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Evidence for adaptive divergence of thermal responses among *Bemisia tabaci* populations from tropical Colombia following a recent invasion

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Abstract

There is an increasing evidence that populations of ectotherms can diverge genetically in response to different climatic conditions, both within their native range and (in the case of invasive species) in their new range. Here, we test for such divergence in invasive whitefly *Bemisia tabaci* populations in tropical Colombia, by considering heritable variation within and between populations in survival and fecundity under temperature stress, and by comparing population differences with patterns established from putatively neutral microsatellite markers. We detected significant differences among populations linked to mean temperature (for survival) and temperature variation (for fecundity) in local environments. A $Q_{ST} - F_{ST}$ analysis indicated that phenotypic divergence was often larger than neutral expectations ($Q_{ST} > F_{ST}$). Particularly, for survival after a sublethal heat shock, this divergence remained linked to the local mean temperature after controlling for neutral divergence. These findings point to rapid adaptation in invasive whitefly likely to contribute to its success as a pest species. Ongoing evolutionary divergence also provides challenges in predicting the likely impact of *Bemisia* in invaded regions.

Introduction

Populations of ectotherms have the potential to diverge genetically in response to stressful thermal conditions. Populations of widespread *Drosophila* species in particular have diverged for a range of traits including tolerance of thermal extremes (Hoffmann *et al.*, 2002; Hoffmann, 2010; Sgrò *et al.*, 2010; Sisodia & Singh, 2010; Rajpurohit & Nedved, 2013) diapause characteristics (Schmidt *et al.*, 2005; Schmidt & Conde, 2006; Schmidt & Paaby, 2008) and fertility (Rohmer *et al.*, 2004). Most of these studies have focused on latitudinal gradients, although some studies have also considered elevation gradients (Parkash *et al.*, 2005; Sørensen *et al.*, 2005; Collinge *et al.*, 2006; Rashkovetsky *et al.*, 2006). However, geographic divergence does not always occur; in some cases, there is limited genetic divergence in some species even if populations are exposed to a range of climatic conditions (Kimura *et al.*, 1994; Sarup *et al.*, 2009) and it also depends on the trait used to measure tolerance (Sørensen *et al.*, 2005; Sgrò *et al.*, 2010).

For other ectotherm groups, data remain fairly limited. However, there is now good evidence of variation in thermal responses in at least some species and examples include variation in resistance of thermal extremes and life-history changes in *Orchesella cincta* springtails along latitudinal gradients (Bahrndorff *et al.*, 2006; Liefting & Ellers, 2008) and changes among populations of the butterfly *Lycaena tityrus* along elevation gradients (Karl *et al.*, 2008). There is also evidence from invasive species that thermal responses can evolve relatively quickly, as in the case of changes in diapause characteristics in the mosquito *Aedes albopictus* (Lounibos *et al.*, 2003).

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Where differences among populations are detected, they are usually interpreted in terms of natural selection, particularly where correlations between traits and environmental conditions follow expectations (e.g. Hoffmann *et al.*, 2002). However, this is not necessarily the case because the thermal conditions experienced by populations might be poorly represented by data from weather stations that normally provide the basis for such comparisons. Moreover, genetic divergence among populations might arise from genetic drift or patterns of gene flow unconnected to local adaptation (Lande, 1992; O'Hara, 2005; Whitlock, 2008; Whitlock & Guillaume, 2009; Volis & Zhang, 2010). This can be tested by comparing patterns of variation in traits with those in neutral markers like microsatellites in a $Q_{ST} - F_{ST}$ comparison (Spitze, 1993; Whitlock, 2008), and/or by comparing the strength of variation along gradients in quantitative traits with those in neutral markers, (Gockel *et al.*, 2001; Volis *et al.*, 2005; Whitlock, 2008; Whitlock & Guillaume, 2009). Some comparisons of this nature have been undertaken for thermal responses (e.g. Kavanagh *et al.*, 2010), but they are still relatively rare.

In this study, we test for adaptive divergence in a group of species rarely considered in these types of comparisons: invasive species in the tropics. Temperature variation in the tropics is often driven by elevation rather than seasonality, resulting in continuous selection pressures favouring responses to different temperatures. Because extreme temperatures cannot be avoided by diapause during unfavourable seasons (c.f. Schmidt & Paaby, 2008), differences among populations should be more easily interpretable (Sgrò *et al.*, 2010).

We consider the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), one of the most economically important agricultural pests in the world. Because there are many biotypes (Oliveira *et al.*, 2001; Perring, 2001) and/or cryptic species composing *B. tabaci*, here we use biotype B to refer to the invasive Middle East-Asia Minor 1 (MEAM 1) clade based on phylogenetic analyses (De Barro *et al.*, 2011). This is the most aggressive species of the *B. tabaci* complex, whose distribution includes areas and seasons with very frequent stressful temperatures up to 42 °C (Oliveira *et al.*, 2001; Perring, 2001). Thermotolerance might therefore be an important component of the colonizing success of this species. *B. tabaci* biotype B was first found in Colombia in 1995 (Quintero *et al.*, 1998, 2001) and has expanded its distribution to almost all agricultural ecosystems in this country. Its distribution covers two important and different regions: the Caribbean region at sea level where temperatures are high, and the Southwest region, which is heterogeneous due to the presence of the Andes Mountains. Although these regions are tropical, temperatures vary across these *B. tabaci* populations (Quintero *et al.*, 2001) leading to the potential for local thermal adaptation.

We address a number of questions. (1) Is there phenotypic divergence among populations for thermal responses? Data were collected on thermal resistance traits in multiple populations from the two regions differing in average temperature. (2) Is there evidence for divergence in plasticity among the populations? We tested whether stress resistance could be increased by hardening and then compared levels of hardening resistance among the populations. (3) Is the phenotypic divergence related to environmental variables, and are they larger than expected based on neutral expectations? We linked thermal responses of populations to environmental conditions and also compared estimates of additive genetic variance within and among populations to patterns obtained from microsatellite markers by a $Q_{ST} - F_{ST}$ analysis.

Materials and methods

Sampling and isofemale lines

Insects were collected in crops in nine locations from two different regions in Colombia (Southwest region: Andes Mountains, and Caribbean region: Atlantic coast) (Table 1 and Fig. 1). These regions are separated by 480 km and they differ in topography, temperature regimens, humidity and host plants for whitefly. The Southwest region covers different elevations and whitefly populations can range between 1000 and 1500 m asl. The Caribbean region is close to the Atlantic coast, representing a flat area near to sea level. Six locations were sampled from the Southwest region from a range of elevations, and three locations were sampled from the Caribbean region. Whiteflies from all populations were previously identified as biotype B or MEAM 1 clade (De Barro *et al.*, 2011) by mitochondrial COI gene sequences following Shatters *et al.* (2009) and Díaz (2013).

Leaves with nymphs were collected in crops, and nymphs were reared to the adult stage in the laboratory. More than 100 adults (males and females) were collected, and the adults were left to mate for 20 days on bean plants (*Phaseolus vulgaris* – ICA Pijao) at 25 °C

Table 1 Colombian populations of *B. tabaci* sampled and host plants.

Population	Code	Latitude, longitude	Host plant of origin
Southwest			
Rozo	ROZ	3.61639, −76.3892	Soybean
Pradera	PRA	3.43333, −76.2333	String bean
Candelaria	CAN	3.33333, −76.3333	Tomato
Cajamarca	CAJ	4.47722, −76.21389	Cucumber
Pavas	PAV	3.675, −76.5836	String bean
Regaderos	REG	3.75889, −76.2153	Tomato
Caribbean			
Ret.Indios	RIN	8.8575, −75.81444	Eggplant
Trementino	TRE	8.81667, −75.4667	Eggplant
Sampues	SAM	9.18361, −75.3817	Eggplant

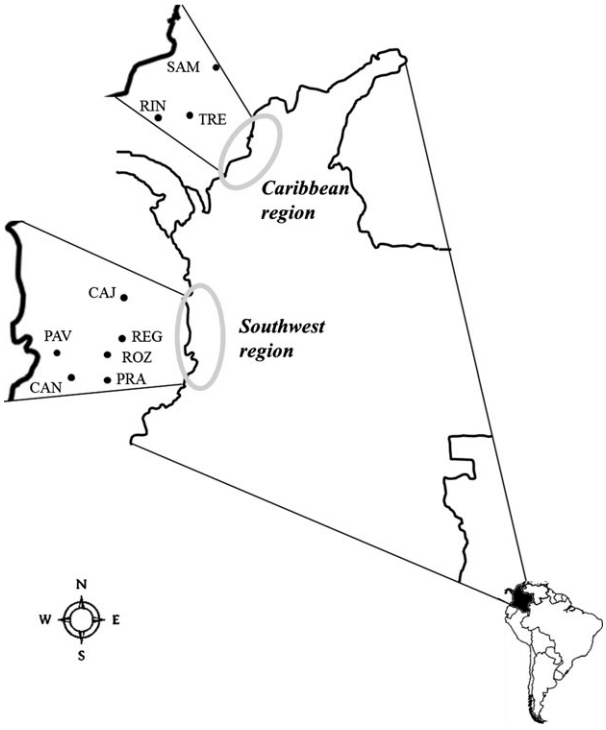

Fig. 1 Populations of the whitefly *B. tabaci* analysed in this study from Colombia. The codes for each sample are as in Table 1.

and 65% RH under a 12L/12D photoperiod. In the offspring generation, pairs of males and females were placed together on new plants to establish isofemale lines. Between 4 and 11 isofemale lines were established per population. Heat-shock experiments were conducted at the third generation of these isofemale lines to generate sufficient insects for experiments and to avoid field-related maternal effects [although persistent cytoplasmic effects were not controlled in this design and these might include endosymbionts in *B. tabaci* (Brumin *et al.*, 2011)].

To assess local temperatures experienced by flies in the field, we used iButton data loggers (DS1923; Maxim Integrated Products, Inc., San Jose, CA, USA). These loggers were left in cloth net bags and placed for 1 year close to the crops where flies were collected. Tropical temperatures in Colombia vary by day rather than across months or seasons, and temperature was measured at hourly intervals. Average daily temperature across the 24 h and temperature variation (maximum – minimum temperature) per day were calculated.

Heat-shock experiments

Adults were heat shocked 1–24 h after emergence under two different treatments, a basal treatment and a hardening treatment. Treatments were applied in a climatic chamber (MLR 351H, Sanyo Electric Co., Ltd., Osaka, Japan), with 25 °C used as the control temperature and RH at around 65%. For the basal thermotolerance treatment, a heat shock of 45 °C for 1 h was applied. For the hardening treatment, a pretreatment of 40 °C for 1 h (followed by 25 °C for 1 h) was applied prior to the heat shock at 45 °C for 1 h.

Two life-history traits related to fitness in *B. tabaci* were measured: survival (for males and females) and fecundity of the females.

Survival

Males and females were placed individually in test tubes (2 × 0.5 cm diameter) covered with netting. Ten individuals per sex were set up for each estimate of survival. Immediately after the heat shock, individuals were placed at 25 °C for 3 h to permit recovery. The number of adults recovered was counted, considering as dead those individuals that were immobile and/or did not walk after tubes were shaken. We scored 5–20 replicates per isofemale line.

Fecundity of females

This was evaluated in the surviving females, 3 h after heat shock. Male–female pairs of *B. tabaci* were placed in clamp cages (1 cm^3) on the underside of bean leaves (*Ph. vulgaris* ICA – Pijao) at 25 °C. Seven days later, pairs were removed and the number of eggs counted. Not all females survived the 7 days period; in those cases, time to death after heat shock (in days) was recorded. All controls survived. We scored 5–26 replicate individuals per isofemale line.

Hardening-basal difference as a measure of phenotypic plasticity

The difference between hardening and basal treatment of an isofemale line was used as indicator of phenotypic plasticity in thermal tolerance and was estimated for both survival and fecundity.

Statistical analyses

Survival and fecundity data were analysed using a General Linear Mixed Model, with regions (Southwest and Caribbean), thermotolerance treatment (basal and hardening) and sex (for survival) as fixed factors. Populations (nested in regions) and isofemale lines (nested in populations) were considered as random factors as well as their interactions with other factors, but not for the plasticity analysis because plasticity was computed based on strain means. Survival data were normalized using angular transformation, whereas fecundity data were untransformed. Both traits then followed a normal distribution and treatment variances were homogenous based on Kolmogorov–Smirnov and Bartlett tests. Statistical analyses were performed with the package GLM in SAS 9.2 software (SAS Institute, Inc., Cary, NC, USA). Total fecundity (number of eggs after the

7 days oviposition period) and the fecundity of females per day were considered in the GLM, but led to similar results, so here we show only the analyses for total fecundity. The difference between hardening and basal treatment for survival and fecundity was used as a measure of phenotypic plasticity. Because environmental temperatures (mean and oscillation per day) are variable for the Southwest region and between regions, we explored the correlation between these measures and thermotolerance of the populations.

Comparing quantitative and neutral genetic differentiation

Historical processes, such as migration and genetic drift, are expected to affect neutral markers across the genome. However, selection affects only genomic regions contributing to the quantitative trait under selection (Whitlock & Guillaume, 2009). To test for the relative contributions of these evolutionary forces influencing the population differentiation in thermotolerance of the whitefly populations, comparisons between the genetic component of quantitative traits and neutral patterns were undertaken. These analyses were performed with a comparison between the quantitative genetic structure estimated by Q_{ST} for each thermotolerance trait (Whitlock, 2008) and the neutral genetic differentiation estimated with microsatellites markers by F_{ST} (Weir & Cockerham, 1984). We analysed the nine populations sampled for Q_{ST}; however, one population (CAN) was not included in the neutral structure analysis. To check the effect of the missing population for quantitative analysis, Q_{ST} was estimated with and without this population.

Neutral differentiation by F_{ST}

The molecular analyses across populations represents part of a broader project and are covered elsewhere (Díaz, 2013), but here we provide a short summary. We used nine microsatellites: BEM18 and BEM25 (De Barro *et al.*, 2003), BT-b69 (Tsagkarakou & Roditakis, 2003), P11 (Delatte *et al.*, 2006), mb02 and mb05 (Fontes *et al.*, 2012), 145 (Dalmon *et al.*, 2008), Btls1.6 (Gauthier *et al.*, 2008) and mb07 (not published, Genbank No: GF109952). DNA was extracted by modifying the method described by De Barro and Driver (1997). We used a system primer introducing a fluorescent dye during the PCR according to the study described by Blacket *et al.* (2012).

All microsatellites were in Hardy–Weinberg equilibrium, and there was no linkage disequilibrium in genotypic frequencies between pairwise comparisons of the loci for each population (Díaz, 2013) according to an exact test (Weir, 1996) performed with Genepop software version 4.2.1 (Raymond & Rousset, 1995). These tests were carried out by the Markov Chain method using 100 batches with 5000 permutations per batch. For these multiple comparisons, a Bonferroni correction was applied with a global significance level $\alpha = 0.05$.

The neutral structure of populations was estimated using θ (allele identity based) according to the study described by Weir and Cockerham (1984), which is appropriate for pairwise $Q_{ST} - F_{ST}$ analysis, because the variance among groups is calculated in the same way as for Q_{ST} (Whitlock, 2008). Alternative measures of population differentiations, F'_{ST} (Hedrick, 2005) and D_{est} (Jost, 2008), have been discussed recently as alternative measures to F_{ST}. These statistics measure population differentiation, but F_{ST} is a fixation measure (Whitlock, 2011). When mutation is important relative to migration, F_{ST} decreases with the population heterozygosity (Whitlock, 2011). However, the same is true for Q_{ST}, which invalidates the other statistics for comparisons with Q_{ST} (Edelaar & Björklund, 2011). Also, we used R_{ST} (allele size based) according to Slatkin (1995), which perform better than F_{ST} for microsatellites following a stepwise mutation model and when mutation is important relative to migration (Slatkin, 1995; Whitlock, 2011). Pairwise population differentiation was estimated using the alternative measures with the package diveRsity version 1.5.5 in R (Keenan *et al.*, 2013) for F'_{ST} (Hedrick, 2005) and D_{est} (Jost, 2008), GenAlEx software version 6.5 (Peakall & Smouse, 2012) and Arlequin software version 3.5.1.3 (Excoffier & Lischer, 2010) for F_{ST}; however, Mantel tests (Mantel, 1967) showed high correlations with F_{ST} (Díaz, 2013). We tested for the mutation effect on genetic structure on these measures and the microsatellite allele sizes (permuting alleles) using diveRsity version 1.5.5 in R (Keenan *et al.*, 2013) and SPAGeDi version 1.4 (Hardy & Vekemans, 2002) following Hardy *et al.* (2003), and those effects were not significant (Díaz, 2013). Taking into account these comparisons, we only used F_{ST} for $Q_{ST} - F_{ST}$ analyses.

Quantitative genetic differentiation by Q_{ST}

Q_{ST}s were estimated for each trait (survival and fecundity) in each thermotolerance treatment (basal and hardening) and also for males and females separately (survival). The statistical model for survival was a Generalized Linear Mixed Model, with binomial distribution and logit as a link function. For fecundity data, we used a General Linear Mixed Model because distribution for this trait was normal, and variances were homogenous based on Kolmogorov–Smirnov and Bartlett tests. In each model, isofemale line (nested in populations) and population were used as random factors. These statistical analyses were performed using the package GLIMMIX in sas 9.2 software (SAS Institute, Inc.). The model was applied for each pair of possible combinations between populations. Variance

components representing between-population genetic variance (V_b) and within-population genetic variance (V_w) were estimated to calculate Q_{ST} as follows (Whitlock, 2008):

$$Q_{ST} = V_b/(V_b + 2^*V_w).$$

V_b was estimated directly from the between-population variance component, and V_w was estimated from the among isofemale line variance component within populations (Hoffmann & Parsons, 1988).

Q_{ST} and F_{ST} analyses by Mantel test

When selection is responsible for phenotypic divergence, genetic variation should be greater for quantitative traits than neutral molecular differentiation ($Q_{ST} > F_{ST}$) (Whitlock, 2008). This analysis was carried out by comparing the average Q_{ST} and F_{ST} for all pairwise comparisons between populations; in addition, the geographic structure of Q_{ST} and F_{ST} values were compared by geographic and environmental distances (mean and oscillations for local temperatures) through Mantel tests (Mantel, 1967) with FSTAT software version 2.9.3.2 (Goudet, 1995). To meet Mantel test assumptions, all correlations were performed linearizing the structure indices (Rousset, 1997) through $F_{ST}/(1 - F_{ST})$ and $Q_{ST}/(1 - Q_{ST})$. To test whether neutral differentiation can explain directly the population structure on quantitative traits, a Mantel test was performed between Q_{ST} and F_{ST} distances. Then, a correlation between F_{ST} and Q_{ST} matrices and geographic distances (Ln km) was performed to test for isolation by distance (IBD). For F_{ST}, this correlation was also performed within regions because we found region effects in the structure of the populations (see Díaz, 2013), and the two regions are separated by a substantial distance. If IBD is principally responsible for neutral differentiation, the correlation between F_{ST} and geographic distances should remain within regions. This comparison was not performed for Q_{ST}s because we did not find region effects on thermotolerance traits (see Results).

The influence of environmental variation on quantitative genetic differentiation was tested by correlating Q_{ST} and environmental distances (Antoniazza *et al.*, 2010). Although Q_{ST} estimates could be correlated with environmental distances, this pattern may be a secondary consequence of neutral processes. To control these processes, we calculate pairwise differences between quantitative and neutral genetic indices ($Q_{ST} - F_{ST}$) to test for correlation between this matrix and the geographical and environmental distances (Antoniazza *et al.*, 2010; Hangartner *et al.*, 2011). If quantitative genetic differentiation is principally due to selection, a correlation will still be evident after the controlling for neutral processes.

Table 2 General linear mixed model analysis of variance for survival as a response to heat shocks.

Effect	d.f. (numerator)	d.f. (denominator)	Mean square	*F*	*P*
Region	1	7.05	2.48	1.65	0.240
Treatment	1	7.26	14.52	87.40	**<0.001**
Sex	1	7.67	2.06	14.78	**0.005**
Region* treatment	1	7.29	0.56	3.40	0.106
Region*sex	1	7.77	0.27	1.96	0.200
Treatment*sex	1	8.97	0.15	4.49	0.063
Reg*treat*sex	1	9.32	0.02	0.74	0.410
Population (reg)	7	15.71	1.71	4.65	**0.005**
Pop*treat (reg)	7	13.92	0.17	2.41	0.077
Pop*sex (reg)	7	8.22	0.15	3.14	0.064
Pop*treat*sex (reg)	7	42.98	0.03	0.66	0.704
Iso [pop(reg)]	47	35.53	0.18	1.74	**0.045**
Iso*treat [pop (reg)]	47	45.90	0.09	1.82	**0.022**
Iso*sex [pop (reg)]	47	47.04	0.06	1.33	0.168
Iso*treat*sex [pop (reg)]	46	13.87	0.05	0.90	0.662

Significant effects are highlighted in bold. Populations (pop) and isofemale lines (iso) were treated as random effects, whereas treatment (treat), sex and region (reg) were fixed effects.

Results

Comparisons between regions and populations

Survival after heat shock

Regions did not differ for survival (Table 2). Hardening significantly increased survival, and sex also had an effect due to higher thermotolerance of females. There were no interactions between region, treatment and sex. Variation due to populations within regions was significant, but there were no interactions between this

Table 3 General linear mixed model analysis of variance for fecundity as a response to heat shocks.

Effect	d.f. (numerator)	d.f. (denominator)	Mean squares	*F*	*P*
Region	1	6.98	7706.2	3.45	0.106
Treatment	1	6.98	1118.5	0.46	0.521
Region*treat	1	6.96	81.6	0.03	0.861
Population (reg)	7	7.87	2230.2	0.84	0.586
Pop*treat (reg)	7	46.66	2445.6	3.58	**0.004**
Iso [pop (reg)]	45	42.90	928.9	1.30	0.198
Iso*treat [pop (reg)]	43	671	716.3	2.22	**<0.001**

Significant effects are highlighted in bold. Populations (pop) and isofemale lines (iso) were treated as random effects, whereas treatment (treat), sex and region (reg) were fixed effects.

Table 4 General linear mixed model analysis of variance for plasticity of survival and fecundity as a response to heat shocks.

Effect	d.f. (numerator)	d.f. (denominator)	Mean squares	*F*	*P*
Survival plasticity					
Region	1	7.20	1245.1	4.47	0.071
Sex	1	9.03	291.6	8.69	**0.016**
Reg*sex	1	8.94	0.0	0.00	0.987
Population (reg)	7	7	289.5	9.29	**0.004**
Sex*pop (reg)	7	94	31.2	0.41	0.893
Fecundity plasticity					
Region	1	7.12	19.7	0.02	0.879
Population (reg)	7	43	800.9	3.07	0.010

Significant effects are highlighted in bold. Populations (pop) and isofemale lines (iso) were treated as random effects, whereas treatment (treat), sex and region (reg) were fixed effects.

factor and treatment or sex. Variation due to isofemale lines within populations and its interaction with treatment were significant, reflecting genetic variation within populations (Table 2).

Fecundity after heat shock

This trait was not affected by hardening or the regions from which whiteflies were sourced (Table 3). While variation due to populations within regions and isofemale lines was not significant, there were highly significant interactions between these random effects and treatment.

Phenotypic plasticity

When the difference between hardening and basal treatment (hardening basal) was used as a measure of plasticity, there was no significant region effect for either survival or fecundity (Table 4). Plasticity was significantly higher for females in the case of survival. Variation due to populations within regions was significant for both measures (Table 4).

Phenotype-environment correlations

Phenotypic plasticity for survival of females was negatively correlated with mean temperature per day, meaning that plasticity of survival decreased with local temperature (Table 5 and Fig. 2). The phenotypic plasticity for fecundity was correlated with local temperature oscillations per day, due to plasticity increasing with the size of the oscillations (Table 5 and Fig. 3). An association between environmental variation and genetic differences among populations was also evident from the Mantel tests comparing Q_{ST} values and temperature distances (Table 6). Plasticity for survival was significantly associated with mean temperature in females, whereas plasticity for fecundity was associated with temperature oscillations. In addition, survival following hardening was associated with mean temperature in females, whereas fecundity at basal treatment was associated with temperature oscillations (Table 6). These correlations were marginally nonsignificant in the population mean comparison (Table 5, Figs 2 and 3).

Table 5 Correlation coefficients (r, *P* value in brackets) between temperature variables and thermotolerance traits of the populations.

Thermotolerance	Temperature mean	Temperature oscillation
Survival – basal		
Males	−0.39 (0.299)	−0.17 (0.656)
Females	−0.29 (0.454)	−0.27 (0.481)
Survival – hardened		
Males	−0.50 (0.175)	−0.03 (0.932)
Females	**−0.60 (0.091)**	−0.27 (0.480)
Survival – plasticity		
Males	−0.46 (0.219)	0.10 (0.800)
Females	**−0.71 (0.032)**	−0.19 (0.621)
Fecundity		
Basal	**−0.63 (0.069)**	**−0.61 (0.082)**
Hardened	−0.32 (0.408)	0.45 (0.218)
Fecundity – plasticity	0.27 (0.482)	**0.84 (0.005)**

Significant and marginally nonsignificant effects are highlighted in bold.

Genetic structure of populations by microsatellites

There was significant neutral genetic structure among whitefly populations, due principally to a region effect (F_{RT} = 0.063, P = 0.011) rather that population differentiation within regions (F_{SR} = 0.015, $P < 0.001$). There was a significant correlation between pairwise estimates of neutral structure by F_{ST} and geographic distance (Mantel test, r = 0.57, P = 0.004); however, this correlation was not significant within regions (Mantel test, r = 0.33, P = 0.300), reflecting the fact that population genetic structure was due principally to the region effect rather than IBD within regions (Díaz, 2013).

Genetic variation in thermotolerance vs. F_{ST}

Q_{ST} values for survival and fecundity at basal and hardening treatments were higher than the average F_{ST} value (F_{ST} = 0.056 ± 0.008). Q_{ST} for survival of males under basal (Q_{ST} = 0.406 ± 0.055) and hardening (Q_{ST} = 0.392 ± 0.056) treatments were seven-fold higher than F_{ST}; it was five-fold higher than F_{ST} for females at basal (Q_{ST} = 0.291 ± 0.054) and hardened (Q_{ST} = 0.286 ± 0.052) treatments. Q_{ST} values for fecundity at basal treatment was three-fold higher than F_{ST} (Q_{ST} = 0.192 ± 0.040) and eight-fold higher for the hardened treatment (Q_{ST} = 0.459 ± 0.069).

The Q_{ST} values for the phenotypic plasticity of survival (Q_{ST} males = 0.132 ± 0.026, Q_{ST} females = 0.131 ± 0.029) and fecundity (Q_{ST} = 0.143 ± 0.043) were more

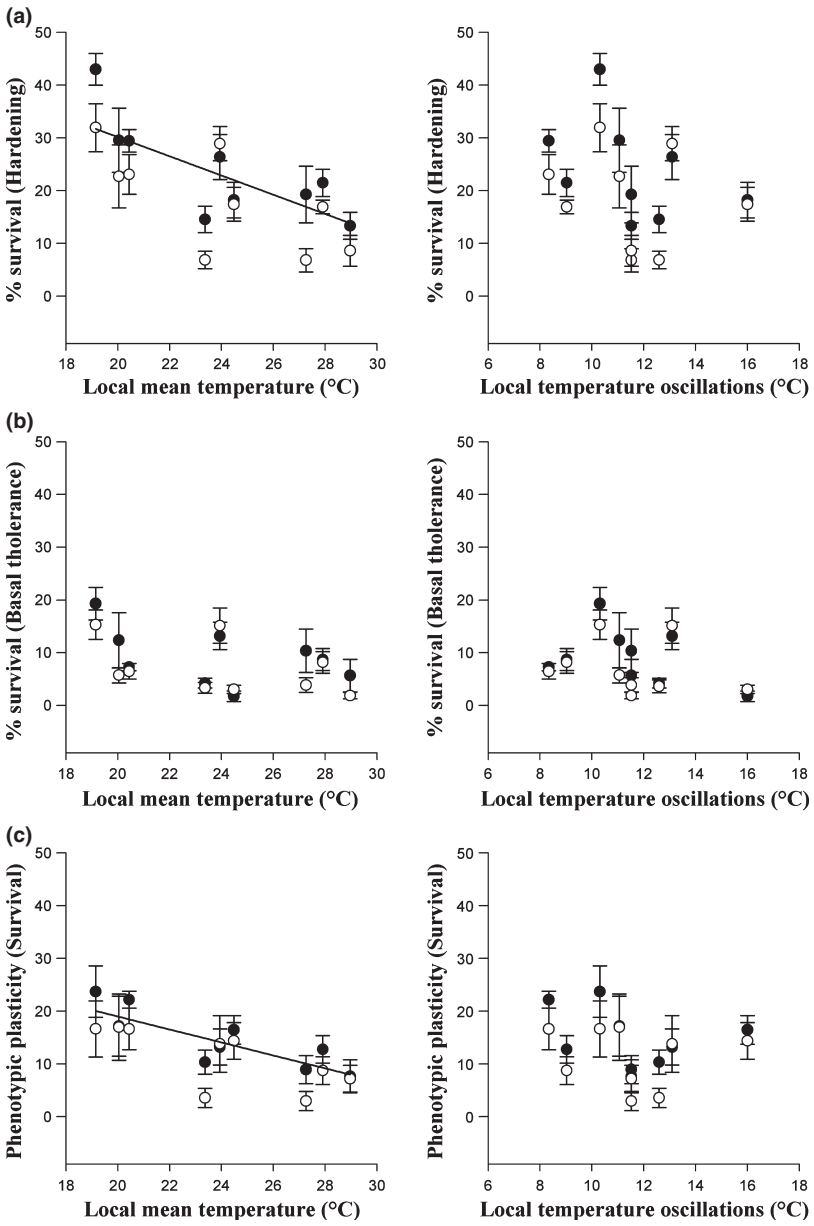

Fig. 2 Associations between survival for (a) Hardening, (b) Basal tolerance, (c) Phenotypic plasticity and local temperatures in °C (open circles for females and closed circles for males). Linear regression lines are included when the correlations were evident from population comparisons or the Mantel tests comparing Q_{ST}. Error bars ± standard errors.

than two-fold higher than F_{ST}. The Q_{ST} estimates without the population for which there was no molecular data (CAN) were different for phenotypic plasticity of fecundity ($Q_{ST} = 0.014 \pm 0.007$), which was four-fold lower than F_{ST}.

Geographic distances were not correlated with the distances for local temperature oscillations (Mantel test, $r = -0.20$, $P = 0.317$). However, geographic distances were correlated with the mean local temperature (Mantel test, $r = 0.74$, $P < 0.001$), and F_{ST} values were also correlated with this variable (Mantel test, $r = 0.62$, $P < 0.001$). With one exception for plasticity of surviving females (Table 6), all other thermotolerance traits were not significantly correlated with F_{ST}, suggesting that the quantitative genetic structure was not explained by neutral differentiation.

As we explained previously, genetic structure for surviving females under the hardened treatment and phenotypic plasticity were correlated with the mean local temperature (Table 6). Fecundity under the basal treatment was significantly correlated with geographic distances and the plasticity for fecundity was correlated with local temperature oscillations (Table 6). Mantel tests performed without the CAN population were different only for phenotypic plasticity of fecundity, with no correlation between quantitative differentiation and

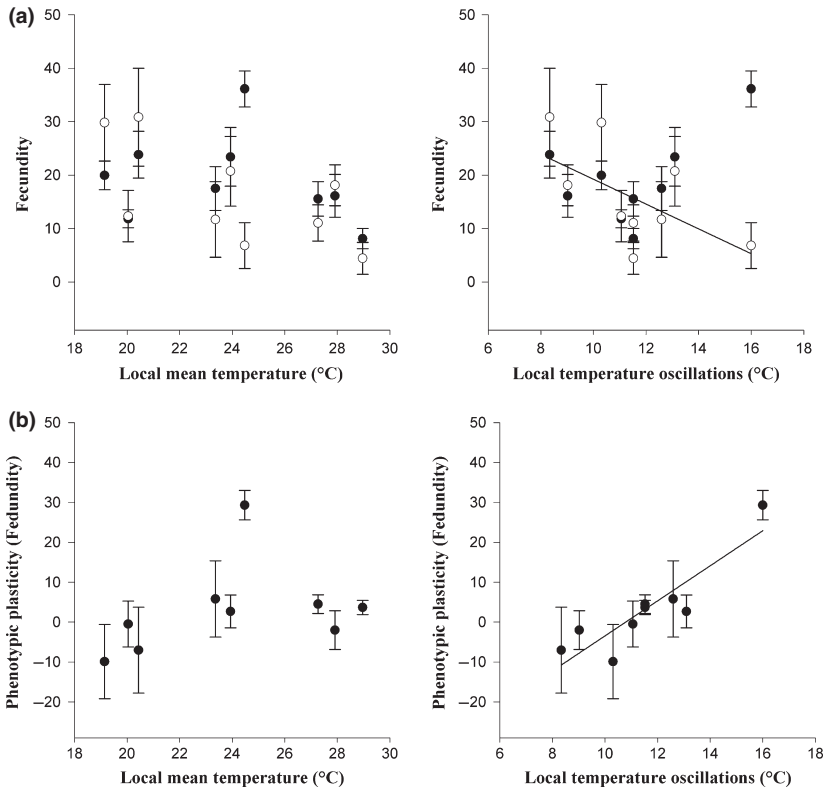

Fig. 3 Associations between (a) Fecundity (open circles for basal tolerance and closed circles for hardening) and (b) Phenotypic plasticity and local temperatures in °C. Linear regression lines are included when the correlations were evident from population comparisons or the Mantel tests comparing Q_{ST}. Error bars ± standard errors.

geographic or environmental distances (Mantel test: geographic distance $r = 0.18$; $P = 0.414$, mean temperature $r = 0.27$; $P = 0.158$, temperature oscillations $r = -0.07$; $P = 0.732$).

After controlling for neutral differentiation using the matrix for pairwise differences between Q_{ST} and F_{ST} ($Q_{ST} - F_{ST}$), the correlation between genetic differentiation for survival of females at the hardening treatment and the mean local temperature was significant (Table 7). These differences for genetic structure of fecundity under the basal treatment and the plasticity for surviving females were marginally correlated with geographic distance and differences in mean local temperature (Table 7).

Discussion

The species MEAM 1 of the *B. tabaci* complex have been distributed in the Colombian Caribbean and Southwest regions since 1995, when this species was found the first time in Colombia (Quintero *et al.*, 1998, 2001). *B. tabaci* showed a high level of tolerance to heat shock, with females surviving and maintaining reproduction after 1 h at 45 °C. Similar results have been obtained in studies with other whitefly populations (Cui *et al.*, 2008a,b; Elbaz *et al.*, 2011). Hardening significantly increased fitness compared with the basal treatment, as was evident from the treatment effect for survival and the interactions between population and isofemale lines by treatment on fecundity, a well-known phenomenon in insects (Parsell & Lindquist, 1993; Dahlgaard *et al.*, 1998) including *B. tabaci* (Salvucci *et al.*, 2000; Muñoz-Valencia *et al.*, 2013). We acclimated whiteflies at 40 °C for 1 h based on Muñoz-Valencia *et al.* (2013) because this was the optimal temperature for increasing thermal tolerance of *B. tabaci*. The increase in heat resistance after heat acclimation may reflect expression of heat shock proteins (Salvucci *et al.*, 2000; Yu & Wan, 2009; Lü & Wan, 2011) or other compounds such as sorbitol (Wolfe *et al.*, 1998; Salvucci *et al.*, 1999; Salvucci, 2000). Males also showed acclimation effects, although their survival after heat shock was lower than for females. Males are haploids and smaller than the diploid females, and this may result in different patterns of gene expression affecting heat resistance (Lü & Wan, 2008).

Despite this relatively recent invasion and high level of resistance and plasticity, we found that there was evidence for population differentiation in the response of *B. tabaci* to heat shock. This was apparent from the population effect evident in the GLM on survival and its plasticity, and the population by treatment interaction evident in the GLM on fecundity. Here, we explore whether this differentiation was the result of adaptive changes or neutral genetic processes by comparing genetic patterns to neutral

Table 6 Mantel tests (*r*, *P* value in brackets) for correlations between quantitative genetic differentiation (Q_{ST} for survival and fecundity at basal and hardening treatments and their phenotypic plasticities) and neutral differentiation (F_{ST}) and geographic distance (Ln km) or environmental (mean and oscillations temperatures) differences. All correlations were performed after linearizing the genetic structure indices by $F_{ST}/(1 - F_{ST})$ and $Q_{ST}/(1 - Q_{ST})$.

	Independent matrix			
Dependent matrix	$F_{ST}/(1 - F_{ST})$	Geographic	Temp. mean	Temp. oscillation
Survival – basal				
Males	0.15 (0.435)	0.16 (0.354)	0.25 (0.138)	−0.13 (0.459)
Females	0.07 (0.726)	−0.26 (0.133)	−0.28 (0.118)	0.06 (0.744)
Survival – hardened				
Males	−0.22 (0.260)	**0.28 (0.095)**	0.17 (0.334)	−0.21 (0.221)
Females	**0.32 (0.098)**	0.18 (0.318)	**0.42 (0.007)**	−0.13 (0.435)
Survival – plasticity				
Males	0.22 (0.248)	0.16 (0.347)	0.03 (0.869)	−0.05 (0.781)
Females	**0.62 (0.006)**	0.24 (0.153)	**0.40 (0.012)**	0.01 (0.968)
Fecundity				
Basal	**0.37 (0.054)**	**0.41 (0.011)**	**0.29 (0.092)**	**−0.29 (0.094)**
Hardened	**0.37 (0.052)**	0.13 (0.504)	0.18 (0.400)	0.32 (0.076)
Fecundity – plasticity	−0.06 (0.775)	0.01 (0.955)	−0.20 (0.246)	**0.59 (<0.001)**

Significant and marginally nonsignificant effects are highlighted in bold.

Table 7 Mantel tests (*r*, *P* value in brackets) for correlations between quantitative genetic structures controlled for neutral differentiation ($Q_{ST} - F_{ST}$ differences for survival and fecundity at basal and hardening treatments and their phenotypic plasticities) and the geographic (Ln km) and environmental (mean and oscillations temperatures) distances. All correlations were performed after linearizing the genetic structure indices by $F_{ST}/(1 - F_{ST})$ and $Q_{ST}/(1 - Q_{ST})$.

	Independent matrix		
Dependent matrix	Geographic	Temperature mean	Temperature oscillation
Survival – basal			
Males	0.22 (0.276)	0.26 (0.190)	−0.17 (0.416)
Females	−0.29 (0.150)	−0.32 (0.111)	−0.12 (0.553)
Survival – hardened			
Males	0.28 (0.157)	0.10 (0.640)	0.01 (0.969)
Females	0.19 (0.362)	**0.40 (0.028)**	−0.05 (0.823)
Survival – plasticity			
Males	0.01 (0.973)	−0.11 (0.590)	0.01 (0.971)
Females	0.16 (0.438)	**0.33 (0.089)**	0.18 (0.363)
Fecundity			
Basal	**0.37 (0.050)**	0.18 (0.374)	−0.09 (0.639)
Hardened	0.12 (0.815)	0.29 (0.190)	−0.22 (0.319)
Fecundity – plasticity	**−0.33 (0.081)**	−0.29 (0.119)	−0.16 (0.433)

Significant and marginally nonsignificant effects are highlighted in bold.

markers and linking patterns of variation to environmental variables.

The narrowsense Q_{ST} values we computed following Pujol *et al.* (2008) were high for all thermotolerance traits and point to genetically based population differentiation. The Q_{ST} was always higher than F_{ST} for the thermotolerance traits, which suggest that selection is probably the most important process contributing to phenotypic differentiation among populations, a pattern noted for many other population comparisons for quantitative traits (Merilä & Crnokrak, 2001; Pujol *et al.*, 2008). We did find a correlation between F_{ST} and Q_{ST} for the plasticity of survival in the females, suggesting that neutral processes affected differentiation among populations for this trait (Merilä & Crnokrak, 2001; Hangartner *et al.*, 2011). Nevertheless, the large Q_{ST} values and environmental correlations point to adaptive differentiation in this invasive species. The correlations between Q_{ST} and environmental variables were tested after we corrected the correlations for neutral differentiation (Antoniazza *et al.*, 2010; Hangartner *et al.*, 2011) by subtracting neutral from quantitative differentiation ($Q_{ST} - F_{ST}$). With this test, a correlation between survival under hardening in females and the local mean temperatures was evident, and phenotypic plasticity for the females was also marginally correlated.

Although logistic constraints prevented us testing more populations and isofemale lines, the population comparisons point to potential associations between resistance levels and local environmental conditions, but patterns were not easy to interpret from an adaptive perspective. Both survival and survival plasticity seemed to be negatively correlated with mean temperature, which suggests that populations from warmer environments were less tolerant of heat stress, contrary to what might be expected. A similar unexpected pattern was observed for *Drosophila simulans* involving a comparison of temperate and tropical populations (Van Heerwaarden *et al.*, 2012) and complex patterns of association between resistance levels and environmental temperature have also previously been documented for heat plasticity for *D. melanogaster* by Sgrò *et al.* (2010) and by Sørensen *et al.* (2001) for *D. buzzatii*. It is possible that the lower thermal tolerance of populations from warmer habitats reflects patterns of regulation for heat-shock proteins. In *D. buzzatii*, Sørensen *et al.* (2001) found higher survival to heat shocks in populations from lower temperatures; they found evidence suggesting that this might reflect the down-regulation of heat-shock protein genes in populations exposed to higher constant temperatures because of costs associated with hardening capacity or phenotypic plasticity. The present results also suggest that in *B. tabaci* plasticity for fecundity may be correlated positively with temperature oscillations. Phenotypic plasticity for fecundity may reflect a decrease in fecundity under the basal

treatment rather than a change under hardening given that these correlations were marginally nonsignificant (Table 5).

The absence of clear associations between some thermal traits and environmental temperature may reflect the fact that the laboratory tests do not relate directly to the stressful conditions encountered in the field. We have found that *B. tabaci* have a very high thermotolerance to heat shocks performed at 45 °C for 1 h and also show a significant increase of this tolerance as a result of hardening capacity using a pretreatment at 40 °C for 1 h. Thermal responses were measured using the upper thermal limits for *B. tabaci* (Cui *et al.*, 2008a; Muñoz-Valencia *et al.*, 2013). The 45 °C limit may be rarely encountered in tropical populations in Colombia, although we have detected ambient temperatures reaching 42–43 °C in the locations sampled.

It is also possible that population differences reflect selection in response to other factors not measured here but genetically correlated with the measured traits. This might include selection associated with host plants, or selection for insecticide resistance (Cardona *et al.*, 2001; Rodríguez *et al.*, 2005), where responses to chemical stress might be correlated with thermal stress. However, at least based on *Drosophila* data, such correlations seem unlikely because responses to thermal stress traits tend to be quite specific rather than being correlated with other traits (Hoffmann *et al.*, 2003). Nevertheless, some genes may have pleiotropic effects on both traits, such as genes involved in protection against oxidative damage that are expressed in response to both thermal stress as well as insecticides (e.g. Yan *et al.*, 2013).

In conclusion, our results suggest that populations of *B. tabaci* have differentiated in Colombia despite a relatively recent history of colonization. The thermal differentiation for this species was due to local temperatures rather than region effects and the $Q_{ST} - F_{ST}$ comparisons point to differences among populations being adaptive, but the selective factors involved remain unclear. Temperature may act as a direct or indirect selective factor for survival but not for fecundity. On the other hand, fecundity in the basal treatment was marginally correlated with geographic distances after controlling for neutral differentiation, and it will be interesting to see how these patterns in quantitative traits and neutral genetic variation change over time into the future. These results point to rapid ongoing adaptation, which suggests that it may be difficult to predict the impact of whitefly in invaded regions or under climate change.

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