Biogents Sentinel traps prior to and after the release period (Ritchie *et al.*, 2013a). Yorkeys Knob is characterized by a higher proportion of commercial residences, including holiday apartments, and also a higher proportion of low set brick houses which are often fully screened. However, the number of yards that are shaded and in poor condition is similar. Mosquito numbers are expected to be higher in areas with raised houses that are not well screened (typically not brick).

In Gordonvale, a linear regression analysis of the number of uninfected larvae onto premises variables indicated an R^2 of 0.424 when all premises features were included in the regression model, with a significant effect of the proportion of brick houses ($P = 0.009$). Forward selection of the variables indicated that, once the proportion of brick houses was included in the model ($R^2 = 0.342$), no other variables contributed to the mean number of uninfected larvae in the blocks. An increase in brick residences in a block led to a sharp decrease in mosquito numbers (Fig. 2). There was no spatial structure in the residuals based

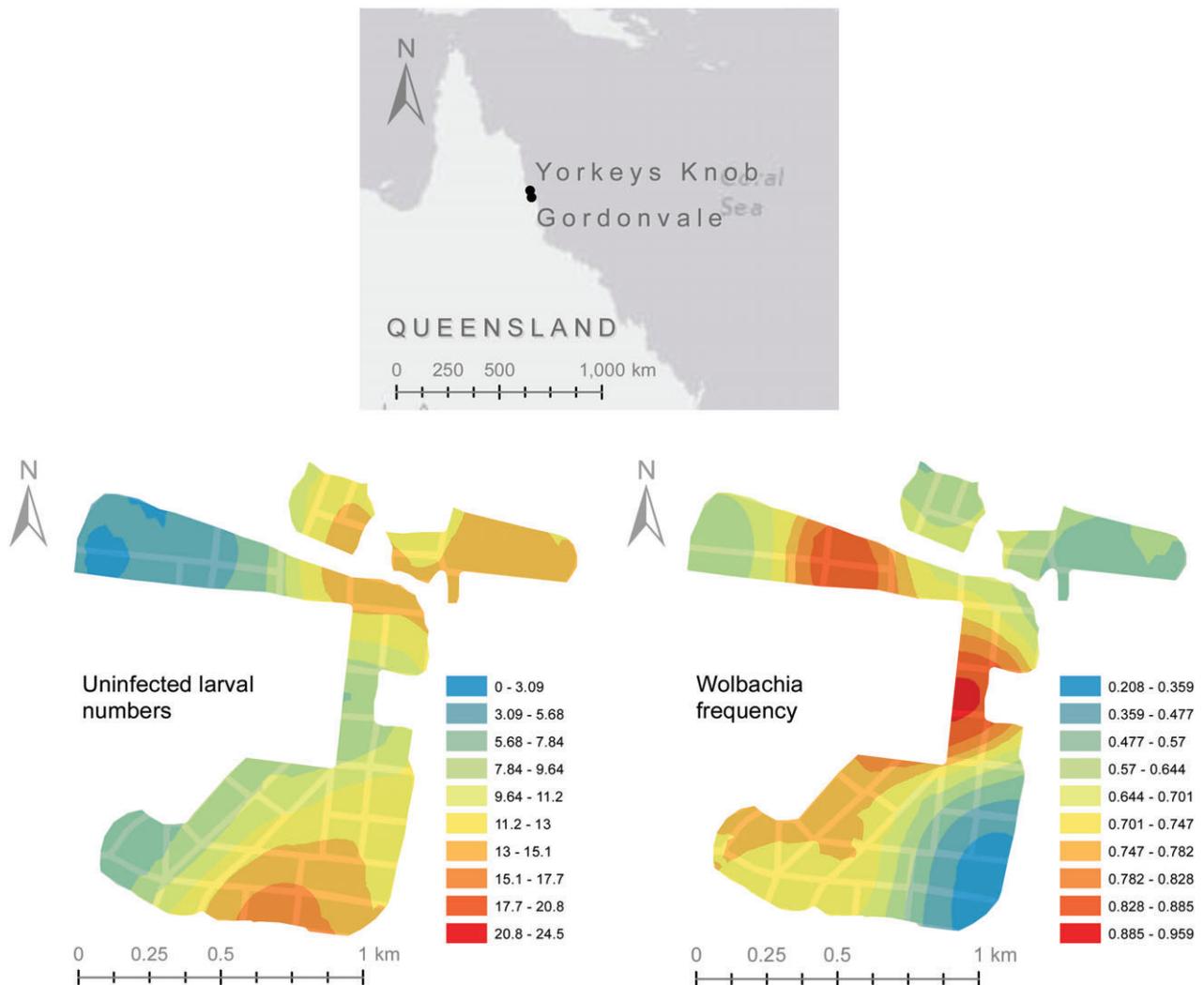


Fig. 1. Maps of the release area at Gordonvale with blocks showing the predicted distribution of uninfected larval numbers (at the start of release) and *Wolbachia* frequencies (during invasion) based on block means. Maps were produced in ArcGIS using ordinary kriging. Inset on top shows location of original release areas discussed in text.

on Moran's I once this predictor variable had been included, and the brick term remained significant in a geographically weighted regression ($P = 0.003$). Note that although brick houses were the best predictor of mosquito numbers, this variable was correlated with others and particularly screening to some extent, with a correlation of 0.63 between these variables based on block values.

For the *Wolbachia* frequency recorded in the blocks, the overall linear regression had an R^2 of 0.415 and there were significant effects of whether houses were brick ($P = 0.012$) and the proportion of premises with shade ($P = 0.037$). Forward selection led to these variables being included in the model ($P = 0.018$ for brick, $P = 0.040$ for shade) along with the presence of breeding sites in the garden ($P = 0.006$). The final regression coefficients were positive for the proportion of brick buildings in a block (0.171 ± 0.069) and negative for the proportion of shaded properties (-0.283 ± 0.128) and the presence of breeding sites in the garden (-0.390 ± 0.133). There was no spatial structure in the

residuals once this model had been fitted based on Moran's I. In contrast to these results, none of the variables had a significant effect on the number of infected larvae in containers at the start of the releases (i.e. at the time the uninfected larvae were scored).

Discussion

The results suggest that local invasion of *Wolbachia* relates to the number of mosquitoes in an area when assessed at the level of residential blocks. In Gordonvale, numbers of mosquitoes were particularly variable with the average number per trap varying by a factor of 4 or more (Fig. 3). This resulted in a difference of around 30% in *Wolbachia* frequencies in the blocks. These patterns can assist in providing guidelines for the release of mosquitoes at a local scale. For instance, it is

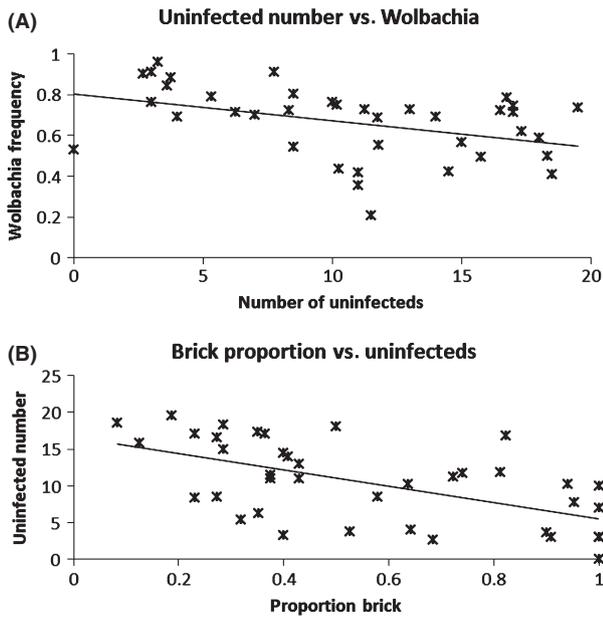


Fig. 2. Relationship between (A) number of uninfected larvae in two traps averaged per residential block at the start of the release and *Wolbachia* frequency per block in Gordonvale, and (B) proportion of buildings in the same block that were brick and number of uninfected larvae in traps in Gordonvale.

suspected that it would be worth doubling release numbers in blocks with relatively high mosquito numbers to counter differences in uninfected mosquitoes. Ovitrap provided a way of making such assessments accurately across a block in spite of the limited number of females contributing offspring to them, which is clearly substantially less than the number of larvae in the containers.

The reasons for this pattern probably reflect the presence of suitable breeding sites and blood-feeding areas, resulting in a higher density of mosquitoes in some parts of Gordonvale. Under a predicted daily mortality of adult mosquitoes of 0.9 (Ritchie *et al.*, 2013a), very few if any of the uninfected adults contributing larvae in the initial ovitrap monitoring periods would have affected *Wolbachia* frequencies of larvae monitored a month later. However, conditions within areas of Gordonvale favourable to breeding and blood feeding presumably would

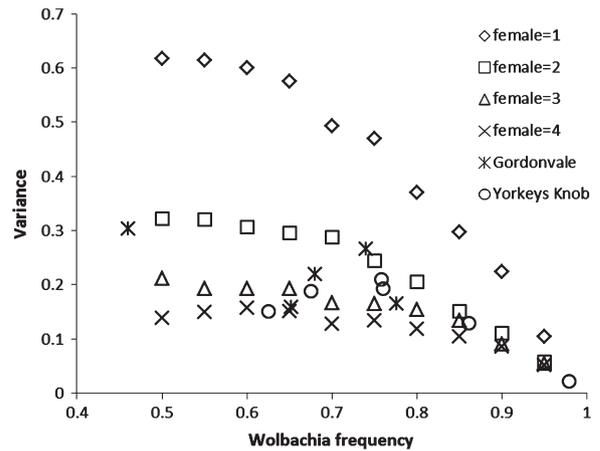


Fig. 3. Impact of different numbers of ovipositing females on variances in *Wolbachia* frequencies among ovitraps. Simulations (indicated by ‘females = 1 females = 4’) reflect 1000 simulated datasets at different frequencies of *Wolbachia*. The observed variances at different trapping times when *Wolbachia* was present at different frequencies are also indicated for Gordonvale and Yorkeys Knob data separately.

have persisted across this period, resulting in the observed negative association between uninfected numbers and *Wolbachia* frequency. Although the main sources of mosquito larvae can change across time, there is also abundant evidence that key households and containers are responsible for much of the production of adults within a local area and that these key households can persist across time (Tun-Lin *et al.*, 1995b; Focks & Chadee, 1997). These effects may be further enhanced by any decrease in the competitive ability of infected larvae relative to uninfected larvae. When in competition with uninfected larvae, *wMel S. aegypti* larvae tend to develop somewhat later and also eclose at a larger size compared with uninfected larvae (Ross *et al.*, 2014), which may decrease their field fitness and slow invasion rates although the infected larvae may then also have a higher fecundity.

At least some of this variability in mosquito numbers and invasion dynamics appears to be associated with premises features. In Gordonvale, there was a high level of variability among blocks in the material used for residences, whether they were low to the ground, and whether they were screened. The number of raised houses lacking screening and made of timber was far higher than

Table 1. Premises features of the 2011 release sites: proportion of premises falling into different categories. Means, minimum values and maximum values per block are given (see text for further details).

Proportions per block	Gordonvale			Yorkeys Knob		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Residential buildings	0.93	0.36	1	0.76	0.23	1
Buildings low to the ground	0.41	0	0.95	0.82	0.67	1
Brick buildings	0.55	0.08	1	0.94	0.75	1
Fully screened buildings	0.29	0	0.875	0.87	0.58	1
Yards with shade	0.78	0.38	1	0.63	0.12	1
Yards with breeding sites	0.16	0	0.50	0.13	0	0.42
Yards with rubbish	0.09	0	0.36	0.08	0	0.33

in Yorkeys Knob, as were the mosquito numbers; previous estimates based on BGS traps suggest that numbers in Gordonvale are around twice as high as those in Yorkeys Knob (Ritchie *et al.*, 2013a). Although the reasons for this difference are not entirely clear, it seems likely that high set houses not constructed from brick may provide additional breeding or adult blood feeding and harbourage sites. In addition, there was an effect of shade and yard features increasing breeding areas and potential resting sites. When all three factors were considered, the resulting multiple regression equation had a substantial impact on relative *Wolbachia* frequencies. For instance based on the results of the multiple regression analysis, changing the percentage of properties in a block constructed with brick, having shade and having potential breeding containers in a yard from 20% to 80% is expected to alter the frequency of *Wolbachia* from 42% to 74%.

Although an association between the number of uninfected larvae and both *Wolbachia* frequencies and some premises features was found, the associations we detected should be regarded as conservative. Numbers of uninfecteds were underestimated at the start because only 20 larvae were sampled over two traps, and not the overall number of larvae in a trap. This also makes it unlikely that the data could have been used effectively to detect areas favourable for both uninfected and infected mosquitoes: because a maximum of 20 larvae were collected from each trap and scored for infection frequency, the null expectation under all traps providing 20 larvae for assessment is a negative association between the number of infected and uninfected larvae. The absence of an association between numbers of infected and uninfected larvae may therefore have masked a positive association between numbers of the two types of mosquitoes.

The estimate of 2–4 females contributing to containers based on variances in *Wolbachia* frequencies among containers is lower than estimates from molecular analyses of containers in domestic environments; for instance Colton *et al.* (2003) estimated an average of 6.2 females contributed offspring to containers in Trinidad, West Indies, whereas Apostol *et al.* (1994) found that many families contributed only 1–2 offspring per container sampled from Puerto Rico. The present findings suggest that in the Cairns region females may engage in ongoing oviposition in the same container rather than skipping actively between containers. This may reflect a limited number of containers available for oviposition. It should be noted however that the ovitraps represent containers that are only present for a short interval in the field. They were left out for a week, but are relatively unattractive to gravid mosquitoes for the first 2 days after addition of the alfalfa pellet.

The local spatial distribution of mosquitoes will influence the spread as well as local dynamics of *Wolbachia* across an area. In the presence of an unstable equilibrium point, *Wolbachia* spread is expected to be dramatically slowed in regions where there is a high density of uninfected mosquitoes but sped up by areas of low density (Barton & Turelli, 2011). The data presented here suggest that these areas could be predetermined through grids of ovitraps [or other traps such as the BGS and the recently developed Gravid *Aedes* trap (Ritchie *et al.*, 2013b)] and also through assessing premises features. Currently there are releases in areas of central Cairns where such assessments could be made. In the long run, premises features and preliminary entomological data could be used to predict optimal release

strategies across an area to ensure that invasion is achieved within a particular time interval.

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Author's declaration of interests

No competing interests have been declared.

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