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The mean annual temperature during the two years of censusing (Jan 2002-Jan 2004) ranged from 20.8 to 20.5°C (minimum temperatures of 2 to 3.2°C and maximum temperatures of 35.2 to 34.6°C), with total annual rainfall of 1,440 to 1,358 mm (rainy period from October to March) and mean relative humidity ranging from 87.8 to 86.9. Weather data were obtained from an automated weather recording station located in PRDTALP / APTA, approximately 250 m from the orchards.

different time periods and is not constrained to match the observed temporal autocorrelation. For each analysis (2 years \times 3 orchards), we simulated 1000 null assemblages, created the histogram of simulated niche overlap values, and estimated the tail probability (one-tailed) of the observed data under the null hypothesis ($p(\text{observed niche overlap} \mid H_0)$).

To insure that the results were not strongly influenced by sampling noise for rare species, we re-ran all null model analyses (niche overlap and co-occurrence) using only the 7 most common species from the collections. Results were qualitatively similar to the analyses of the complete samples that we present here.

Co-occurrence Analyses

We analysed species co-occurrence patterns using both the original abundance data (counts of the abundance of each species in a sample) and incidence data (presence or absence of each species in a sample). The abundance data were analyzed with the program CoOccurrence Version 2.0 [30]. We used the IT null model, which resamples the matrix cells proportional to observed row and column totals until the total row and column abundances are achieved [31]. Abundance patterns of co-occurrence were quantified with the “checker” and “anodf” indices. These indices are analogous to measures used in presence-absence analysis, and quantify respectively the number of checkerboard units, and the degree of nestedness in the abundance matrix [31].

The data from each orchard collected in a year were also organized into binary presence-absence matrices in which each row represents a species, each column represents a sample, and entries indicate the presence (1) or absence (0) of a species at a sampling period [32]. For each matrix, the co-occurrence pattern for each unique species pair was summarized as the C score [33], which measures the degree of aggregation (low C-score) or segregation (high C-score) for a species pair.

The statistical significance for each species pair was calculated with the software package PAIRS [34], using the fixed-fixed null model. This algorithm randomizes the occurrence of presences and absences, but preserves the row and column sums of the original matrix, and has good statistical properties [35]. Because row totals are preserved, inherent differences in the commonness and rarity of species are incorporated into the analysis. Because the column totals are preserved, inherent differences in the suitability of different time periods (due possibly to differences in temperature, humidity, or other common environmental effects) are also incorporated into the analysis. In some cases, these procedures may be overly conservative, but they prevent many Type I statistical errors (incorrectly rejecting a true null hypothesis), which is important for the analysis of non-experimental data. See Gotelli and Ulrich 2012 [36] for additional discussion.

In our analysis, with n species in the matrix, there are $(n)(n-1)/2$ possible pairs of species, and many of these pairs may not be biologically or statistically independent from each other [37]. The PAIRS software adjusts for the large number of pairwise significance tests in the analysis. We used the Empirical Bayes Confidence Limit method in PAIRS to adjust the p -values for each pair of species [34]. As in any statistical analysis, it is difficult to detect significance if both members of a species pair are very rare.

Finally, once statistically significant pairs of aggregated or segregated species were identified, we carried out additional tests [38] to determine whether the pattern of aggregation or segregation could be attributed to seasonal or thermal associations. For each segregated pair, we compared with a t-test the calendar (Julian) date and the air temperature during sampling of samples that contained one of the two species (10) to samples that contained the other species (01). If the Julian dates were statistically different for these two groups of samples, the species

pair was segregated by season. Within each set of yearly samples, we analysed the average sampling dates as circular data, using the 'aov.circular' function in the R package 'circular'. This analysis ensures that average dates are calculated correctly for samples that may overlap seasonally from December and January. If the air temperatures were statistically different for samples that contained each species occurring by itself, the species pair was segregated by temperature. If neither temperature nor Julian date were significantly different, the species pair was segregated in time, but the presence and absence sequence formed a temporal checkerboard that could not be attributed to seasonality or temperature differences.

For each aggregated pair, we carried out an analogous set of calculations, but this time comparing the calendar (Julian) date and the temperature of samples that contained both species (11) with those that contained neither species (00). These analyses revealed whether species aggregations could be attributed to seasonality or similar temperature affinities. Data for the association of *A. obliqua* and *C. capitata* in the loquat orchard illustrate how measures of air temperature and sampling date were analyzed for each significant species pair (Fig 1). In this example, the two species were collected at similar air temperatures throughout the year (temperature test; $p = 0.42$), but once *C. capitata* began to occur in the second half of the year, the occurrence of *A. obliqua* was greatly reduced (seasonality test; $p < 10^{-9}$).

Data Sources and Graphics

Original data matrices for all analyses are given in [S1a–S1c Table](#). All graphs were created with R Version 3.0.1 [39], and R script files for the graphs are given in [S1 Text](#).

Results

For the guava, loquat, and peach orchards sampled in 2002 and 2003, we collected 106 samples, and captured 25,872 individuals of the exotic Mediterranean fruit fly *Ceratitis capitata* (21,252 females and 4,620 males) and 89,958 individuals of 21 congeneric species of the genus *Anastrepha* (48,041 females and 41,916 males; [Table 1](#)).

In all orchards, air temperatures reached minima during June and July, which also corresponded to low capture rates of flies in traps (Fig 2). Each year, fruit fly abundance reached a peak as green fruit ripened, although on guava trees, fruit fly abundance was also high when

For co-occurrence analysis of individual species pairs, there was a total of 388 possible species pairs that could be formed from the data matrices. Of these 388, 18 pairs (11 segregated and 7 aggregated) were statistically significant using the empirical Bayes confidence limit criterion (Table 4).

Figs 4 and 5 illustrate the temporal pattern of the 18 species pairs with significant positive or negative associations. In the guava orchard in first year, there were three non-random pairs of species, of which two were segregated (*C. capitata* x *A. obliqua* and *C. capitata* x *A. grandis*) and one was aggregated (*A. obliqua* x *A. discolor*). In the second year, the pattern was very similar, with two pairs of segregated species (*C. capitata* x *A. obliqua* and *C. capitata* x *A. sororula*) and one pair of aggregated species (*A. obliqua* x *A. sororula*).

In the loquat orchard in the first year, there were four significantly segregated species pairs (*C. capitata* x *A. obliqua*, *C. capitata* x *A. grandis*, *A. bisriga* x *A. psuedoparallela* and *A. sororula* x *A. psuedoparallela*) and two aggregated species pairs (*A. sororula* x *A. bisriga* and

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Table 5. Analysis of average air temperatures and Julian calendar dates (= season) for time periods of species presences and absences in statistically significant species aggregations and segregations (see Table 4).

	Guava		Loquat		Peach	
	2002	2003	2002	2003	2002	2003
Aggregated species pairs						
A. obliqua x A. distincta	\					
A. obliqua x A. sororcula		↔		↔		
A. obliqua x A. grandis			↔			
A. bistrigata x A. sororcula			Δ			
C. capitata x A. sororcula					↔	↔
Segregated species pairs						
C. capitata x A. sororcula		↔				
C. capitata x A. obliqua	↔	↔	↔	↔		
C. capitata x A. grandis	↔		↔			
C. capitata x A. bistrigata					↔	
A. bistrigata x A. pseudoparallela			\			
A. sororcula x A. pseudoparallela			\			
A. obliqua x A. pseudoparallela				\		

Legend:

Temp. Season

↔ = N.S. S.

Δ = S. N.S.

\ = N.S. N.S.

□ = Species pair association N.S.

S. = Significant

N.S. = No significant

Significant results indicate a pair of species in which aggregation or segregation was associated with non-random patterns of air temperature or seasonality (see Fig 1). ↔ = significant seasonal difference, no temperature difference; Δ = significant temperature difference, no seasonal difference; \ = significant species aggregation or segregation without associated differences in air temperature or seasonality; white = segregation or aggregation not statistically significant.

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guava during this study [44]. Fruit flies species began to attack fruits of guava, loquat and peach in the intermediate ripening stage of fruits and the population of flies showed an increase tendency when full fruits were completely ripe [44]. *Anas repha grandis* is a quarantine species, which attacks melon and cucurbits, and *A. distincta* is a minor pest, which develops in Fabaceae species that are not of economic importance.

It is important to note that the co-occurrence analyses, by definition, depends on covariation of species presences and absences. For this reason, the widespread polyphagous *A. fraterculus* did not exhibit any statistical associations because it was present in all samples in this study due to host succession [50].

In summary, understanding the causes of species associations in spatially replicated “natural experiments” is challenging because habitat associations and dispersal limitation as well as species interactions can lead to non-random co-occurrence patterns [38]. Temporal patterns of co-occurring fruit flies represent a system in which dispersal limitations and habitat variation are minimal, so that patterns of species aggregation and segregation are more likely to represent the direct and indirect effects of species interactions.

Supporting Information

S1 Table. Original data matrices for all analyses of fruit flies species collected in orchards of (a) loquat, (b) peach and (c) guava, Monte Alegre do Sul, São Paulo, Brazil (2002–2003).
(XLS)

S1 Text. R script files for the graphs.
(TXT)

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Author Contributions

Conceived and designed the experiments: GNL MFSF RAZ. Performed the experiments: GNL MFSF. Analyzed the data: GNL NJG. Contributed reagents/materials/analysis tools: GNL LJUL. Wrote the paper: GNL NJG WACG RAZ.

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