Phenotypic Structure of Colombian Populations of Anastrepha fraterculus Complex (Diptera: Tephritidae)

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Abstract

Anastrepha fraterculus (Wiedemann) (Diptera: Tephritidae) is one of the principal quarantine pests in Colombian fruit production. Studies have shown that the nominal species is a complex of cryptic species and that the more common biological entity that exists in Colombia, the "Andean morphotype," is a species, different from others on the continent. The bioecological information available for this morphotype is scarce, yet it is essential for establishing integrated pest management programs and for defining the insect's quarantine status. Knowledge of the pest's population structure, whether biological, genetic or morphological, is a valuable tool in making management decisions. The goal of this study was to gain information on the phenotypic structure of the Colombian populations of this species complex through the morphometric study of the eggs, larvae, and adults. Populations of *A. fraterculus s.l.* from 2 fruit species widely known as hosts for this insect (coffee, *Coffea arabica* L. [Rubiaceae], at medium altitudes; feijoa, *Acca sellowiana* (O. Berg) [Myrtaceae]) at high altitudes) were collected at 9 Colombian localities from the south of the country (Nariño) to the northeast (Santander). Adults were obtained from the collected fruits, which were then used to rear insects in feijoa fruits. Significant variables were selected using a discriminant analysis, and the differences between the populations were analyzed using a multivariate analysis of variance. The variability of each significant variable was studied with a Kruskal-Wallis nonparametric analysis. The morphological variables studied were 21 for adult females, 7 for adult males, 24 for third-instar larvae, and 2 for the eggs. Intraspecific variability was found in the Colombian populations of the *A. fraterculus* Andean morphotype. In populations from higher altitudes, the adults and larvae are larger, whereas the eggs are smaller, compared to populations from lower altitudes. Variability might have a genetic component and be due to the limited movement of the individuals, which may favor the application of management activities in selected areas.

Key Words: South American fruit fly; morphometry; adults; larvae; eggs; altitude

Resumen

Anastrepha fraterculus es una de las principales plagas cuarentarias de la fruticultura colombiana. Diferentes estudios han mostrado que la especie nominal es en realidad un complejo de especies crípticas y que la entidad biológica predominante en Colombia, denominada "Morfotipo Andino", es una especie particular diferente de las demás especies del continente. Es muy poca la información bio-ecológica existente de este morfotipo, lo cual es esencial en el establecimiento de programas de manejo integrado de plagas o en la definición de estatus cuarentenario del insecto. El conocimiento de la estructura poblacional de una plaga, ya sea biológica, genética o morfológica es una herramienta de ayuda en la toma de decisiones de manejo. El presente trabajo busca dar a conocer la estructura fenotípica de poblaciones colombianas del complejo, a través del estudio de variables morfométricas de huevos, larvas y adultos. Se colectaron poblaciones provenientes de 2 especies de frutos ampliamente conocidos como hospederos del insecto (café, *Coffea arábiga* en altitudes medias y feijoa, *Acca sellowiana* en altas altitudes), de 9 localidades colombianas, desde el sur de país (Nariño) hasta el Nor-Oriente (Santander). De los frutos colectados se obtuvieron los adultos, los cuales fueron utilizados para criar insectos en frutos de feijoa. Utilizando el análisis discriminante se seleccionaron las variables significativas y mediante un análisis de varianza multivariado se estudió la diferencia entre poblaciones; usando el análisis no paramétrico de Kruskal-Wallis se estudió la variabilidad de cada una de las variables significativas. Se estudiaron 21 variables morfológicas de hembras adultas y 7 de machos, 24 variables morfológicas de larvas de tercer instar y 2 mediciones de huevos. Se encontró variabilidad intraespecífica en las poblaciones colombianas del morfotipo andino de *A. fraterculus*, siendo que las poblaciones provenientes de mayores altitudes son de mayor tamaño para adultos y larvas, pero con huevos de menor tamaño. La variabilidad puede tener un componente genético y ser debida al poco movimiento de los individuos, lo cual puede favorecer el establecimiento de medidas de manejo en áreas seleccionadas.

Palabras Clave: Mosca suraméricana de las frutas; morfometría; adultos; larvas; huevos; altitud

Approximately 300 species of *Anastrepha* are known (Norrbom et al. 2013, 2016). The South American fruit fly *A. fraterculus* (Wiedemann) (Diptera: Tephritidae) is a very important fruit pest in several South American countries (Norrbom et al. 2013; Hendrichs et al. 2015). *Anastrepha fraterculus s.l.* is distributed in many countries in the American Continent, from the United States to Argentina (Hernández-Ortiz & Aluja 1993; Steck 1991; Zucchi 2000). In South America, this species is spread along 2 bands, 1 along the west coast, including the highlands

and coastal shorelines of the Andes, and the other along the eastern coast of the continent (Vera et al. 2006). South America is its center of diversity, and this fruit fly has been reported to infest approximately 110 plants, including fruit of great economic importance (Norrbom et al. 2013; Hendrichs et al. 2015).

Recently *A. fraterculus s.l.* was demonstrated, using various techniques, to be a complex of cryptic species (Steck 1991; Selivon & Perondini 1998, 2007; Selivon et al. 1999, 2004, 2005; Smith-Caldas et al.

2001; Hernández-Ortiz et al. 2004, 2012; Vera et al. 2006; Cáceres et al. 2009). Hernández-Ortiz et al. (2012, 2015) reported the existence of 8 morphotypes, which might correspond to distinct species, apparently with different host status and therefore of different economic importance.

According to Hernández-Ortiz et al. (2012, 2015), the *A. fraterculus* Andean morphotype is distributed in the highlands of the Colombian and Venezuelan Andes. Population studies of this species from 9 regions of Colombia have shown that only the Andean morphotype exists in the Colombian highlands (Hernández-Ortiz et al. 2015); this morphotype is also known to be reproductively isolated from the other morphotypes (Devescovi et al. 2014). The biological and ecological aspects (including the hosts) of *A. fraterculus* are practically nonexistent for Colombia or Venezuela. Thus, despite being referred to as a quarantine species in Colombia (ICA 2010), this morphotype is largely unknown. The strategy of defining areas of low prevalence of *A. fraterculus* has been prioritized in Colombia to enable non-chemical management of this pest.

To make methods for the control of fruit flies more efficient and safe, greater knowledge is needed on the structure and dynamics of the populations, as well as on the biogeography of the target species (Matallanas et al. 2013, Hendrichs et al. 2015). Current knowledge suggests that, in Colombia, *A. fraterculus s.l.* is distributed mostly at an elevation of about 900 to 2,500 masl (Castañeda et al. 2010; NA Canal, unpublished data), using mainly coffee at the intermediate altitudes, and feijoa, mulberry, and banana passion fruit above 2,000 masl (NA Canal, unpublished data).

Altitude, as a physical factor, may affect the morphology, ecophysiology, behavior, reproduction, diversity and abundance of insects (Cárdenas et al. 2013). A series of ecosystems exist in the tropical Andes, characterized by having different physiologically adapted plants and animals, wherein particular adaptations can be observed even within altitude gradients as small as 500 m (Cárdenas et al. 2013).

Little is known about the intraspecific variability of *Anastrepha* species, or tephritids in general, attributable to the differences in altitude. The Andean morphotype of *A. fraterculus*, offers a unique opportunity in this respect. Thus, the objective of this study was to determine if Colombian specimens of the Andean morphotype of the *A. fraterculus* complex show morphological variability in different locations and at 2 different altitudes. We hypothesized that there are differences among the populations of this species that could provide information on population movements if recolonization of sites occurs when management practices are applied. In addition to being informative concerning the adaptations of fruit flies to different habitats, this study provided additional ecological information about *Anastrepha*. This genus contains a group of pests of great economic importance, for which more information is needed to apply pest management programs effectively.

Materials and Methods

SPECIMEN SAMPLING

Specimens for this study were retrieved from fruits collected in crops at 9 locations from southern to northeastern Colombia (Table 1, Fig. 1). The selected locations were known for their abundance of *Anastrepha*, which allowed collection of large numbers of individuals from small sampling areas. Previous studies (Castañeda et al. 2010) indicated that insects were mainly distributed at intermediate and high altitudes, and that the most commonly used hosts were coffee at intermediate altitudes (1,300–1,900 m), and feijoa at high altitudes (over 2,000 m); these crops do not overlap in their distribution, and due to weather conditions, fruits are available continuously and flies always are present in overlapping generations. In southern Colombia, the Andes mountain chain splits into 3 mountain ranges (western, central and eastern), divided by 2 deep valleys (Cauca and Magdalena) with varying biogeographic characteristics (Kattan et al. 2004). Collections were made from populations in all 3 mountain ranges; apparently these populations are geographically isolated (Fig. 1).

Collected fruits were packed in trays or boxes with vermiculite and transported to the laboratory of entomology at the University of Tolima in Ibagué, Colombia. After 2 wk, the fruits were dissected and the fly larvae and pupae were transferred to bottles for emergence (1-L plastic containers with moistened vermiculite and covered with tulle).

After 2 wk, the adults emerged and were transferred to rearing boxes (aluminum-framed structures of 30 × 30 × 30 cm, with a polypropylene base and tulle walls), fed with an adult diet (Núñez-Bueno & Guzmán-Dueñas 1999) and provided water until they reached sexual maturity. These populations were considered the parental generation, F_0.

Insects may reflect size variations based on food consumption or temperature conditions during development. For this reason, to minimize external effects the insects were reared in feijoa fruits in the lab at 22 °C with 12:12 h L:D photoperiod, and 65 ± 5% relative humidity. Washed and disinfected fruits were offered for 72 h to insects located in the boxes; the fruits then were removed and conditioned in moistened vermiculite for 12 d, after which they were dissected to obtain the larvae and pupae. These larvae and pupae were transferred to emergence chambers that were similar to the chambers previously described but smaller (250 cc) until emergence. Five-d-old adults from the first generation were retrieved, sacrificed and fixed in 75% alcohol for use in measurements. The Ibagué population studied consisted of a 4-yr-old lab colony to which field material was added once a yr to reduce the rearing effect; the insects used in this study were 2 generations after introduction of wild material.

Larvae were obtained from the insects reared inside the feijoa fruits, from the 3rd until the 5th generations, according to the availability of material. Larvae were prepared as indicated by Frías et al. (2006)

Table 1. Information on specimen collection of the Andean morphotype of the *Anastrepha fraterculus* complex in Colombia.

| Host	Department	City	Latitude	Longitude	Altitude (m)
Acca sellowianae	Caldas	Pensilvania	5.3676°N	75.1582°W	2091
Coffea arabica	Cundinamarca	Anolaima	4.7504°N	74.3836°W	1850
Coffea arabica	Tolima	Ibaguè	4.4148°N	75.3140°W	1433
Coffea arabicae	Valle del Cauca	Roldanillo	4.3849°N	76.2223°W	1764
Acca sellowiana	Boyacá	Duitama	5.8249°N	73.0749°W	2569
Coffea arabica	Valle del Cauca	Sevilla	4.2888°N	75.9064°W	1556
Coffea arabica	Nariño	La Unión	1.6149°N	77.1315°W	1704
Psidium acutangulum	Putumayo	Sibundoy	1.2014°N	76.9135°W	2136
Coffea arabica	Santander	Florida Blanca	7.1021°N	73.0703°W	1558
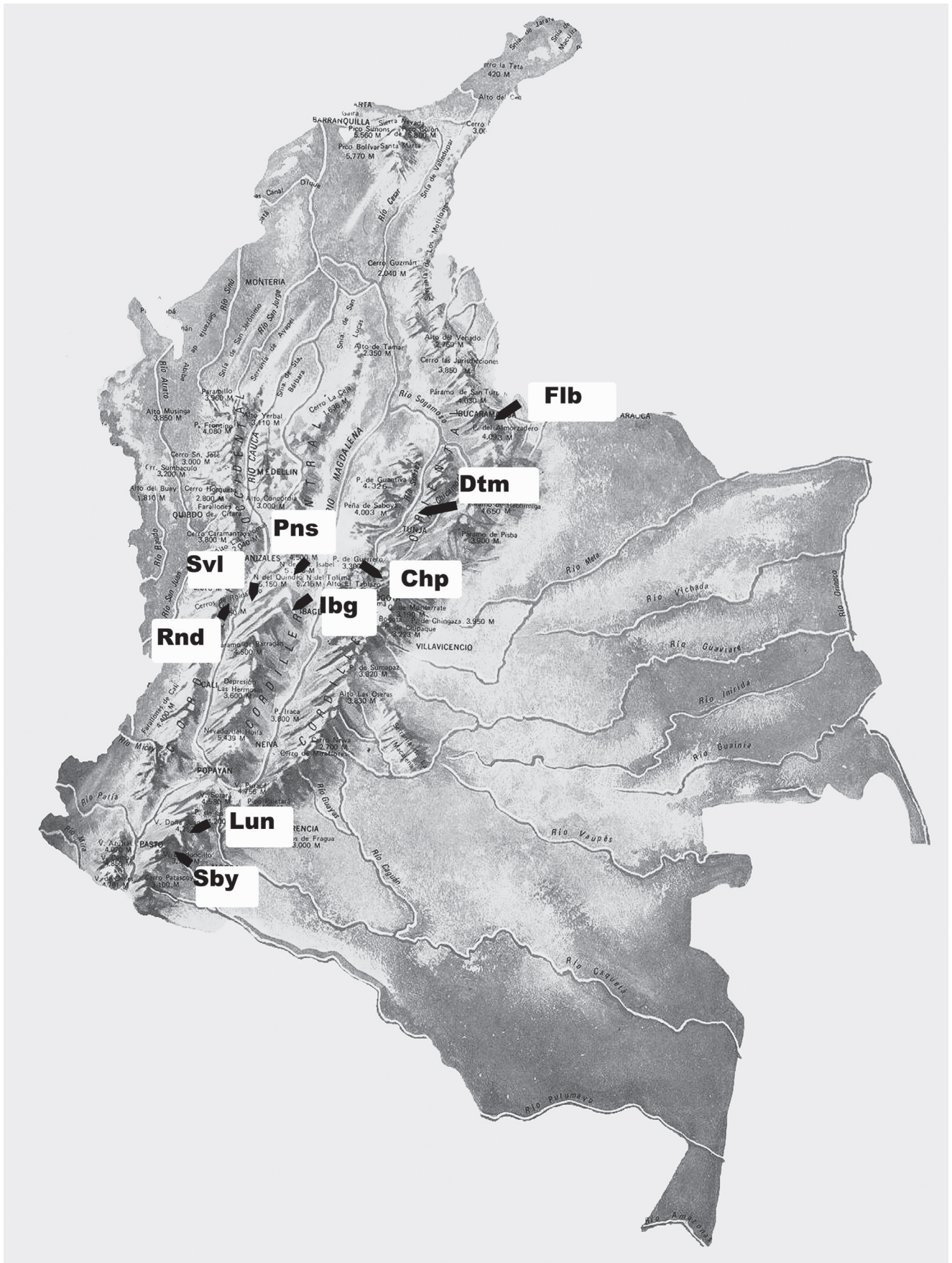
Fig. 1. Geographic distribution of populations of the *Anastrepha fraterculus* Andean morphotype collected in Colombia. Chp: Cachipay; Dtm: Duitama; Flb: Florida Blanca; Ibg: Ibagué; Lun: La Unión; Pns: Pensilvania; Rnd: Roldanillo; Sby: Sibundoy; Svl: Sevilla.

and Canal et al. (2015), i.e., sacrificed in boiling water for 30 to 60 s and fixed in 70% commercial alcohol. Larvae were photographed using a stereoscopic microscope connected to a Moticam 10 camera (Motic Incorporation Ltd., Hong Kong, China) and then placed in KOH overnight for the subsequent removal of the cephalopharyngeal skeleton.

Eggs were collected for the morphometric studies. Oviposition domes made from fabric, paraffin wax, and dye were placed in the rearing chambers. About 20 eggs in good condition were obtained. Eggs were collected from populations cultured for at least 2 yr, which were from approximately the 10th generation. Populations were reared following the methods of Núñez-Bueno & Guzmán-Dueñas (1999).

MORPHOMETRY OF THE ADULTS

Photographs and measurements were taken of the thorax and wings of the adults. Morphometric measurements were taken from females according with the methods of Hernández-Ortiz et al. (2012, 2015), with selection of the features based on their importance in the taxonomy of the genus. The ovipositor and right wing were extracted from 20 females. The ovipositor was placed in 10% KOH overnight, after which it was removed and mounted, along with the wing, on final plates in Canada balsam. The ovipositors were photographed with

a Canon Power Shot G10 coupled to a Zeiss Primo Star microscope (Carl Zeiss MicroImaging GmbH, Gottingen, Germany), and the wings were photographed under a stereoscopic microscope with an attached Moticam 10 camera. All pictures were edited with the software Adobe Photoshop CS5 Extended® 12.0.4 (Adobe System Incorporated, San Jose, California, USA). The ovipositors were measured using the same software, whereas for the wings, TPS files were built using the software TPS-UTIL (James Rohlf, Stony Brook, New York, USA), and wing measurements then were taken with the TPS-DIG2 software (James Rohlf, Stony Brook, New York, USA). Photographs of the thorax were taken with a camera attached to a stereoscopic microscope (Fisher Scientific Micromaster, Shanghai China). To achieve this, each thorax was air-dried, mounted with entomological micropins from beneath, and placed on a base.

The measurements suggested by Hernández-Ortiz et al. (2012 Figs. 2–4, 2015 Figs. 1–3) were taken, with the exceptions of the W5 and W6 variables. *Aculeus*: A1, total length of the aculeus; A2, width at the end of the sclerotized margin on the ventral side; A3, width at the beginning of the serrated section, measured between the apices of the second pair of teeth; A4, length of the basal end of the aculeus (from the margin of the sclerotized area on the ventral site at the beginning of the serrated section); A5, length of the apex of the aculeus (length

Fig. 2. Box-plots of the significant linear variables and Kruskal-Wallis analysis of means comparing females among populations of the *Anastrepha fraterculus* Andean morphotype in Colombia. Means topped by the same letter are not significantly different at the 5% significance level.
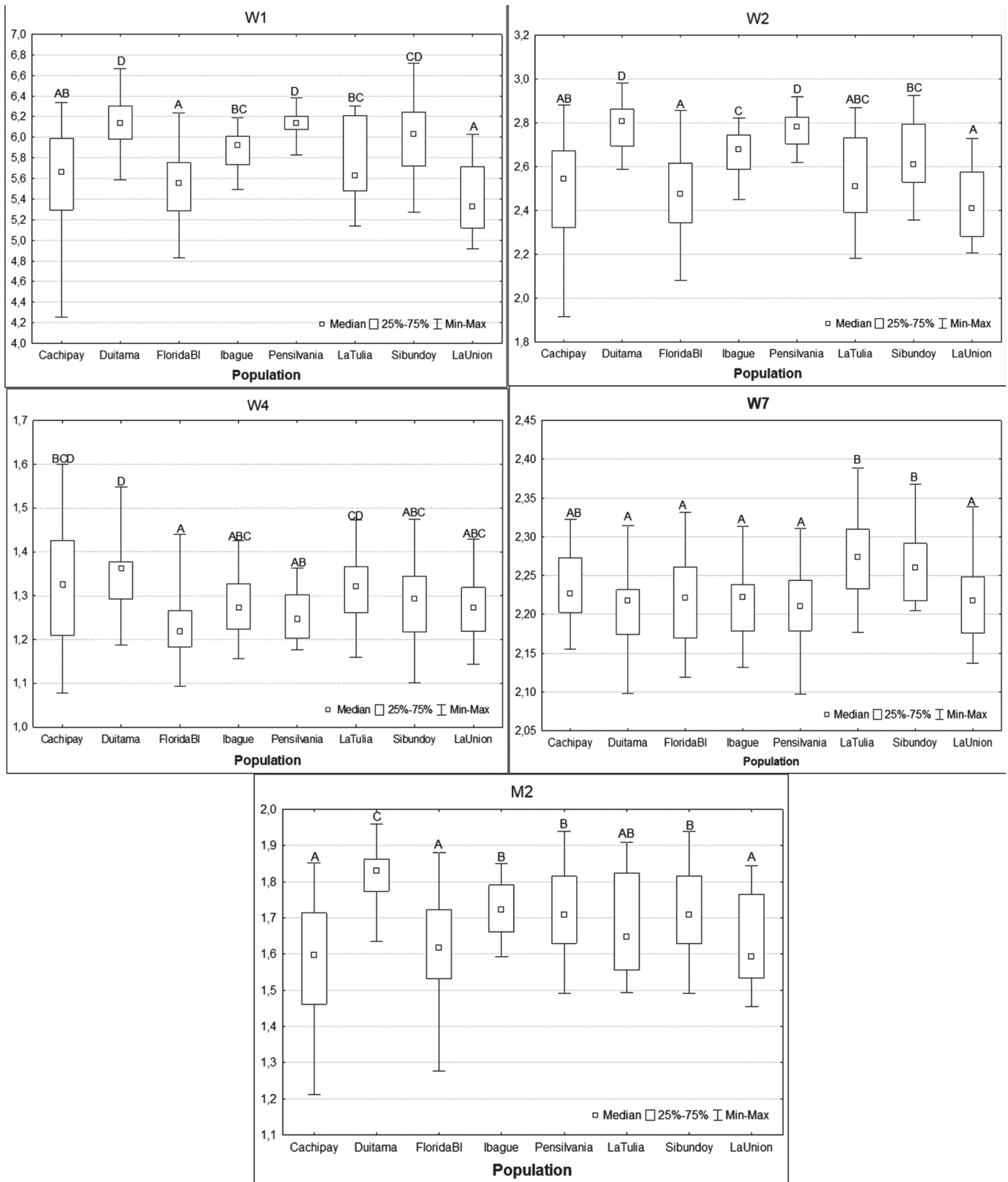
Fig. 3. Box-plots of the significant linear variables and Kruskal-Wallis analysis of means comparing males among populations of the *Anastrepha fraterculus* Andean morphotype in Colombia. Means topped by the same letter are not significantly different at the 5% significance level.

of the serrated section); A6, length of the lateral right side from the base of the sclerotized area; A7, average number of teeth per side; A8, length of the apex (A4+A5); A9: proportion of the non-serrated

and serrated areas of the apex (A4/A5); A10, proportion of the length of the tip of the aculeus and total aculeus length (A8/A1); and A11, proportion of the non-serrated area of the apex and total apex length
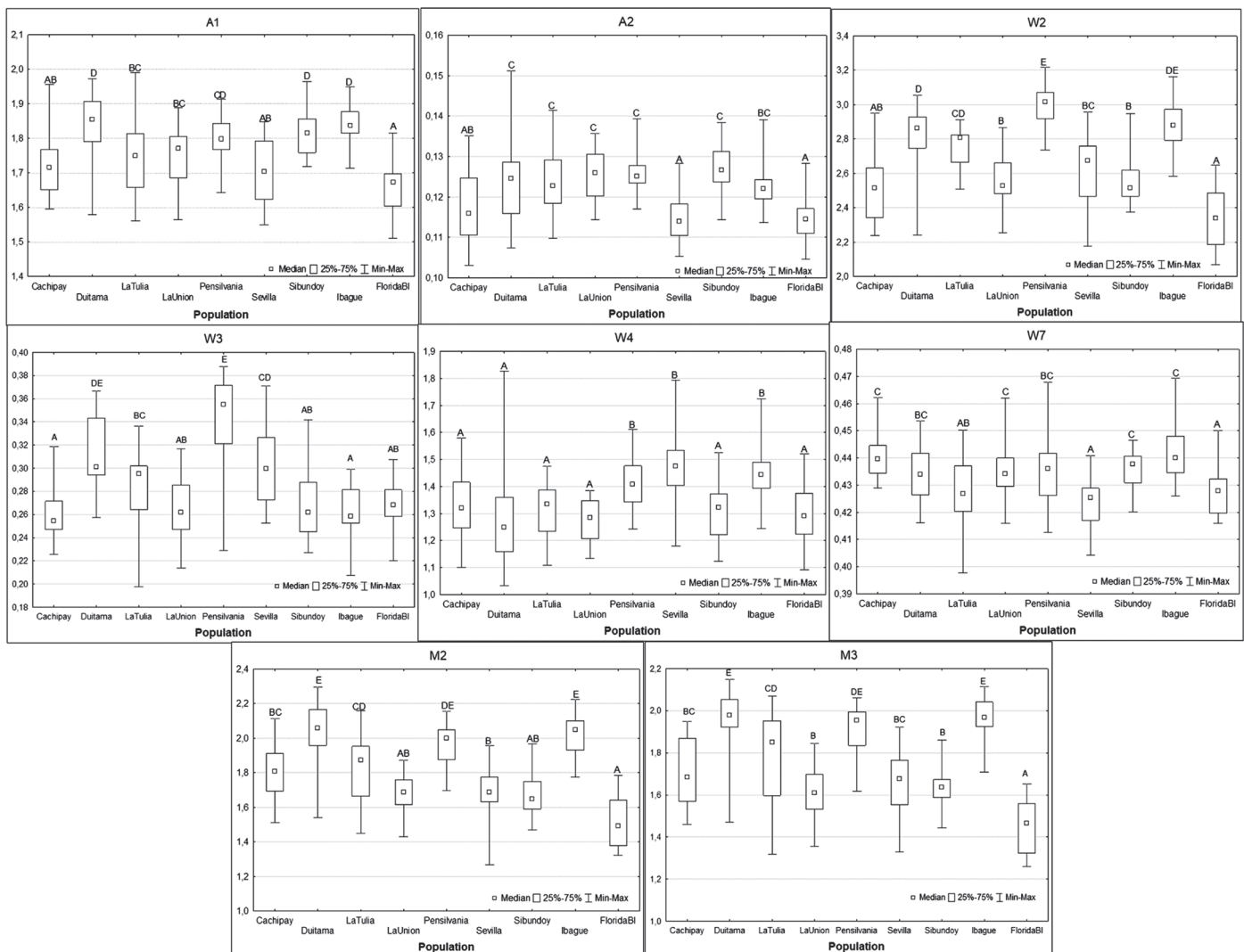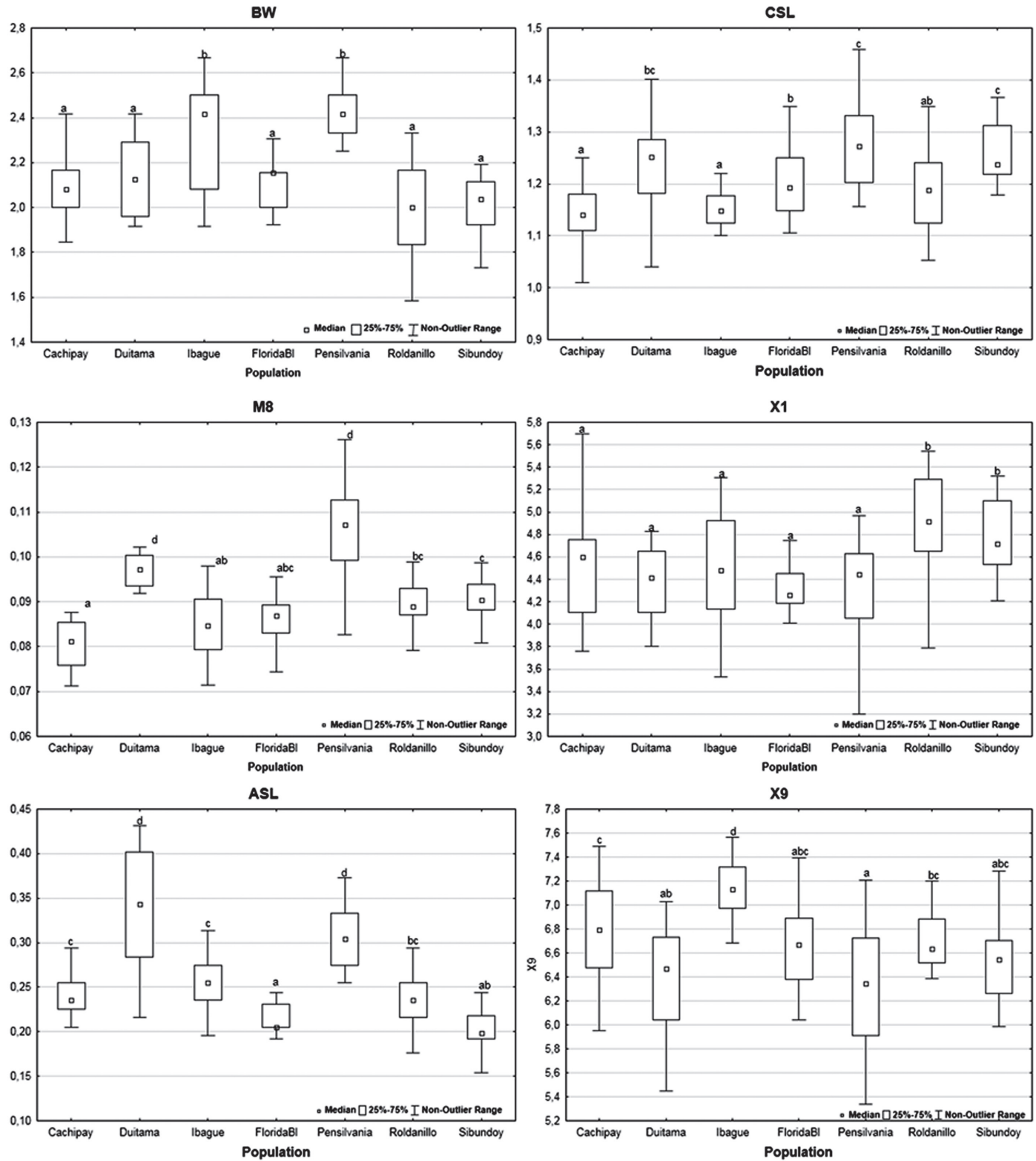
Fig. 4. Box-plots of the significant linear variables and Kruskal-Wallis analysis of means comparing larvae among populations of the *Anastrepha fraterculus* Andean morphotype in Colombia. Means topped by the same letter are not significantly different at the 5% significance level.

(A4/A8). *Wing*: W1, length of the wing from the basal extreme of the costal margin to the apex; W2, wing width at the apex of the R1 vein; W3, width of the S band from the union of the S band and the R4+5 vein perpendicular to the costa; W4, width of the base of the proximal branch of the V band; and W7, W1/W2. *Thorax*: M1, maximum length; M2, width at the level of the postsutural supra-alar seta; and M3, diagonal distance from the postsutural supra-alar seta to the apex of the scutellum. In addition, the X1 ratios were used, proportion of the total

length of the aculeus and maximum length of the thorax (A1/M1); X2, proportion of the total aculeus length and wing length from the basal extreme of the costal vein to the apex (A1/W1); and X3, proportion of the maximum thorax length and maximum wing length (M1/W1).

LARVAL MORPHOMETRY

To study size variation in the larvae, measurements used by Canal et al. (2015 Fig. 2) were taken. Cephalopharyngeal skeletons were mounted in side view and photographed with a Moticam 10 camera attached to a Zeiss Primo Star microscope. Images were edited with Photoshop CS5 extended 12.0.4 (Adobe 2010) software and measured with the Motic Image Plus 2.0 software (Motic China Group CO., Ltd., Xiamen, China). The measurements used were BL: body length; BW: body width at the 6th abdominal tergite; CSL: cephalopharyngeal skeleton length, from the anterior apex of the mandible to the end of the ventral cornua, at lower end of the dorsal cornua; HSL: hypopharyngeal sclerite length, from mouth hook joint to the rear distal point; and HSH: height of the hypopharyngeal sclerite at the anterior base of the hypopharyngeal bridge, perpendicular to the upper edge. The measurements of the mouth hook were M1: length from the apex to the ventral apodeme; M2: length from the apex to the dorsal most tip of neck; M3: length from the apex to the anterior base of the dorsal apodeme; M4: height from the apex of the ventral apodeme to the anterior base of the dorsal apodeme; M5: depth of ventral concavity from line M1 to tip of nub; M6: thickness of mouthhook at posterior base of nub by the posterior notch; M7: distance between the posterior base of nub and dorsal most tip of neck; and M8: width of the ventral apodeme at the base of the neck, in a line parallel to M1. ASL: width of the left anterior spiracle between the apices of the most extreme tubules; AST: number of tubules of the anterior spiracles; X1: BL/BW; X2: M1/M4; X3: M2/M4; X4: M1/M5; X5: M2/M5; X6: M3/M4; X7: CSL/HSL; X8: CSL/M3; and X9: CSL/M1.

MORPHOMETRY OF THE EGGS

Eggs were placed on carved slides and photographed with a Moticam 10 camera attached to a microscope. The maximum lengths and widths of the eggs were measured with Motic Image Plus 2.0 software.

STATISTICAL ANALYSIS

To identify the morphological traits with highest contribution to the differentiation of populations, a canonical discriminant analysis was performed for the measurements of the adults, larvae, and eggs, grouping individuals *a priori*, according to the origin of the populations. Hernández-Ortiz et al. (2015, see Figure 11a) showed the results of discriminant analysis of the same populations used in our study and no different groups were found; these data were not included in this paper. Larval discriminant analysis was similar to that of adults, and also was not included. To know the inter-populations variability, according to our objectives, Wilks's Lambda test was used to measure the statistical significance of the discriminant variables, and only the significant variables were used in the remaining analyses. Those significant variables were included in a comparison of populations using a nonparametric analysis of variance. Wilks, Pillai, Lawley-Hotelling, and Roy statistics were used to study the significance of the model. Each of the significant variables was compared between the different populations using a Kruskal-Wallis test.

Nonparametric analyses of variance were used to study the variability between populations, between the sexes, and according to altitude. To study the variability caused by altitude, analyses were performed considering 2 groups: the populations collected below 1,900 m and those collected at higher altitudes. The 1st group corresponds to the populations obtained from the coffee fruits and the 2nd to those collected from the feijoa. All analyses were conducted with the software Statistica 12 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

VARIABILITY IN ADULT SIZE

Discriminant analysis of the variables for females showed that significant differences exist among the populations (Wilks's Lambda = 0.02; approx. *F* = 4.9; df = 152,1148; *P* < 0.0001). Nine variables were significant in the model: 2 from the ovipositor, 5 from the wing, and 2 from the thorax (Table 2). Wing size variables showed the highest

Table 2. Discriminant function analysis for the morphometric variables of females (F) and males (M) from the Andean morphotype populations of *Anastrepha fraterculus* in Colombia. (* Significant variables).

	Wilks's Lambda		Partial Lambda		F-remove		P-level	
	F	M	F	M	F (8,153)	M (7,145)	F	M
A1	0.028		0.854		3.249		**0.002***	
A2	0.027		0.880		2.593		**0.011***	
A3	0.026		0.913		1.820		0.077	
A4	0.026		0.924		1.582		0.134	
A5	0.024		0.983		0.327		0.955	
A6	0.026		0.937		1.290		0.253	
A7	0.025		0.970		0.592		0.783	
A8	0.025		0.962		0.747		0.650	
A9	0.025		0.938		1.255		0.271	
A10	0.025		0.953		0.935		0.490	
A11	0.025		0.960		0.795		0.608	
W1	0.032	0.239	0.737	0.877	6.832	2.915	**0.000***	**0.007***
W2	0.031	0.241	0.764	0.868	5.902	3.149	**0.000***	**0.004***
W3	0.031	0.231	0.763	0.908	5.941	2.098	**0.000***	**0.047***
W4	0.031	0.273	0.776	0.768	5.509	6.263	**0.000***	**0.000***
W7	0.028	0.241	0.854	0.868	3.269	3.149	**0.000***	**0.004***
M1	0.030	0.219	0.796	0.955	4.901	0.969	0.388	0.456
M2	0.025	0.287	0.956	0.729	0.875	7.687	**0.003***	**0.000***
M3	0.028	0.220	0.860	0.949	3.110	1.107	**0.002***	0.362

contribution to the model. In the multivariate analysis of variance that included only the significant variables, populations from the south (La Unión and Sibundoy) showed no significant differences, whereas all other populations were significantly different from each other ([Wilks's = 0.04; F = 9.55; df = 72; P < 0.0001]; [Pillai = 2.29; F = 7.58; df = 72; P < 0.0001]; [Lawley-Hotelling = 4.85; F = 10.87; df = 72; P < 0.0001]; [Roy = 2.22; F = 41.88; df = 9; P < 0.0001]) (Table 3).

A Kruskal-Wallis test conducted for each of the variables separately showed that individuals from Florida Blanca were the smallest in 6 of the significant variables, because they significantly differed in the variables related to body size (the 2 aculeus variables, wing, and thorax size). Ibagué individuals were the largest in 5 variables, sharing the first rank in 3 of the variables with individuals from Duitama (Fig. 2).

For males, the discriminant analysis showed significant differences among the populations (Wilks's Lambda = 0.2; approx. F (56,786) = 4.7; P < 0.0001). Six variables were significant (Table 2). The multivariate analysis of variance showed differences between groups ([Wilks's = 0.29; F = 4.91; df = 42; P < 0.0001]; [Pillai = 1.02; F = 4.43; df = 42; P < 0.0001]; [Lawley-Hotelling = 1.52; F = 5.25; df = 42, P < 0.0001]; [Roy = 0.86; F = 18.76; df = 7; P < 0.0001]) (Table 3), and the Kruskal-Wallis analysis for the significant variables found differences in 5 variables. The Duitama population's individuals were larger in all the variables, and for 2 of the variables (regarding wing size), individuals of this population did not differ from those of Pensilvania (Fig. 3). Individuals from Florida Blanca and La Unión were the smallest.

Size was analyzed according to sex, using wing and thorax variables. The multivariate analysis of variance showed significant differences between the sexes, with females being bigger than males ([Wilks's = 0.03; F = 1649.35; df = 7; P < 0.0001]; [Pillai = 0.97; F = 1649.35; df = 7; P < 0.0001]; [Lawley-Hotelling = 37.12; F = 1649.35; df = 7; P < 0.0001]; [Roy = 37.12; F = 1649.35; df = 7; P < 0.0001]) (Table 4).

The multivariate analysis for females ([Wilks's = 0.68; F = 9.07; df = 9; P < 0.0001]; [Pillai = 0.32; F = 9.07; df = 9; P < 0.0001]; [Lawley-Hotelling = 0.48; F = 9.07; df = 9; P < 0.0001]; [Roy = 0.48; F = 9.07; df = 9; P < 0.0001]) and for males ([Wilks's = 0.68; F = 11.74; df = 6; P < 0.0001]; [Pillai = 0.32; F = 11.74; df = 6; P < 0.0001]; [Lawley-Hotelling = 0.46; F = 11.74; df = 6; P < 0.0001]; [Roy = 0.46; F = 11.74; df = 6; P < 0.0001]) grouped by altitude, and when only the significant variables from the multivariate analysis were considered, showed that individuals living at higher altitudes were bigger (Table 5).

The significant variables were related to body sturdiness, with differences according to sex. The aculeus showed variations among populations for just 2 (total length and width) of the 11 variables studied. Females showed wing width variability, whereas in males, the wing varied in length and width. The coloration pattern of females varied in the width of the S band at the intersection with the R_{4+5} vein, in the an-

terior part of the wing; in males, the variability was significant for the width of the base of the V band, in the posterior part of the wing. Females showed significant variability in thorax width, whereas in males the significant variable was length.

LARVAL SIZE VARIABILITY

The analysis of larvae was performed on 7 populations because 2 populations (Sevilla and La Unión) were not reared in the laboratory in adequate numbers to obtain larvae and adults. The discriminant analysis indicated that the populations were different (Wilks's Lambda = 0.1; approx. F = 6.55; df = 102,662; P < 0.0001). From the 24 variables used, 17 were part of the model, and 7 were significant (Table 6). The multivariate analysis of variance indicated that the populations from Roldanillo and Cachipay did not differ from each other, but they did differ from the remaining populations, which are, in turn, all different from each other ([Wilks's = 0.04; F = 13.21; df = 42; P < 0.0001]; [Pillai = 1.99; F = 9.24; df = 42; P < 0.0001]; [Lawley-Hotelling = 5.63; F = 16.53; df = 42; P < 0.0001]; [Roy = 3.13; F = 58.05; df = 7; P < 0.0001]) (Table 7). The Kruskal-Wallis analysis showed significance for 6 variables: the Ibagué and Pensilvania individuals showed greater body width; populations from higher altitude showed larger cephalopharyngeal sclerites and larger ventral apodemes; the anterior spiracles from the Duitama and Pensilvania individuals were longer; and those of Sibundoy were smallest (Fig. 4). The analysis of larval size in relation to the host of origin showed that the larvae from populations collected at the higher altitudes were largest ([Wilks's = 0.51; F = 17.77; df = 7; P < 0.0001]; [Pillai = 0.49; F = 17.77; df = 7; P < 0.0001]; [Lawley-Hotelling = 0.96; F =17.77; df = 7; P < 0.0001]; [Roy = 0.96; F = 17.77; df = 7; P < 0.0001]) (Table 8).

EGG SIZE VARIABILITY

The discriminant analysis showed that the 2 variables studied were significant (data not shown). Multivariate analysis of variance showed that Sibundoy individuals were smaller than individuals from Pensilvania, and these 2 populations were significantly smaller than the other populations, which did not show significant differences ([Wilks's = 0.41; F = 12.33; df = 12; P < 0.0001]; [Pillai = 0.62; F = 9.86; df = 12; P < 0.0001]; [Lawley-Hotelling = 1.39; F = 14.97; df = 12; P < 0.0001]; [Roy = 1.34; F = 29.25; df = 6; P < 0.0001]) (Table 9). The results from the Kruskal-Wallis test for length did not show significant differences between the Sibundoy and Pensilvania populations, but these 2 populations are significantly different from the others. With regard to width, individuals from the Sibundoy population were the narrowest, being significantly different from the others. Furthermore, Pensilvania, Flori-

Table 3. Comparison of population multivariate means by the Hotelling test with Bonferroni correction for females (F) and males (M) from 9 Colombian origins of the *Anastrepha fraterculus* Andean morphotype. Different letters indicate significant differences among males or females in the populations at the 5% level.

| | A1 | A2 | W1 | | W2 | | W3 | | W4 | | W7 | | M2 | | M3 | | |
Population	F	F	F	M	F	M	F	M	F	M	F	M	F	M	F	F	M
Roldanillo	1.75	0.12	6.26	5.77	2.76	2.55	0.28	0.26	1.31	1.32	0.43	2.27	1.84	1.69	1.79	A	A
Pensilvania	1.80	0.13	6.90	6.13	3.00	2.78	0.34	0.26	1.41	1.26	0.44	2.21	1.96	1.72	1.91	B	B
Sevilla	1.71	0.12	6.19		2.62		0.30		1.47		0.42		1.69		1.65	C	
Ibague	1.84	0.12	6.40	5.87	2.87	2.66	0.26	0.24	1.45	1.28	0.44	2.21	2.02	1.72	1.96	D	BCD
Cachipay	1.72	0.12	5.76	5.59	2.52	2.50	0.26	0.26	1.33	1.33	0.44	2.23	1.80	1.59	1.70	E	A E
Duitama	1.84	0.12	6.47	6.14	2.83	2.64	0.32	0.25	1.28	1.28	0.44	2.26	2.04	1.72	1.97	F	D
Florida Blanca	1.66	0.11	5.35	5.51	2.34	2.49	0.27	0.24	1.30	1.23	0.43	2.22	1.52	1.62	1.45	G	E
La Unión	1.75	0.12	5.80	5.40	2.55	2.44	0.26	0.25	1.28	1.28	0.44	2.22	1.68	1.62	1.61	H	E
Sibundoy	1.82	0.13	5.82	5.97	2.56	2.64	0.27	0.25	1.31	1.28	0.44	2.26	1.67	1.72	1.64	H	C

Table 4. Comparison of multivariate means by the Hotelling test with Bonferroni correction for sex differences among 8 Colombian populations of the *Anastrepha fraterculus* Andean morphotype, using 6 morphometric variables of the wing and thorax. Different letters indicate significant differences between males or females in the populations at the 5% level.

Sex	W1	W2	W3	W4	W7	M2	M3	n	
Female	5.98	2.63	0.28	1.33	0.44	1.82	1.75	160	A
Male	5.80	2.60	0.25	1.66	0.46	2.65	1.69	159	B

da Blanca, and Duitama form a second group (Fig. 5). The multivariate analysis of variance for the host of origin showed a significant difference, with the eggs from medium altitude populations being larger than those from the high altitude ([Wilks's= 0.66; F = 35.3; df = 2; P < 0.0001]; [Pillai = 0.34; F = 35.3; df = 2; P < 0.0001]; [Lawley-Hotelling = 0.52; F = 35.3; df = 2; P < 0.0001]; [Roy = 0.52; F = 35.3; df = 2; P < 0.0001]) (Table 10).

Discussion

Hernández-Ortiz et al. (2012) compared Latin American populations of *A. fraterculus s.l.* through linear morphometry and found that Colombian and Venezuelan highlands populations corresponded to the same morphotype, which they called the Andean morphotype. Hernández-Ortiz et al. (2015) compared the same populations used in our study with other populations from Central America and northern South America, using linear and geometric morphology, and found that all of them belong to the Andean morphotype of the complex. However, genetic tools used by Sutton et al. (2015) showed variability in Colombian specimens belonging to the Venezuelan or Peruvian morphotypes (those collected at low altitudes) and to the Andean morphotype (those from medium to high altitude); 4 specimens in the Andean morphotype showed a small polymorphism.

This study of the intraspecific variability in the Andean Colombian populations revealed statistically significant differences in individual size for all males, females, and immature stages (Tables 2, 6, 9). The individuals studied in the present study were reared in feijoa fruits under the same conditions. The size differences were found to exist despite being reared under identical environmental conditions and in the same host. Similarly, there are sex differences, with females being bigger (Table 4). Moreover, interspecific variability was more evident in females than in males (Table 3). All populations were different at the level of larvae, and showed differences in egg size (Tables 6, 9). Individuals collected at high altitudes were bigger than individuals collected at lower altitude, when compared in the larval and adult stages (Tables 5, 8, 10); however, eggs from higher altitudes were smaller.

Altitude variation influences richness and the population structure of insects (Hodkinson 2005; Körner 2007; Maveety et al. 2011), but additionally, at an intraspecific level, it influences their morphology, physiology, behavior and reproduction (Hodkinson 2005; Cárdenas et al. 2013). However, the effect is not always the same; for example, in some groups the influence of altitude on size results in bigger individuals (Reeve et al. 2000; Gilchrist et al. 2004), whereas in others, the result is opposite (Mousseau 1997; Brehm & Fiedler 2004). Adults and larvae of the Colombian *A. fraterculus* complex were bigger at higher altitudes, and females showed greater morphological local adaptation whereas males are more similar among the different collection sites. Eggs, however, were smaller at higher altitudes.

Dujardin et al. (2009) made a thorough revision of the interspecific morphological variation in Triatominae, which were found to persist in populations collected from different habitats. This suggests that ac-

Table 5. Comparison of multivariate means by the Hotelling test with Bonferroni correction for differences among individuals from Colombian populations of the *Anastrepha fraterculus* Andean morphotype, collected at 2 altitudinal gradients. Different letters indicate significant differences among males or females in the populations at the 5% level. F: females, M: males.

Altitude	A1	A2	W1 F	W1 M	W2 F	W2 M	W3 F	W3 M	W4 F	W4 M	W7 F	W7 M	M2 F	M2 M	M3 F	M3 M	n F	n M	F	M
Medium	1.74	0.12	5.96	5.63	2.61	2.53	0.27	0.25	1.36	1.29	0.43	2.23	1.76	1.65	1.69		120	100	A	A
High	1.82	0.13	6.40	6.08	2.80	2.73	0.31	0.26	1.33	1.30	0.44	2.23	1.89	1.76	1.84		60	60	B	B

Table 6. Discriminant function analysis for morphometric variables of 3rd-instar larvae of 7 populations of the *Anastrepha fraterculus* Andean morphotype in Colombia. (* Significant variables)

	Wilks's Lambda	Partial Lambda	F-remove (-6,115)	*P*-value
ASL	0.039	0.482	20.568	0.000*
M1	0.020	0.918	1.712	0.124
BW	0.021	0.876	2.718	0.0167*
X1	0.021	0.878	2.659	0.019*
M8	0.025	0.738	6.811	0.000*
X7	0.021	0.901	2.100	0.058
M4	0.020	0.956	0.875	0.516
X5	0.021	0.880	2.610	0.021*
X8	0.020	0.941	1.205	0.309
X9	0.022	0.868	2.922	0.011*
HSL	0.020	0.912	1.854	0.095
CSL	0.021	0.897	2.208	0.047*
BL	0.020	0.924	1.568	0.162
HSH	0.020	0.925	1.556	0.166
ND	0.020	0.940	1.220	0.301
M6	0.020	0.946	1.084	0.376
X6	0.020	0.950	1.016	0.418

commodation mechanisms or genetic assimilation may occur, which could lead to genetic differentiation, and represent cases of true disruptive selection. In contrast, the variation in the physical conditions that exist due to altitude variation (temperature, atmospheric pressure, humidity, presence and quality of hosts, among others), which could also be enhanced by topography, become true barriers to the movement of insects (Hodkinson 2005; Körner 2007). Therefore, populations are isolated in these geographic areas, which can become sites of microevolution and adaptation (Körner 2007).

No studies have been conducted on the morphological variability of Tephritidae in association with different latitudinal or altitudinal geographic environments. Only the studies by Hernández-Ortiz et al. (2004) refer to some morphological variability in the different Mexican populations of *A. fraterculus*, which are present in different ecological conditions, and in various Central American populations of the same morphotype of the complex. Studies of genetic structures have been performed on fruit flies of various genera, among which *A. fraterculus* has been studied only in Argentina (Alberti et al. 1999) and *A. ludens* in Mexico (Molina-Nery et al. 2014). Both cases showed high levels of homozygosity, indicating little breeding among individuals from separate populations, which in turn might suggest low displacement of these insects. Few results exist on the movement capability of *A. ludens* flies, and those indicate that these flies could move up to 240 m (Hernández et al. 2007) or several kilometers (Fletcher 1989; Thomas

Table 8. Comparison of multivariate means by the Hotelling test with Bonferroni correction for differences in the larvae of Colombian populations of the *Anastrepha fraterculus* Andean morphotype collected at 2 altitudinal gradients. Different letters indicate significant differences among populations at a 5% level.

Altitude	ASL	BW	X1	M8	X5	X9	CSL	n	
Medium	0.24	2.13	4.59	0.09	3.58	6.85	1.17	78	A
High	0.28	2.18	4.51	0.10	3.64	6.44	1.25	60	B

& Loera-Gallardo 1998), which ultimately depends on the availability of the hosts (Fletcher 1989), although the exact causes are unknown (Fletcher 1989; Hernández et al. 2007).

In Argentina, sympatric population of *A. fraterculus* showed microdifferentiation due to host use, which was revealed in morphometric and genetic variability (Gómez-Cendra et al. 2016). Colombian populations of the Andean morphotype of this species show microevolutionary processes like Argentinian populations; however, Colombian populations are allopatric. The Argentinian specimens were adults collected from the field; in contrast, our specimens were reared in the laboratory under same conditions, and we studied adults and immature stages. Our data strengthened findings of Gómez-Cendra et al. (2016).

Results of this study show that in Colombia, populations of the *A. fraterculus* Andean morphotype might have little migratory movement (sensu Aluja 1993), keeping populations partially isolated, but our hypothesis is that the altitude and the heavily rugged topography are the most important barriers that impede those movements. Because morphological variability remained in individuals reared under the same conditions, it is possible that genetic factors may be involved in the expression of these phenotypes, as was observed in Argentinian host-related populations. However, other tools such as genetic studies should be conducted to assess possible microevolutionary processes or differentiation processes, such as was observed in the triatomines (Dujardin et al. 2009). In this regard, Raba et al. (2003) conducted a genetic study of Colombian *A. fraterculus* using 12 populations and 10 oligonucleotides for use in random amplified polymorphic DNA (RAPD); they found polymorphisms among all populations, and the results were similar to ours.

In our study, females show the most differentiated adaptation processes. However, too little is known about the ecology of this species to infer the causes of those variations. The reason for the difference in the variables between the males and females is most likely related to reproductive processes, although nothing is certain in this regard. Females searching for hosts for oviposition probably undergo further displacement (of a trivial type sensu Aluja 1993), and the variability in size could be caused by the need for flight adaptations in the physical conditions of the atmosphere at higher altitudes (Körner 2007). Adaptations in males are perhaps more related to courtship, as noted by

Table 7. Comparison of population multivariate means by the Hotelling test with Bonferroni correction for the morphometric variables of larvae from 7 origins of the *Anastrepha fraterculus* Andean morphotype based on 9 morphometric variables. Different letters indicate significant differences among populations at a 5% level.

Population	ASL	BW	X1	M8	X5	X9	CSL	n	
Ibague	0.26	2.33	4.51	0.08	3.65	7.20	1.15	19	A
Pensilvania	0.31	2.42	4.35	0.11	3.55	6.31	1.28	20	B
Sibundoy	0.20	2.00	4.80	0.09	3.71	6.55	1.26	20	C
Duitama	0.34	2.13	4.39	0.10	3.66	6.46	1.23	20	D
Florida Blanca	0.21	2.10	4.37	0.09	3.63	6.70	1.21	20	E
Roldanillo	0.23	2.01	4.94	0.09	3.54	6.72	1.19	19	F
Cachipay	0.24	2.08	4.53	0.08	3.50	6.79	1.14	20	F

Table 9. Comparison of population multivariate means by the Hotelling test with Bonferroni correction for morphometric differences in eggs from 7 Colombian origins of the *Anastrepha fraterculus* Andean morphotype. Different letters indicate significant differences among the populations at the 5% level.

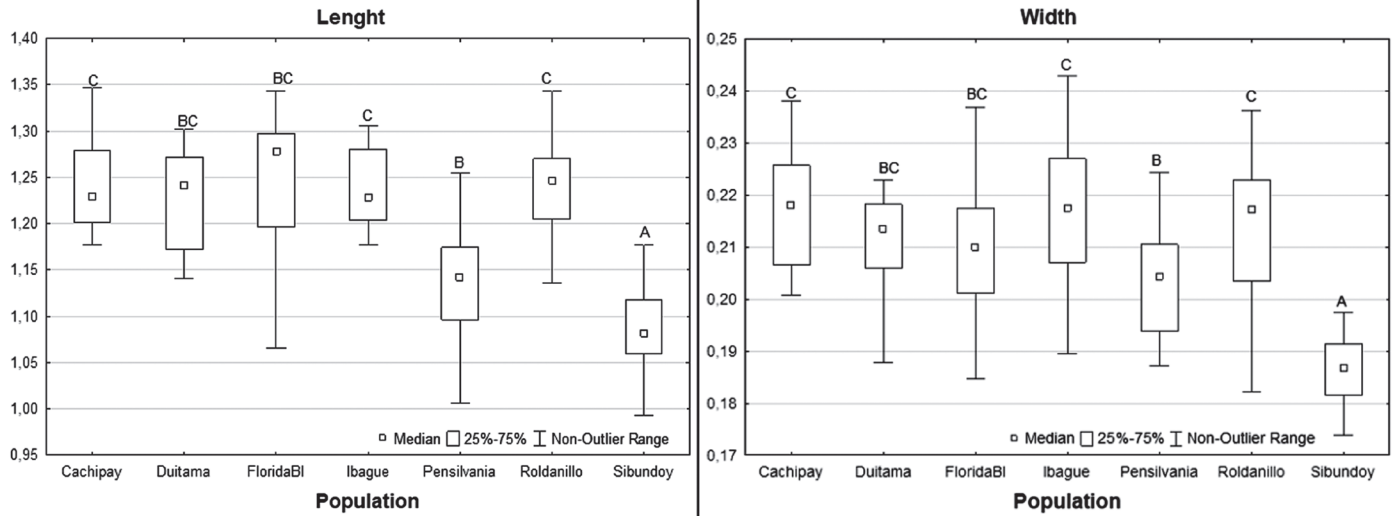| Population	Length	Width	n	
Sibundoy	1.09	0.19	20	A
Pensilvania	1.14	0.20	21	B
Roldanillo	1.24	0.21	20	C
Ibague	1.25	0.22	20	C
Cachipay	1.25	0.22	19	C
Duitama	1.23	0.21	18	C
Florida Blanca	1.25	0.21	20	C

Fig. 5. Box-plots of the significant linear variables and Kruskal-Wallis analysis of means comparing eggs among populations of the *Anastrepha fraterculus* Andean morphotype in Colombia. Means topped by the same letter are not significantly different at the 5% significance level.

Marsteller et al. (2009) for *Blepharoneura*. In the case of the Andean morphotype of *A. fraterculus*, however, the development of further adaptations may not have been necessary. Insect adaptations to the different altitudes are due to physical changes in the environment. For example, bigger size may be due to longer development periods (Hodkinson 2005), which could be the cause of greater sizes in larvae. Körner (2007) also indicates that the egg shells in some birds become more porous to facilitate the exchange of oxygen for proper development of the embryo. By extension, in Colombian populations of *A. fraterculus* the smaller eggs may be adaptive because they require a lower amount of oxygen. In the same manner, larger anterior spiracles of larvae allow them to take in greater amounts of oxygen.

Anastrepha fraterculus is considered one of the most important pest species to fruit producers in Colombia (ICA 2015). The practical significance of this work indicates the importance of an awareness of pest migratory processes in pest management programs (Fletcher 1989; Aluja 1993), along with the need to establish control measures in selected areas. This insect pest is primarily associated with coffee and some secondary hosts at intermediate altitudes; at high altitudes, it infests crops such as feijoa, banana passion fruit, and mulberry (Castañeda et al. 2010; NA Canal, unpublished data). In selected production areas that suffer important economic damage, measures to eliminate the pest could be implemented. These measures, together with quarantine measures, would lead to reduced opportunity for re-infestation due to the low rate of natural movement of external populations toward the areas under treatment.

Another important aspect is the role that the Colombian topography can play in the displacement of introduced species. Adults of the Mediterranean fruit fly, *Ceratitis capitata* (Weidenmann) (Diptera: Tephritidae) can travel several kilometers (Fletcher 1989); this pest was detected in southern Colombia in 1987 and, until 1993, it had been detected in a few areas (mostly urban and suburban) in 5 departments, probably by passive transport (Velez 1997). According to epidemiological information from the national plant protection organization, by 2010 this insect remained in the same areas and had barely moved toward the urban zones of a neighboring state (ICA 2010). This suggests that despite the migration ability of the Mediterranean fruit fly, it can only passively migrate in Colombia, perhaps due to topographical barriers. These barriers seem to be the cause of isolation of the *A. ludens* (Loew) (Diptera: Tephritidae) populations in Mexico (Molina-Nery et al. 2014) and might have the same effect on other native or introduced tephritids, perhaps not only in Colombia but in other regions as well.

In summary, the Colombian populations of the Andean morphotype of *A. fraterculus* from different localities differ in morphometry, suggesting that populations remain partially isolated. In addition, minimal migration is thought to occur between the different areas, which is favorable for the establishment of management systems.

Acknowledgments

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Table 10. Comparison of multivariate means by the Hotelling test with Bonferroni correction for differences in egg size in Colombian populations of the *Anastrepha fraterculus* Andean morphotype collected at different altitudes. Different letters indicate significant differences among populations at the 5% level.

Altitude	Length	Width	n	
Medium	1.25	0.21	79	A
High	1.15	0.20	59	B

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