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Fine Scale Microevolutionary and Demographic Processes Shaping a Wild Metapopulation Dynamics of the South American Fruit Fly *Anastrepha fraterculus*

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Abstract
Anastrepha fraterculus (Wiedmann) is an important American pest species. Knowledge of its population dynamics is of particular interest for ecology, evolutionary biology, and management programs. In the present study, phenotypic, genotypic, and spatial data were combined, within the frame of landscape genetics, to uncover the spatial population genetic structure (SGS) and demographic processes of an Argentinian local population from the Yungas ecoregion. Eight simple sequence repeats (SSR) loci and six morphometric traits were analysed considering the hierarchical levels: tree/fruit/individual. Genetic variability estimates were high ($H_E = 0.72$, $R_A = 4.39$). Multivariate analyses of phenotypic data showed that in average 52.81% of variance is explained by the tree level, followed by between individuals 28.37%. Spatial analysis of morphological traits revealed a negative autocorrelation in all cases. SGS analysis and isolation by distance based on SSR showed no significant autocorrelation for molecular coancestry. The comparison between phenotypic (P_{ST}) and molecular (F_{ST}) differentiation identified positive selection in different fruits for all traits. Bayesian analysis revealed a cryptic structure within the population, with three clusters spatially separated. The results of this study showed a metapopulation dynamics. The genetic background of the components of this metapopulation is expected to change through time due to seasonality, repopulation activities, and high gene flow, with an estimated dispersal ability of at least 10 km. Effective population size (*Ne*) of the metapopulation was estimated in around 800 flies, and within subpopulations (clusters) *Ne* was associated with the levels of genetic drift experienced by the founding lineages.

Keywords Demographic processes · fruit flies · population genetics · quantitative genetics · spatial analysis · SSR markers

Introduction

The genus *Anastrepha* (Diptera: Tephritidae) is endemic to the Neotropical region, where it is widespread. It comprises over 300 species (Troya et al. 2020) including several economically important fruit pests. The South American fruit fly *A. fraterculus* (Wiedmann 1830) is known to infest over 170 fruit species in 12 Latin American countries (Hernández-Ortiz et al. 2019). It is widely distributed from central Argentina in the south to the Rio Grande Valley in the north of Mexico (Steck 1991, 1999), and according to Hernández-Ortiz et al. (2012, 2019) it has been recorded even in southern Texas. Currently, *A. fraterculus* is considered a cryptic species complex rather than a single biological species, with at least eight morphotypes (Hernandez-Ortiz et al. 2012, 2015). In particular, the morphotype "Brazilian-1" or *Anastrepha sp*.1 *aff. fraterculus* (Yamada and Selivon 2001) is widely distributed, including Southern Brazil and Argentina (Hernández-Ortiz et al. 2012).

Recent studies suggest that, at the southern edge of its range, *A. frater*culus has been expanding southwards and westwards from an area located in the Paraná forest for about 2500 YBP (Giardini et al. 2020; Vilardi et al. 2021). This indicates that the species was able to thrive along different

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ecoregions in a short period of time thanks to its wide dispersal ability and high gene flow between populations. Nowadays, in Argentina the species is mostly present in the subtropical north-east (NEA) and north-west (NOA) regions, where the weather is warm and humid. These two regions are separated by the bio-geographical province of Chaco, a very arid region where *A. fraterculus* is normally absent (Alberti et al. 2002; Cladera et al. 2014).

Insect populations usually undergo strong seasonal variation due to biotic and abiotic factors at both local and regional scales (Begon et al.1996). Movement between wild and cultivated hosts is an important process that strongly affects survival of several major fruit fly species (Aluja et al. 2014). This cyclic movement throughout the year generates constant colonisation-extinction events whose dynamics is scarcely known. In addition, patches of secondary unmanaged forests provide suitable refuges for fruit flies, as an *A. fraterculus* habitat. In this sense, the Yungas ecoregion in north-west Argentina is an interesting scenario for developing integrated genetics and ecological studies. These approaches may facilitate the design of better control strategies (Schliserman et al. 2016). Nowadays, the only method available so far to control *A. fraterculus* populations is chemical pesticide applications, but more environmentally friendly options, such as the sterile insect technique (SIT) and parasitoids, are being explored (Vera et al. 2013; Schliserman et al. 2016). The successful implementation of these methods relies heavily on knowledge regarding the movement of individuals as well as the neighbourhood sizes, as defined in Wright (1946) and Ryberg et al. (2013), for different populations and regions (Estoup and Guillemaud 2010). Such data would be relevant for making decisions about the quantity, frequency, and releasing sites of controlling agents (sterile flies, parasitoids, even predators).

Significant insight on population structure and dynamics may be produced by landscape genetic approaches. Molecular markers have proven to be essential tools for exploring changes in genetic variability. For example, Alberti et al. (2008), using a fragment of the *A. fraterculus* mtDNA COII gene, found no correlation between haplotypes and geographic distribution in Argentina. However, Vilardi et al. (2021), combining the information of the same mtDNA region with geographic and environmental data, were able to identify two population clusters in the southern edge of *A. fraterculus* range that may be considered two different management units. Inter simple sequence repeats (ISSR) markers coupled with chemical analyses allowed Oroño et al. (2013) to arrive at the conclusion that adaptation to plant chemistry may contribute to genetic differentiation between populations exploiting alternative synchronic hosts.

Simple sequence repeats (SSR) markers for *A. fraterculus* have been developed (Lanzavecchia et al. 2014), providing an invaluable tool to analyse population structure and dynamics. In addition to information about historical and contemporary population dynamics provided by neutral molecular markers, it is crucial to understand adaptive processes that may affect the heterogeneity among local populations under the underlying ecological constraints. Therefore, the parallel analysis of molecular and quantitative trait variation is relevant given that the latter is frequently associated with different fitness components, such as mating success, developmental rate, and dispersal ability. In this regard, Rodriguez et al. (2019) performed a fine-scale genetic diversity and population structure analysis that provided evidence of cryptic genetic structure of *A. fraterculus.* They also confirmed that females were able to disperse widely from the pupal emergence site before reaching maturity and starting mating and ovipositing activities. However, more precise information on the dispersal ability of the species and the quantification of microevolutionary processes is still needed.

In the present study, we integrated quantitative trait and molecular marker data to improve our understanding of the processes governing population dynamics of *A. fraterculus,* assessing the relative contribution of genetic and environmental factors. The comparison of the levels of population variation at putatively selective traits with respect to neutral genetic markers contributes to disentangling adaptive from neutral phenotypic differentiation (Leinonen et al. 2008). Integration between these analyses may give an account of adaptive strategies in response to environmental changes of the species (Freeland 2006). In the present study, we complemented the approach previously applied in Rodriguez et al. (2019) by including the geographic coordinates of the sampled trees, to evaluate the spatial distribution of genetic and morphometric variability as the result of a balance between stochastic and deterministic evolutionary processes.

Our main goal was to contribute to the knowledge of the biology, dispersal ability, and behaviour of *A. fraterculus,* and to unravel the evolutionary-demographic processes that may be shaping its populations' dynamics. The interest of this approach is twofold because (1) *A. fraterculus* represents an excellent model for the development and evaluation of evolutionary hypotheses and (2) the information about colonising strategy and internal structure of a pest species may be helpful in the design of a suitable management strategy. In particular, the success of biological and/or genetic control techniques is dependent on demographic characteristics of the target population. These features include its dispersal ability; the co-occurrence of several management units in the same area, which could have a different response to the controlling agents; and the effective population size (*Ne*). The latter parameter is roughly related to the number of effective breeding individuals in the population. Additionally, *Ne* allows to estimate the rate of inbreeding and loss of genetic variation in wildlife and could be used to predict the census size (*N*) (Frankham 1995).

Our specific objectives were to (1) characterise the genetic diversity and fine-scale spatial genetic structure (SGS) of a local wild population; (2) estimate the effective size of the population (and subpopulations if they exist); (3) evaluate the connectivity between subpopulations and the effect of genetic drift; and (4) integrate molecular and morphometric information to infer selective mechanisms. We applied a hierarchical sampling of *A. fraterculus* and analysed eight microsatellite markers and six quantitative traits, to address the following questions: (1) is there cryptic genetic structuring in an apparently single population? (2) How is the variation distributed at different hierarchical levels (overall, tree, fruit, individual)? (3) Is there any evidence of isolation by distance for molecular differentiation? (4) What is the scale at which selective forces that influence morphological differentiation could be acting on?

Material and methods

Study area

The wild *Anastrepha fraterculus* population analysed is sited near San Pablo City, Tucumán, Argentina, a location in the southernmost edge of the Yungas ecoregion (Morello et al. 2012) (Fig. 1). The studied area ranges from 27°4′ to 26°52′S and from 65°30′ to 65°20′W and it is located within a foothill forest remnant ranging between 400 and 700 m above the sea level in the eastern slope of the San Javier Hill. The climate is classified as "humid warm-temperate" with a rainy-warm season from October through April, and a dry-cold season from May through September. Mean annual rainfall ranges between 1,300 and 1,600 mm, with a mean annual temperature of 18 °C (Köppen 1918). The landscape is heterogeneous, characterised by disturbed secondary vegetation (exotic and native plant species combined). The sampling site involves a small number of guava trees (*Psidium guajava L.*) confined to a narrow strip, limited by the Yungas edge (A in Fig. 1), the densely populated (12,000 inhabitants) city of San Pablo (B in Fig. 1) and a suburban area (C in Fig. 1). There is no ongoing pest management program which might affect fly population properties or individual survival in this site.

Sample collection

To evaluate the distribution of morphological and genetic variance in the population of *A. fraterculus*, fruits with oviposition holes were collected in the wild, and a nested (hierarchical) design was applied considering three levels: tree, fruit (nested in tree), and individuals (within fruit).

In March 2016, guava trees were chosen in the study area, and their spatial coordinates were recorded (Fig. 1) with a GPS device (Garmim® eTrex). The minimum distance between sampled trees was about 300 m (pair 2–3) and the maximum pairwise distance was 27,500 m (pair 1–7) (Table 1). Trees were selected among the healthiest ones, with the caveat that the distance between them should be larger than the GPS resolution and that they were spread along the whole orchard. Due to the uncertain probability of survival of the flies after transport from the sampling site to processing site, a major effort was carried out. Thus, 250 fruits (between 30 and 40 per tree) with evidence of infestation by tephritids (oviposition holes) were collected from seven trees, a number considered representative of the small orchard.

Sampled guava fruits were placed in individual containers on sand covered with a piece of gauze, and kept at 20–25 °C. Each container was checked daily for emerged third instar larvae and pupae until the fruits started to dry. All pupae from each fruit were transferred to a Petri dish with vermiculite and sent to the Laboratorio de Genética de Poblaciones Aplicada, at the University of Buenos Aires. There, they were kept in flasks with vermiculite at room temperature and daily checked for adult emergence. Emerged adults were labelled according to the fruit and tree they had come from, and stored in Eppendorf tubes at − 20 °C.

As our hierarchical design required a balanced number of fruits per tree and individuals per fruit (to obtain unbiased variance estimates), the five fruits that hosted the largest number of recovered adults were selected for each tree. Undamaged adult individuals were randomly sampled from each fruit, trying to keep a balanced design with three individuals per fruit and a 1:1 total sex ratio. The total 105 individuals were processed for morphometric and molecular analyses as described below. All statistical analyses were conducted with different packages of the software R ver. 4.0.3. (R Core Team 2020), unless otherwise stated.

Morphometric analysis

The chosen individuals were dissected and photographed following the protocol by Rodriguez et al. (2019). Six traits related to body size, head shape, and flying ability were selected for morphometric analysis (Fig. 2): thorax length (THL), maximum head width (HW), minimum face width (i.e. minimum distance between the eyes) (FW), eye length (EL), wing length (WL), and wing width (WW). A Leica EZ4HD stereoscopic microscope with a built-in 3MP camera was used for photography. THL, WL, and WW were measured at 16×; HW, EL, and FW at 35×. Measurements were obtained with a specifically created macro for the *ImageJ* image system. WW was defined as the distance between the point where the sectoral branch of the radial vein intersects the wing border and the point where the first branch of the anterior cubital vein joins the external border

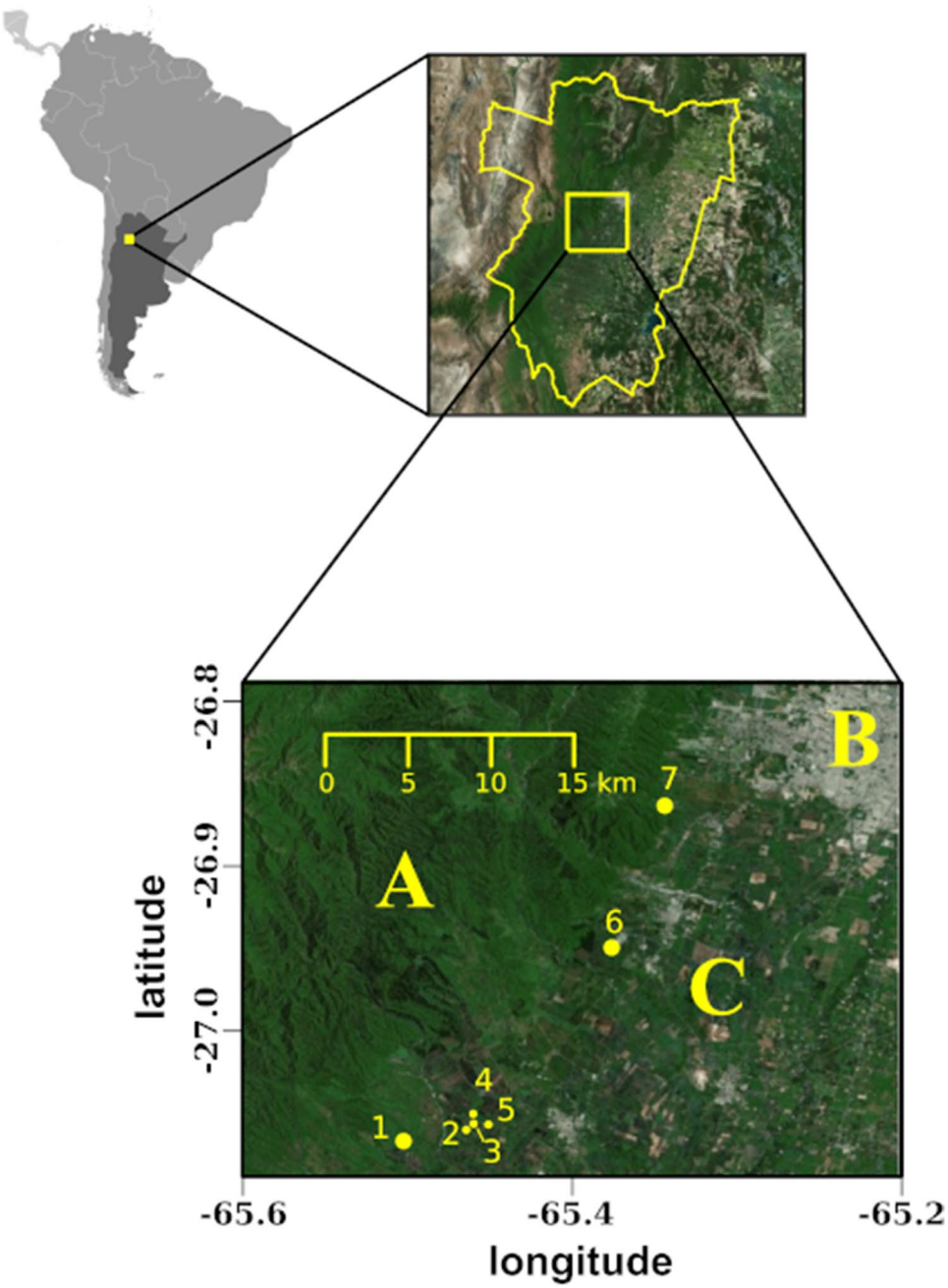

Fig. 1 Geographical location of the sampled *Anastrepha fraterculus* population. The location of Argentina and the province of Tucumán are detailed. The sampled guava trees are identified with circles 1–7 in the enlarged picture. The study area is at the foot of the San Javier Hill, surrounded by the edge of the Yungas forest (**A**), the San Pablo city (**B**), and suburbia (**C**)

Table 1 Pairwise geographic distances in metres between *Anastrepha fraterculus* sampling sites (trees)

Guava tree	1	2	3	4	5	6
2	4036					
3	4279	289				
4	4479	605	327			
5	5030	1000	805	797		
6	18,085	14,813	14,526	14,211	14,154	
7	27,519	24,612	24,330	24,007	24,040	10,064

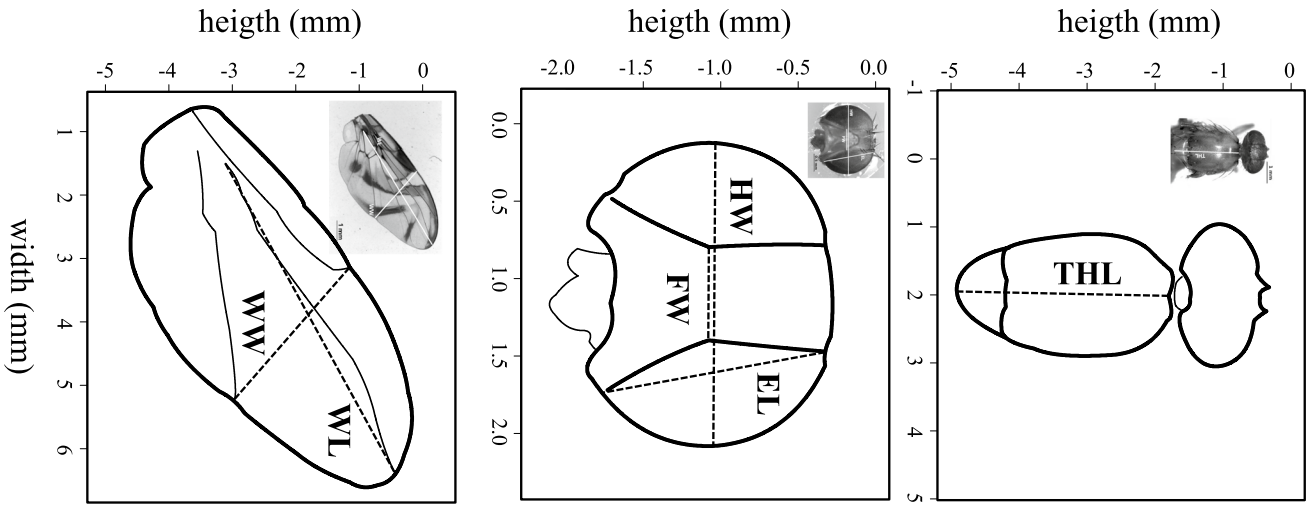

Fig. 2 Morphometric traits measured in *Anastrepha fraterculus*. WL, wing length; WW, wing width; EL, eye length; FW, face width; HW, head width; THL, thorax length

(D13 segment in Selivon et al. (2005)). WL was defined as the distance between the point R4 + 5 sectoral branch of radial vein intersecting the external border and the point where the medial vein joins CuA1 (distance between points 4 and 8 in Selivon et al. (2005)).

Components of variance and morphological differentiation

Differences between left and right wings were evaluated by a paired *t*-test (*base* package), and as they were not significant ($P = 0.6304$ and 0.2554 for WL and WW respectively), only one wing (the left one, unless damaged or missing) was used in the rest of the analyses. In order to apply a multivariate analysis including all traits, all data were standardised for males and females separately, to mean = 0 and variance = 1.

The distribution of morphometric variability was evaluated by two different approaches. The first one was a univariate analysis applying restricted maximum likelihood (REML) using the package *lme4* (Bates et al. 2013). The linear model follows the following general expression:

$$y_{ijk} = \mu + t_i + f_{ij} + e_{ijk} \quad (1)$$

where y_{ijk} represents the observation (measurement) of the trait for the individual k from the fruit j of tree i, μ is the overall mean, t_i is the random effect of the tree i, f_{ij} represents the random effect of the fruit j nested in tree i, and e_{ijk} is the random residual error. Significance of each variance component was assessed by comparing different models by analysis of variance. Confidence intervals (*CIs*) for the variances were obtained by bootstrap (2,000 resamplings).

The second approach was a multivariate analysis conducted with the package *MCMCglmm* (Hadfield 2010) which uses Bayesian inference and estimates covariance matrix components at each hierarchical level. The estimates were based on 200,000 iterations, with a thinning of 200, and burning of 30,000. Based on the deviation information content (*DIC*), three models were compared: (1) the variance between trees (V_t) and the variance between fruits within trees (V_{ft}) are both different from zero, (2) $V_t > 0$ and $V_{ft} = 0$, (3) $V_t = 0$ and $V_{ft} > 0$. The confidence intervals (95%) of variance components were calculated with the highest density interval (HDI) method using the function *HPDinterval* of the *coda* package (Plummer et al. 2006).

The population structure based on components of phenotypic variance was quantified by P_{ST} (Brommer 2011; Pujol et al. 2008), estimated according to the expression:

$$P_{ST} = \frac{\sigma_B^2}{\sigma_B^2 + 2\sigma_W^2} \quad (2)$$

Variance estimates were taken from the univariate analysis, where $\sigma_B{}^2$ is the variance between groups and $\sigma_W{}^2$ is the variance within groups. The analysis was performed for the fruits set (P_{fT}) and the tree level (P_{tT}) (with $\sigma_B{}^2$ corresponding to between fruits and between trees variance estimates respectively).

The metric of Eq. 2, which relies on phenotypic rather than additive genetic data, is a raw approximation to Spitze's (1993) quantitative index of population genetic divergence (Q_{ST}). Confidence intervals (95%) of P_{ST} estimates were obtained by bootstrap (1,000 resamplings) using the package *boot* (Canty and Ripley 2017).

DNA extraction and genotyping

The individuals previously measured for morphometric analysis were genotyped for eight SSR loci developed by Lanzavecchia et al. (2014): A115, D105, A120, A7, C103, A10, A112, and A122. Total genomic DNA extraction followed the protocol specified by Baruffi et al. (1995) with modifications (Lanzavecchia et al. 2014). DNA was amplified under the following PCR conditions: one cycle at 95 °C (2 min), 30 cycles at 95 °C (30 s), 58 °C (30 s), and 72 °C (30 s), and final elongation at 72 °C (10 min). Amplification was performed in a thermal cycler Ivema T21, using a final volume of 30 µl for the reaction mix.

PCR products were sequenced by the Macrogen Sequencing Service (KOREA) with GS 500 LIZ marker and processed using *GeneMarker*® v.2.4 (SoftGenetics Llc., www.softgenetics.com). The presence of null alleles and correct scoring of allele sizes were evaluated using *Micro-Checker* v2.2.3 (Van Oosterhout et al. 2004).

Molecular diversity and population structure estimators

The hypothesis of an independent distribution of the analysed loci was evaluated by means of the index of association *Ia* (Brown et al. 1980) and the standardised index of association *rD* (Agapow and Burt 2001) estimated with the *poppr* package (Kamvar et al. 2015). The significance of both indices was obtained by a permutation test with 1,000 replicates. The function *divBasics* of the package *diveRsity* (Keenan et al. 2013) was used to find the number of alleles (A) and the allelic richness (R_A) in each fruit. The observed (H_O) and the unbiased expected (H_E) heterozygosities per fruit, and the total expected heterozygosity (H_T) per locus according to Nei and Chesser (1983) were calculated using the package *hierfstat* (Goudet 2006).

The excess/deficiency of heterozygotes in each locus within each fruit was evaluated by the fixation index (F_{IS}) (with the package *hierfstat*), and the U score (Rousset and Raymond 1995) estimated with the package *HWxtest* (Engels 2016).

The genetic structure of the whole sample was analysed considering trees, fruits, and individuals as nested levels, and applying two methods: hierarchical F statistics (Wright 1951; Weir and Cockerham 1984) and analysis of molecular variance (AMOVA) (Excoffier et al. 1992). F statistics were estimated with the package *hierfstat* (Goudet 2006) and their significances were evaluated by the *test.within* and *test.between* functions. Confidence intervals (95%, based on 1,000 replicates) were calculated with the function *boot.vc* of the same package. The AMOVA (Φ statistics) was performed with the package *poppr*. The significance of each level was obtained with the function *randtest.amova* of the *ade4* package with 1,000 permutations. Based on the genetic structure analysis, we used an indirect method to estimate the number of ovipositing females per fruit as: $N_F = 1/(4\ F_{ST})$ (Rodriguez et al. 2019).

Neutrality test

To assess the relative importance of selection and drift as determinants of genetic differentiation for quantitative traits, the comparison between P_{ST} and F_{ST} estimates was performed following Brommer (2011). Assuming that F_{ST} is estimated from neutral genetic markers, according to Merilä and Crnokrak (2001) there are three possible outcomes from the comparisons of F_{ST} and Q_{ST} indices, each of which has a unique interpretation. The same logical criteria can be applied to P_{ST}—F_{ST} comparisons:

- $P_{ST} > F_{ST}$: directional natural selection must be involved favouring different phenotypes in different populations
- $P_{ST} = F_{ST}$: no need to invoke natural selection, the degree of differentiation does not differ from the expected by genetic drift alone
- $P_{ST} < F_{ST}$: stabilising selection favours the same phenotype in different populations.

The difference between these coefficients was considered significant when their *CI*s (95%) do not overlap.

Cluster analysis

For the purpose of identifying cryptic population structure in the *A. fraterculus* population, a Bayesian model–based spatial cluster analysis was conducted using the *Geneland* package (Guillot et al. 2005). The integrative landscape analysis implemented in *Geneland* helps inferring and locating genetic discontinuities within populations in space. To identify clusters of individuals, the information of geographic coordinates, the multilocus molecular genetic data, and morphometric information are combined. In the present study, a correlated allele frequencies model was used. Different sets of parameters (number of repetitions, thinning, and burn-in) were applied in different test runs, in order to find the optimal parameters. As a result, the best runs were chosen based on their likelihood, setting the Markov chain Monte Carlo (MCMC) repetitions at 1,000,000, with a thinning of 100, and a burn-in period of 2,000. The number of clusters (K) to be tested was set at 1–10 with coordinates uncertainty = 10. After 10 different runs, the definitive one was chosen on the basis of the highest average posterior probability. The genetic differentiation between the identified clusters was evaluated by the F_{ST} as described above, using the assigned clusters as the grouping factor.

Isolation by distance (IBD) and spatial autocorrelation (SA)

In order to test isolation by distance (IBD), pairwise matrices of genetic and geographic distances between trees were constructed and compared by Mantel tests with 5,000 replicates using the function *mantel.randtest* of the package *ade4* (Chessel et al. 2004). Geographic distances were calculated from the coordinates (UTM) of sampled trees with the function *dist* of the *stats* package. A pairwise matrix of Nei's genetic distances was obtained with the *poppr* package. An UPGMA tree was constructed from the Nei's genetic distances using the function *aboot* from the same package, with 1,000 bootstrap replications.

For the characterisation of fine-scale spatial genetic structure (SGS), several analyses were performed using the package *EcoGenetics* (Roser et al. 2017). For genetic data, correlograms based on Loiselle's *Fij* (Loiselle et al. 1995) kinship (coancestry) measures were obtained with the function *eco.malecot* applying the global method. Minimum and maximum distances were set to zero and 27,000 m respectively with four distance classes. For each morphometric trait, spatial analysis (SA) was conducted using Moran's *I* method (Moran 1950). For both SGS and SA, the spatial weight matrix was obtained with the k-nearest neighbour method (KNN) using the function *eco.weight*. The parameter *k*, which corresponds to the number of neighbours for the nearest neighbour distance, was set at 20, because this number is higher than the number of individuals at each coordinate.

A global SA test was conducted, which produces a single SA estimate across all the analysed area. The multivariate analysis was performed with the function *eco.gsa* with 10,000 simulations. Finally, local SA analysis was applied to uncover local clusters of autocorrelated observations based on the computation of a local SA measure. They were carried out for each trait with the function *eco.lsa*, which computes a SA statistic for each individual, using the weights object specifying the spatial relations among individuals. The Markov chain Monte Carlo (MCMC) repetitions were set at 1,000.

Genetic drift, gene flow, and effective population size

The demographic history of the clusters identified by *Geneland* was investigated under the admixture *F*-model (AFM) with the package *RAFM* (Karhunen and Ovaskainen 2012). The approach assumes that the subpopulations (clusters) evolved from a single ancestral population comprising several independent lineages which partially admixed, migrated, and underwent genetic drift throughout the differentiation process. The method allows to estimate gene flow and pure random drift parameters to evaluate their relative influence on the level of differentiation. The effect of drift is quantified by the alpha parameter vector ($\boldsymbol{\alpha}$), and the contribution of each lineage to each local subpopulation by the kappa matrix ($\boldsymbol{\kappa}$), which is an estimate of gene flow. The relative contribution of the *i* lineage to the whole population may be obtained by the sum of the $\boldsymbol{\kappa}_{ij}$ values over all subpopulations (*j*):

$$\sum\nolimits_j \kappa_{ij} \tag{3}$$

The parameters for AFM were set in 20,000 iterations, burn-in of 10,000 and thinning of 10, with the clusters identified by Geneland as the grouping factor. Finally, we recalculated F_{ST} between clusters, defined through probability of identity by descendent as:

$$F_{ST} = \frac{\theta^W - \theta^B}{1 - \theta^B} \tag{4}$$

where θ^W and θ^B are, respectively, the average pairwise coancestry between individuals of the same or different subpopulations.

The effective population size (*Ne*) represents the population size of an idealised population with the same inbreeding rate as the actual population under study. This value usually is smaller than the census size (*N*), and closer to the actual number of breeding individuals. In this work, *Ne* was assessed by a molecular coancestry single-sample estimator (Nomura 2008) implemented in *NeEstimator* V2.1 (Do et al. 2014). Confidence intervals for *Ne* were based on a jackknife method implemented in the same software. This approach was applied first to the whole population and then to the clusters found by *Geneland*, which were considered subpopulations.

Results

Components of variance and morphological differentiation

Components of variance for the univariate and multivariate approaches are summarised in Table 2. Results from the univariate analysis showed that in all traits the variation between individuals within the same fruit was the highest component. The tree level is significant to explain the variation only for both wing traits (WW, WL). Finally, for the remaining traits, the variation between fruits from which the individuals emerged was significant, but the tree level was non-significant.

The multivariate analysis indicated that the model 2 ($V_t > 0$ and $V_{ft} = 0$) was the best supported ($DIC = 1003.1$), in comparison with model 1 ($V_t > 0$ and $V_{ft} > 0$, $DIC = 1090.5$) and model 3 ($V_t = 0$ and $V_{ft} > 0$, $DIC = 1071.4$). This means

Table 2 Components of phenotypic variance of six morphometric traits in *Anastrepha fraterculus* emerged from guava fruits collected from seven trees in San Pablo, Tucumán, Argentina. Confidence intervals (95%) of each variance component are indicated in brackets

Trait	REML			MCMC mult		
	Tree	Fruit:tree	Individual	Tree	Fruit:tree	Individual
THL	0.10	0.29*	0.59*	1.38	0.54	0.68
	[0.00–0.62]	[0.23–0.76]	[0.64–0.90]	[0.28–3.08]	[0.26–0.90]	[0.47–0.89]
EL	0.17	0.24*	0.59*	1.36	0.48	0.67
	[0.00–0.73]	[0.16–0.71]	[0.53–0.89]	[0.30–3.40]	[0.23–0.80]	[0.48–0.91]
FW	0.07	0.14*	0.79*	1.29	0.51	0.84
	[0.00–0.51]	[0.00–0.61]	[0.75–1.03]	[0.30–2.82]	[0.21–0.84]	[0.58–1.11]
HW	0.12	0.28*	0.59*	1.34	0.49	0.68
	[0.00–0.63]	[0.22–0.76]	[0.56–0.89]	[0.29–3.21]	[0.22–0.81]	[0.47–0.91]
WW	0.21*	0.13	0.68*	1.34	0.43	0.75
	[0.00–0.77]	[0.00–0.59]	[0.69–0.97]	[0.32–3.19]	[0.12–0.71]	[0.52–1.01]
WL	0.20*	0.15	0.66*	1.40	0.44	0.75
	[0.00–0.77]	[0.00–0.61]	[0.66–0.94]	[0.27–3.22]	[0.20–0.75]	[0.52–0.98]

Univariate analysis applying restricted maximum likelihood (REML), multivariate analysis by means of a Bayesian approach which approximates the estimates by Markov Chain Monte Carlo simulations (MCMC mult). * indicates significant values. Acronyms for traits are defined in Fig. 2

that the variance between fruits within trees is not significant. For all traits, on average the contribution of fruits within trees to total variance was 18.82%. The greatest proportion of the variance, 52.81%, is explained by the tree level, followed by the residual level (inter-individuals), which explains 28.37% of total variance. The pairwise correlations between traits were all significant (data not shown).

Molecular diversity and population structure estimators

The eight analysed loci showed high polymorphism, with 8 to 16 alleles per locus, and an allelic richness per fruit (R_A) ranging from 2.68 to 5.60 (Table 3). All individuals exhibited different multilocus genotypes (MLG). The analysis of linkage disequilibrium in the whole sample was non-significant ($Ia = 0.073$, $P = 0.063$; $rD = 0.025$, $P = 0.058$) suggesting that the analysed loci are independent. In most loci heterozygote deficiency was observed ($H_O < H_E$). F_{IS} estimates for 5 loci and the overall average loci were positive (Table 3) for the fruits set. However, the 280 U scores obtained for the global population for all fruit × loci combinations results were non-significant (data not shown), indicating that genotype frequencies do not depart significantly from Hardy–Weinberg expectations at the fruit level.

The genetic differentiation among all fruits evaluated by Wright's F statistics was not significant ($F_{if} = 0.0244$, $P = 0.44$, $CI_{95} = 0.0090–0.0393$). Based on this estimate, the number of ovipositing females per fruit was ~ 10. The differentiation between fruits within trees was not significant either ($F_{ft} = 0.013$, $P = 0.56$). When the same analysis was conducted to estimate the differentiation among trees, the F_{ST}, although low, was significant ($F_{tT} = 0.011$, $P = 0.03$, $CI = -0.0025–0.0230$, based on 1,000 permutations).

The AMOVA results showed high consistency with the F statistics (Table 4), indicating that the larger variation occurs within individuals, the variance between individuals was mainly represented within fruits, and the variance between trees was the lowest component. The variation between fruits within trees was low and non-significant (1.548%, $\Phi = 0.015$,

Table 3 Diversity estimates and population structure statistics in *Anastrepha fraterculus*. The number of alleles in the population (A), average allelic richness per fruit (R_A), observed heterozygosity (H_O), expected heterozygosity within fruits (H_E) and for the whole populations (H_T), Wright's fixation indices (F_{IS}) and (F_{ST}) of each locus are shown

Loci	A	R_A	H_O	H_E	H_T	F_{ST}	F_{IS}
D105	10	3.085	0.4095	0.5376	0.5764	0.0672	0.2383
A115	13	4.228	0.4143	0.7471	0.7764	0.0377	0.4455
A7	14	3.709	0.6929	0.6827	0.7003	0.0251	−0.0149
A120	14	5.549	0.7381	0.8515	0.8918	0.0452	0.1332
C103	16	4.643	0.8667	0.7784	0.7878	0.0120	-0.1134
A112	15	5.603	0.8810	0.8635	0.8576	−0.0069	−0.0202
A122	11	4.353	0.5738	0.7513	0.7740	0.0294	0.2362
A10	8	2.683	0.5643	0.5740	0.5662	−0.0137	0.0169
Overall	12.62	4.395	0.6426	0.7233	0.7413	0.0244	0.1116

Table 4 Analysis of molecular variance (AMOVA) based on eight SSR loci considering trees, fruits, and individuals

Source of variation	Df	SS	MS	Est Var	%	Φ	P
Between trees	6	52.147	8.691	0.053	0.900	0.008	0.028
Between fruits within trees	28	197.308	7.047	0.092	1.548	0.015	0.082
Between samples within fruit	70	454.965	6.499	0.676	11.340	0.116	0.000
Within samples	104	540.306	5.146	5.145	86.212	0.138	0.000
Total	208	1244.726	5.956	5.968	100	-	-

Df degrees of freedom, *SS* sum of squares, *MS* mean squares, *Est Var.* estimated variability, % proportion of genetic variability, Φ fixation indices, *P* significance level

Table 5 P_{ST} estimated for six quantitative traits and F_{ST} from eight SSR loci in *Anastrepha fraterculus* which had emerged from different guava fruits collected in the Yungas ecoregion, Argentina. P_{fT}: among fruits P_{ST}. P_{tT}: among trees P_{ST}. Confidence intervals ($CI_{95\%}$) of each variance component are indicated in brackets. CI values in bold do not overlap F_{ST} estimates. Acronyms for traits are defined in Fig. 1

Trait	P_{fT}	P_{tT}
THL	0.20	0.06
	[0.21–0.60]	[0.00–0.18]
EL	0.17	0.10
	[0.17–0.59]	[0.00–0.25]
FW	0.07	0.03
	[0.08 – 0.49]	[0.00–0.15]
HW	0.19	0.07
	[0.20– 0.60]	[0.00–0.20]
WW	0.09	0.13
	[0.11–0.55]	**[0.02–0.28]**
WL	0.10	0.13
	[0.12– 0.54]	**[0.02–0.26]**
F_{ST}	0.024 [0.009–0.03]	0.011 [0.00–0.02]

$P=0.082$), while structure between trees, although low, was significant ($\Phi=0.008$, $P=0.028$).

Neutrality test

Estimates of quantitative genetic divergence (P_{ST}) of the six morphometric traits were compared with the hierarchical F_{ST} estimated from molecular markers (Table 5). Quantitative differentiation among fruits (P_{fT}) was significantly higher than expected under neutrality for all traits. The magnitude differed between traits, and the higher differentiation was thorax length (THL). Tree-level analysis revealed possible evidence of directional selection in both wing traits (WW, WL) with similar magnitude (Table 5).

Cluster analysis

Since the AMOVA showed that the highest components of genetic variance corresponded to the within and among individual levels, data were sent to *Geneland* analysis where no prior group information is given, and spatial, morphometric, and molecular data are combined to uncover cryptic structure. The run with the highest average posterior density yielded a modal number of groups $K=3$ (Fig. 3a). Cluster 1 included all the flies obtained from trees 2, 3, 4, and 5, a total of 61 individuals (Figs. 3b and 4). Cluster 2 consisted of all the individuals (29 in total) from trees 6 and 7 (Figs. 3c and 4), while tree 1 alone formed Cluster 3, with 15 individuals (Figs. 3d and 4). All flies from the same tree tend to belong to the same cluster (Fig. 4). The genetic structure between these clusters, quantified by the F_{ST}, was significant ($F_{ST}=0.011$, $P=0.014$, $CI=0.000$–0.029 based on 1,000 permutations).

Isolation by distance (IBD) and spatial autocorrelation (SA)

The UPGMA tree based on Nei's genetic distances (Fig. 5) showed that individuals from different trees are not grouped according to geographic distances. The correlation between geographic and genetic distances was positive but non-significant ($r=0.21$, $P=0.22$), indicating that genetic differentiation between flies among trees does not fit the IBD model.

No significant spatial genetic structure was detected for molecular coancestry. Local analysis failed to identify SA for any of the six morphometric traits. Regarding the global spatial autocorrelation, all traits showed a negative autocorrelation with distance, but the SA was significant only for THL, EL, and WL (Fig. 6).

Genetic drift, gene flow, and effective population size

The differentiation among the clusters obtained by *Geneland* was analysed by means of an admixture *F*-model. The resulting F_{ST}, which represents the probability of identity by state (Eq. 4), was significant ($F_{ST}=0.015$, $P=0.014$). The analysis showed that clusters have been experiencing gene flow due to migration from the ancestral population. The contribution of each lineage, estimated by the parameter κ, varies among clusters (Table 6). The lineage 2 was the most

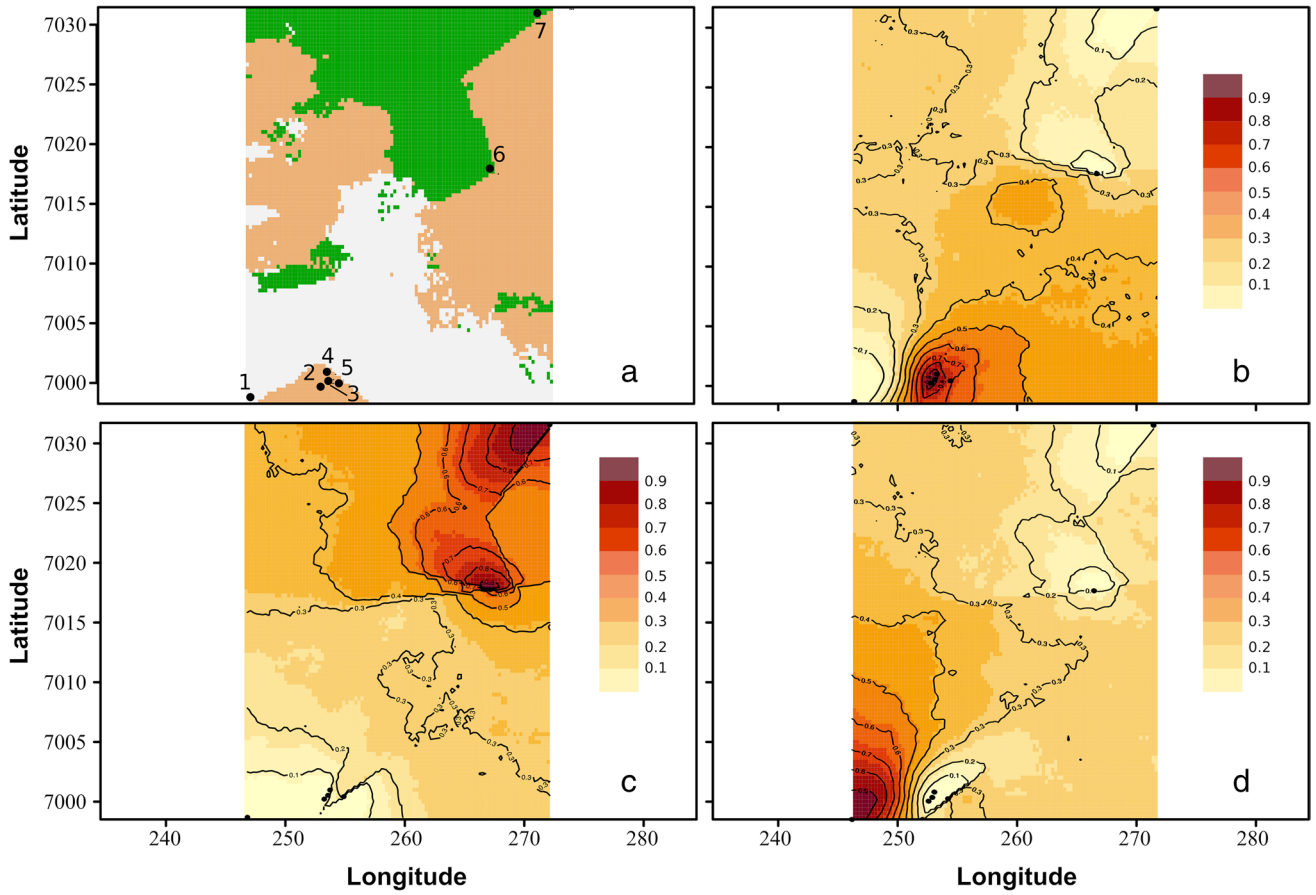

Fig. 3 Landscape Bayesian analysis combining molecular, morphometric, and spatial data (*Geneland*). Under a correlated frequencies model, flies were grouped into three clusters. (**a**) Probable distribution map of each cluster. (**b**), (**c**), and (**d**) represent respectively the membership probabilities for clusters 1, 2, and 3 of each point of the map. Clusters are indicated by areas with different intensities of colour. Darker-coloured areas indicate a higher probability that individuals belong to that cluster (the maps show the location of the sampled trees as black dots, and population codes are the same as in Table 1)

widely spread, as the sum of its contribution ($\Sigma\kappa_2$) is 1.19, and the least expanded would be the lineage 3 ($\Sigma\kappa_3 = 0.812$). Regarding drift (measured by the parameter $\boldsymbol{\alpha}$), the greatest effect was observed for lineage 1, followed by 3 and 2 (Table 6).

These results are consistent with the information from the *Ne* calculated with NeEstimator (Table 7), as the cluster with the highest *Ne* (Cluster 2) has the largest contribution of the lineage with the lowest drift effect. For the whole population, the result for *Ne* was 166.2 consistent with the sum of the aforementioned *Ne* for each cluster (173.3).

Discussion

Pest species can modify native biodiversity, directly or indirectly shape new interspecific interactions, and induce changes within the community that may even lead to the exclusion of native species (Devescovi et al. 2015). Hence, knowledge on the biology of pest species is essential for management programs and ecosystem restoration. In this study, we characterised the genetic diversity, genetic structure, and dynamics of a local population of *Anastrepha fraterculus*, an important pest of cultivated and wild fruit plants. Little is known of ecologically influenced evolution in the genus *Anastrepha* (and most other economically important fruit fly genera) (Ruiz-Arce et al. 2019); therefore, the results obtained in this study may contribute to discuss the possible interaction among different evolutionary processes in related species with similar colonising strategies.

As many other species of the genus, *A. fraterculus* is highly polyphagous with a great ability to acclimate to different environments, and is considered an opportunistic species. In the area studied, its population dynamics shows a marked seasonality, with two annual peaks in March–April and September-December respectively (Altamirano 2017). The first peak coincides with the fruiting of the guava trees (*Psidium guajava*, Myrtaceae), which is one of the preferred

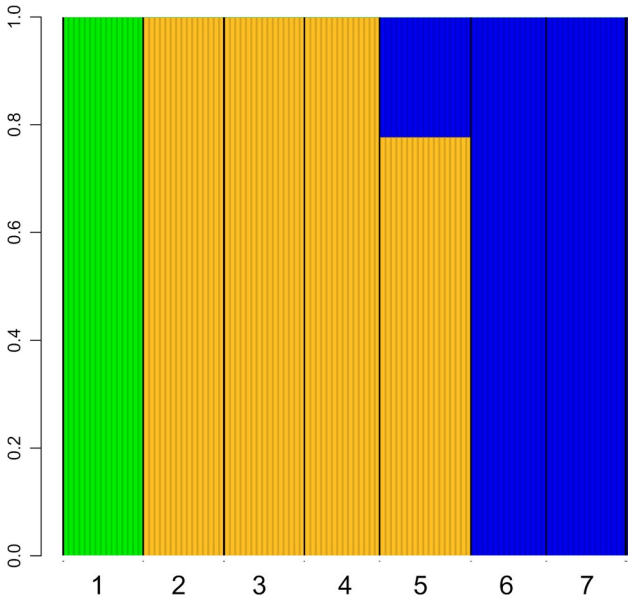

Fig. 4 Membership probability for each individual for the three identified clusters (*Geneland*). Trees 2, 3, 4, and 5 were assigned to cluster 1; 6 and 7 to cluster 2, and tree 1 to cluster 3

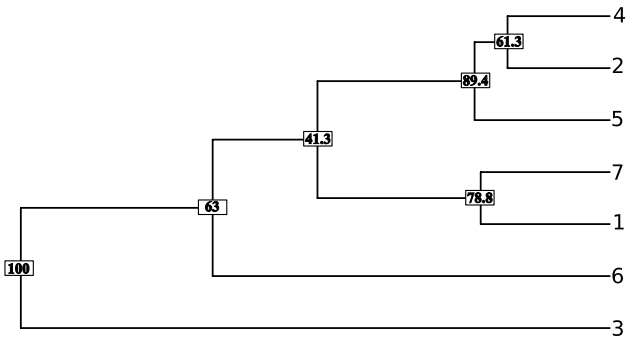

Fig. 5 UPGMA based on Nei's genetic distances between *Anastrepha fraterculus* sampling sites studied. Numbers over branches represent bootstrap support for each node. Subpopulations are not grouped according to geographic distances

hosts of the *A. fraterculus* complex and the main multiplier host for *A. fraterculus* in the Austral Yungas (Schliserman et al. 2016). During this period, the species reaches its highest biotic potential and population density. During the rest of the year, mortality factors predominate, and flies migrate in search of alternative hosts (Altamirano 2017). The marked seasonality coupled with the holometabolic cycle of this species gives place to a metapopulation dynamics (Hanski and Gilpin 1991). Subpopulations occurring in particular patches continually go extinct and have a high population turnover, but the metapopulation persists as long as the colonisation rate is equal to the extinction rate (Balkenhol et al. 2015).

Population dynamics is a function of historical and recent evolutionary processes. Genetic drift, gene flow, inbreeding, natural selection, and mutation, as well as environmental and anthropogenic factors, shape population structure, genetic variation, and connectivity among and within populations (Barrett and Kohn 1991; Slatkin 1993; David et al. 2003). The evaluation of population structure at both local fine scale and large geographic scale levels is crucial for gaining insight into the target species history. This would be a key factor for pest management because it improves the ability to predict how populations will react to environmental changes (Wei et al. 2015). In the present work, we focused on a local population dynamics and structure. Although the sampling is limited, the analysis conducted allowed us to reveal signatures of recent demographic and selective processes. This approach, applied to other populations of *A. fraterculus,* would contribute to a better understanding of the adaptive strategy of this species.

Environmental changes, including physical factors as well as resource properties, are expected to affect adaptive loci, which would be either directly or indirectly reflected in the phenotype and individual fitness. Moreover, ecological mechanisms such as spatial heterogeneity and differences in productivity among substrates that stimulate the diversification of individuals within fruit fly species of the family Tephritidae have been recorded in many species.

Fig. 6 Global spatial autocorrelation analysis. Through the obtention of a single SA statistic (Moran's I) across all the analysed areas, all traits showed a negative autocorrelation with distance. The SA was significant for thorax length (THL), eye length (EL), and wing length (WL) (shown in red, $P < 0.05$)

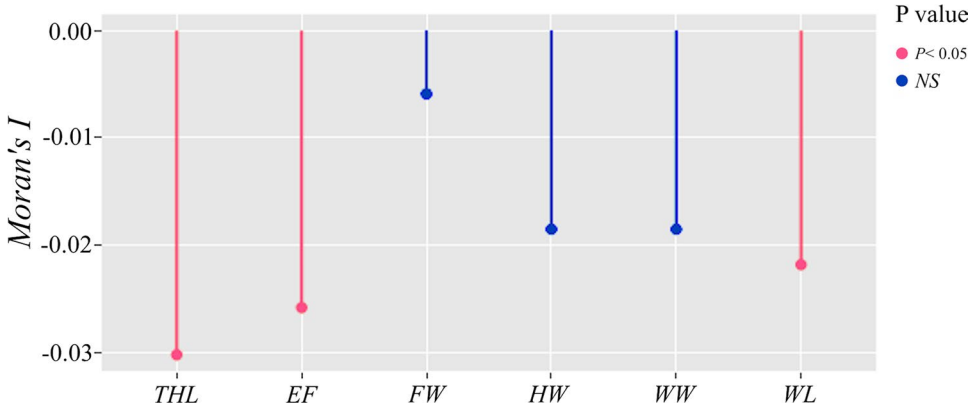

Table 6 Contribution of different lineages to the sampled clusters. Three lineages with differential contributions were found. Subpopulations were labelled following clusters found by *Geneland.* Σ κ represents the sum of each lineage contribution. Lineage-specific drift is represented by *α*

Subpopulation	Lineage 1	Lineage 2	Lineage 3
1	0.564	0.227	0.209
2	0.333	0.589	0.077
3	0.100	0.374	0.526
Σ κ	0.997	1.19	0.812
α	4.02	1.19	2.20

Table 7 Effective population size (*Ne*) estimates obtained by multilocus coancestry (Nomura 2008) for the current subpopulations, which were labelled following clusters found by *Geneland*

Subpopulation	*N*	*Ne*	*CI* [95%]
Cluster 1	61	10.5	3.4–21.5
Cluster 2	29	155	0.2–778.0
Cluster 3	15	7.8	3.8–13.2
Sum	105	173.3	0.2–778.0
Whole pop		166.2	0.2–834.4

N sampled individuals, *CI* confidence intervals

An example is *A. obliqua,* where they influenced the use of alternative resources (Ruiz-Arce et al. 2019). Similarly, it has been recorded in *A. fraterculus* that chemical differences between host plants may have led to population differentiation (Oroño et al. 2013), insects may adapt to exhibit different phenotypes in particular resources, and these specialised forms would allow the colonisation of diverse ecological niches. Therefore, estimating quantitative trait genetic parameters is essential to quantify adaptive genetic variation.

One of the objectives of this paper was to unveil the differences in morphometric and genetic variances at different hierarchical levels. A methodological constraint for the analysis conducted comes from the unpredictability of the number of individuals that may be obtained from each fruit and the restricted area occupied by the host plant in the sampled site. As a consequence, although the sampling involved a large number of infested fruits, the effective data set was relatively small. However, we believe that our results are trustworthy because we made efforts to reduce sample imbalance and applied statistical methods and generalised models that are not demanding in terms of assumptions. The morphometric analyses revealed that the greatest proportion of the variance was explained by the individual level. Tree level was significant only for wing traits (WL, WW). Body size and head shape traits (THL, EL, FW, HW) exhibited significant variance at the fruit level. When the distribution of variance components is the result of demographic factors, the same trends should be recorded for all traits because processes such as drift-migration interaction are not trait dependent. This is the expected scenario when traits are selectively neutral. By contrast, the different trends observed in the present case for different traits are compatible with selective causes. Additional evidence for this hypothesis can be obtained from the comparison with genetic structure parameters estimated from neutral markers.

As for the molecular analyses, the employed SSR markers proved to be highly polymorphic generating different multi locus genotypes for each individual. The population analysed in the present paper showed higher genetic diversity than that recorded by Rodriguez et al. (2019) using the same markers in another population from the Yungas. Among the possible reasons, we found a marked difference in the number of female founders per fruit, since we estimated 10 ovipositing females per fruit, a value three times higher than that found by those authors. Although the accuracy of this estimate relies in assumptions that are not usually met in population studies, the high genetic variability among individuals recovered from single fruits is in line with a relatively high number of founders. *Anastrepha fraterculus*, as well as many other fruit flies, such as *Ceratitis capitata*, deposit host-marking pheromones (HMPs) on the fruit surface after oviposition (Prokopy et al. 1978), which are expected to inhibit egg laying by other females of the same species (Devescovi et al. 2015). The same effect was described for faeces of the ovipositing female (Bachmann et al. 2020). The response to those deterrents in tephritids is a good example of oviposition plasticity (Devescovi et al. 2015). Responsiveness to HMPs seems to be variable and changes according to egg load, fruit size, the experience of the female in terms of proportion of infested/non-infested hosts encountered during foraging, and the time since the last host was encountered (Roitberg and Prokopy 1984; Aluja et al. 2001). Altogether, these studies suggest that, when the time needed to find an optimal host is long, females are more prone to accept already occupied hosts (Papaj et al. 1989). More ovipositing females per breeding site could lead to the higher diversity we found. This feature may contribute to their ability to colonise novel habitats (Karsten et al. 2013), because the population evolutionary potential (or capacity to adapt to environmental changes) is highly dependent on the genetic diversity (see Lavergne and Molofsky 2007; Steeves et al. 2017).

Along with high levels of genetic diversity, both *F* and *Φ* statistics indicated limited genetic differentiation among trees, which suggested high gene flow patterns. The high within tree genetic diversity and low among tree genetic differentiation may be due to high gene flow within the metapopulation. In this sense, little is known of the dispersion of *A. fraterculus* in natural populations. The study of dispersal ability is fundamental for the design of trapping

and monitoring programs, and knowledge of the dispersal ability of the released sterile flies is essential to carry out an effective control by SIT (Utgés 2012). Previous studies based on the release of artificial populations of *A. fraterculus* revealed an average maximum distance reached by flies of 150–160 m (Utgés 2012). However, direct estimates are typically measured under natural or semi-natural conditions at a specific time point and may exclude the movement of individuals under extreme conditions, over longer time scales or multiple generations (Karsten et al. 2013).

Population structure Bayesian methods can be used to detect dispersers and offspring with mixed ancestry from two or more genetic groups documenting evidence of gene flow (Manel et al. 2005). In this work, the Bayesian analysis using the *Geneland* package gave evidence of a cryptic structure with three clusters. They seem to be natural groups as the analysis conducted with RAFM demonstrated that the genetic differentiation (F_{ST}) among them is significant. It has been proposed that Bayesian methods may help to delineate population boundaries when those boundaries are uncertain (Pritchard et al. 2000). The assignment test showed that eight microsatellite loci are sufficient to reveal population differentiation. *Geneland* analysis also suggested that flies are able to widely disperse through distances of the same order as the distances between neighbour sampling sites (trees). The maximum distance between trees involving the same cluster was 10 km (distance between trees 6 and 7). Therefore, this suggests a dispersal ability of *A. fraterculus* of at least 10 km, which could be even higher because some admixture is observed between clusters 1 and 2 in tree 5, which was separated by 14 km from the nearest tree (6). These findings suggest a natural dispersal ability higher than that of *C. capitata,* estimated between 7 and 8 km (mainly flight) (Karsten et al. 2013).

Differences between UPGMA (Fig. 5) and *Geneland* (Figs. 3 and 4) results may be explained because the datasets they incorporate are different. The UPGMA uses an estimated pair-wise distance matrix and does not include spatial information. On the other hand, *Geneland* analysis is broader since it provides information on the spatial genetic structure and combines geographic coordinates, molecular, and morphometric data. This analysis is more sensitive and integrative, which makes it better for populations presenting subtle structuring, as is our case. From the evidence retrieved from this approach, we then carried out an analysis to contrast natural selection and genetic drift in the population structure of quantitative traits, to dissociate genetic and environmental factors shaping population dynamics.

The lack of significant correlation between geographic and genetic distance showed that the differentiation among populations did not fit the IBD model. This may be explained by assuming that populations have not reached the migration-drift equilibrium (Hedrick 2005a, b a,b) due to successive colonisation-extinction processes, a result also consistent with the lack of molecular coancestry autocorrelation. As for quantitative traits, we found a negative spatial correlation for all traits, although significant only for WL, THL, and EL. Negative spatial autocorrelation occurs when similar observations are regularly more spaced than random expectations, so that nearby observations are on average more dissimilar than more distant ones. These results are consistent with the high number of female founders within fruit, which would have led to high morphometric diversity. Thus, quantitative and molecular analyses were congruent and showed evidence of high gene flow and gave support to our estimate of the dispersal ability of *A. fraterculus.*

Within this scenario, individual movement between patches is perhaps the most important defining feature of a metapopulation. We performed a modelling of the demographic history of the found subpopulations (clusters) under the admixture *F*-model (AFM), which contains both gene flow and pure random drift as factors influencing the level of differentiation. Given that each possible host is not available in all seasons, flies are forced to shift hosts along the year. For example, in neighbouring areas to the studied site, *A. fraterculus* moves from peaches to guava from December to April (Oroño et al. 2013). Therefore, successive fly generations will use different resources. Each generation is composed of different lineages coming from different places/hosts. The contribution of each lineage to each sampled subpopulation (cluster) is expectedly uneven. That is precisely what we found, with patterns also indicating that clusters are repopulated through time. Garnas et al. (2016) suggested that this could generate an increase in genetic diversity in metapopulations over time, which would lead to rapid evolution and consequent difficulties to manage populations.

In addition to identifying ecological features that shape population dynamics, it is also important to estimate population size. Regarding this issue, Wright (1931) described *Ne* as the number of breeding individuals in an idealised population that contribute genetically to the next generation. Life-history aspects and reproductive biology are thus major determinants of *Ne*, and it is almost always smaller than the census population size (*N*) or the number of individuals estimated from field or other surveys. Among the reasons for this, unequal sex ratio, assortative mating, population size fluctuations, and overlapping generations and/or developmental stages are all examples of demographic factors that make *Ne* smaller than *N* (Balkenhol et al. 2015; Frankham 1995). The ratio between the effective and the census size (*Ne*/*N*) is quite variable, even within the same species. For example, for *Drosophila melanogaster* there are records ranging from 0.004 to 0.98 (Frankham 1995). Nevertheless, it has been reported that 0.1 is a generalised result across different species.

Although periodic estimates of *Ne* are useful for evaluating existing adaptive potential or monitoring changes in the strength of genetic drift and the population viability (Hare et al. 2011), to our knowledge there was no previous available estimation of *Ne* of *A. fraterculus*. If the 0.1 ratio is applicable to our population, the estimated *Ne* of 800 flies obtained here would suggest a *N* close to 8,000–10,000 flies. This result is very important for genetic programs, as the damage to crops is directly proportional to *N*, and an eventual SIT would need to release a number of sterile flies proportional to the reproductive wild ones. Additionally, the *Ne* itself would be important for the determination of inbreeding and would allow predictions about gene drift and gene flow.

Our results showed that the three identified clusters have different *Ne*. This can be explained by the different levels of genetic drift present on the founder lineages revealed by AFM. Genetic drift can be influenced by the number of breeding individuals and the variance in reproductive success among breeders. We found that the cluster formed mainly by the lineage with the lowest drift parameter (*alpha*) had an Ne one order of magnitude higher than the other two clusters. From an ecological point of view, the increase and decrease in the number of fruit flies may be associated to different factors such as the availability, susceptibility and location of the host plants, the intervention of natural enemies, variations in temperature and humidity, and by the general constitution of the landscape surrounding the host orchard area (Schliserman et al. 2014), which determines the relative success of local pest species populations (Kurota and Shimida 2002). Looking to the future, the success of management programs in decreasing pest population size should involve a decrease in genetic diversity of the target population (Karsten et al. 2013). Also, as *Ne* decreases, the ability to adapt to the environment is reduced when compared with the effect of genetic drift.

In line with the variance component results discussed above, neutrality tests indicated signatures of directional selection (local adaptation) for all traits among fruits and for wing traits at tree level. Since adaptation is an inherently multivariate process, natural selection is expected to act on functionally related traits, as our results indicate. The phenotypic differentiation among patches (fruits or trees) is higher than expected under a neutral model based only on the interaction between genetic drift and mutation. This indicates that different local optima are favoured in different patches. In our case, given the seasonality of the hosts, the resource availability would represent environmental changing pressures. We found a high differentiation at the fruit level which suggests local adaptation or acclimation mediated by phenotypic plasticity which would reduce intraspecific competition. For example, smaller body size traits would be associated with lower quality diets. Rodriguez et al. (2019) have found evidence of directional selection acting on wing traits as a possible response to environmental challenges in different fruits. The spatial information included in the present analysis showed that geographically nearby flies experience different selective pressures and of different magnitudes. This fact is a good example of the ecological related variability of natural selection coupled with high phenotypic plasticity in a generalist species. The fact that different phenotypes are favoured in different patches (fruits) without reproductive barriers between flies emerged from different fruits represents a diversifying selection example that allows keeping high genetic variation for quantitative traits.

Taken as a whole, our results show clear cut evidence of ecological and microevolutionary processes including drift associated with cryptic genetic structure and directional selection, playing the main role in micro spatial genetic dynamics of the South American fruit fly, *Anastrepha fraterculus*. Although our analysis is based on a restricted patch of a widely distributed species, the results are useful to understand the internal structure and dynamics of *A. fraterculus* populations. This species is not distributed as a continuum, but as many patches of internally fragmented semi-isolated populations that must face environmental changes across time and space. The modern robust statistical methods employed in this study have proven to be an essential tool that allowed the quantification of evolutionary change processes shaping demographic events for the first time in this species. Further studies on regional and continental scales within landscape genetics may unravel the influence of these various mechanisms at different scales, which would allow gaining a complete view of the genetics and ecology of this species.

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Author contribution LIF, PGC, and JCV designed experiments and collections. LIF, AIR, and PGC processed all the biological material; DF and LIF collected molecular data; and PGC collected morphometric data. AIR contributed to the molecular and morphometric data collection. DF, LIF, JCV, and PGC analysed the data. DF drafted the initial version of the manuscript. All the authors commented and edited later versions of the manuscript and approved its final version.

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Availability of data and material All data discussed in the paper will be made available to readers.

Code availability Software applications are mentioned in the methods, and programs are publicly available.

Declarations

Conflict of interest The authors declare no competing interests.

Consent for publication The authors consent to the publication of all submitted data.

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