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Research

Assessing the Use of Wing Morphometrics to Identify Fall Armyworm (Lepidoptera: Noctuidae) Host Strains in Field Collections

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Abstract

The fall armyworm (*Spodoptera frugiperda*) (J. E. Smith) (Lepidoptera: Noctuidae), a major agricultural pest in the Western Hemisphere, has recently become established in Africa and Asia. This highly polyphagous species has potential to economically harm multiple crops. Contributing to this host range are two fall armyworm populations historically called 'host strains' that differ in host specificity. Understanding behaviors of the two strains is crucial to effective management of this pest. A major difficulty in such studies is that strains have long been considered morphologically indistinguishable, with molecular markers the only reliable means of identification. However, studies of fall armyworm in Colombia reported strain differences in wing morphology sufficiently large to potentially provide a more economical alternative method to determine strain. This study tested whether a similar phenotypic difference was present in Florida populations using geometric morphometric analysis of 15 anatomical landmarks on forewings of 182 specimens from three habitats associated with different host plants. Principle component and linear discriminant analyses identified significant differences in wing size and shape in comparison of strains from different habitats, but not between strains within the same habitat. Data indicate that apparent strain distinctions in wing phenotype are most likely a secondary consequence of differences in developmental growth patterns on different host plants combined with strain-biased host choice. Furthermore, Florida specimens showed much larger phenotypic overlap than observed for strains from Colombia. Together these findings suggest that wing morphology is probably not a reliable indicator of strain identity in field populations where different host plants are available.

Key words: *Spodoptera frugiperda*, geometric morphometrics, wings, host strains, pest

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), commonly called fall armyworm, is native to the Western Hemisphere where it is the primary insect pest of corn in the southeastern United States, the Caribbean, and South America (Andrews 1988). Populations were first detected in western Africa in 2016 (Goergen et al. 2016), followed by subsequent observations of substantial infestations throughout sub-Saharan Africa(Cock et al. 2017, FAO 2017, du Plessis et al. 2018), India (Ganiger et al. 2018, Shylesha et al. 2018, Swamy et al. 2018), and most recently southeastern Asia (Li et al. 2019). Economic damage in the Eastern Hemisphere has so far been primarily in corn, *Zea mays* L. with costs estimated to be in the billions USD (Stokstad 2017). The potential for losses in other crops has yet to be determined but is expected to be significant given that fall armyworm is capable of feeding on over 80 plant species, periodically causing significant economic damage to rice, *Oryza* spp.; sorghum, *Sorghum* spp.; millet, *Panicum* spp.; soybean, *Glycine max* (L.) Merr.; wheat, *Triticum* spp.; alfalfa, *Medicago sativa* L.; cotton, *Gossypium* spp.; turf, and feed grass crops (Luginbill 1928). This broad host range is in part due to the existence of two populations that differ in their distribution between host plants (Pashley 1986, Pashley and Martin 1987).

The two groups were historically designated 'host strains' and have long been considered morphologically indistinguishable. How they differ biologically remains uncertain. A variety of strain-specific characteristics are reported that include differences in female pheromone, mating behaviors, and developmental rates on different host plants (Pashley and Martin 1987, Pashley 1988, Groot et al. 2008, Lima and McNeil 2009, Schöfl et al. 2009, Groot et al. 2010, Rios-Diez and Saldamando-Benjumea 2011, Schöfl et al. 2011, Rios-Diez et al. 2012). However, fall armyworm exhibits substantial variability between geographical populations independent of strain differences that have made characterizing the two populations problematic (e.g.,

see Unbehend et al. 2014). Productive mating between the strains can occur as observed in the laboratory and suggested by genetic studies in the field, but is more restrictive and less frequent than mating within a strain (Pashley and Martin 1987, Schöfl et al. 2009, Groot et al. 2010, Nagoshi 2010, Schöfl et al. 2011, Kost et al. 2016, Nagoshi et al. 2017a).

The strains were originally identified by genetic marker differences between larval specimens collected from rice and corn plants, which gave rise to the designation 'rice-strain' and 'corn-strain' (Pashley et al. 1987). Subsequent studies showed the rice-strain had variable specificity to rice (Juárez et al. 2012, Murúa et al. 2015). Because the description of host plant specificity continues to evolve and the rice-strain designation is potentially inaccurate, we will herewith refer to the two subpopulations as the C-strain and R-strain. Field studies have demonstrated that the C-strain is preferentially found in corn, sorghum, and cotton while the R-strain predominates in pastures and millet (Pashley et al. 1987, Nagoshi et al. 2007b, Juárez et al. 2012, Murúa et al. 2015). Other crops, such as peanuts, *Arachis* spp., are only sporadically infested by fall armyworm and the preference of the two strains to such hosts have generally, not been described. Genetic markers remain the most consistent determinant of strain identity. The best characterized markers are single base substitutions found as haplotypes, with those most useful for our studies derived from portions of the mitochondrial *Cytochrome oxidase sSubunit I* gene (*COI*) (Levy et al. 2002) and the nuclear *Triosephosphate isomerase* gene (*Tpi*) (Nagoshi et al. 2006a, Nagoshi 2010).

Host plant preference is the most consistent behavioral phenotype distinguishing the two strains with the correspondence between markers and host plant observed throughout the Western Hemisphere (Nagoshi and Meagher 2004a,b; Nagoshi 2010). Yet even here the correspondence is not absolute, with on average about 20% of larvae from corn expressing R-strain markers and sporadic observations of more substantial divergence from expectations in field surveys (Nagoshi and Meagher 2004a, Prowell et al. 2004,Nagoshi, 2010 #1052, Juárez et al. 2014). This lack of correspondence suggests either inaccuracy in the markers or plasticity in host plant choice. Despite this variability, the association of the *COI* (and *Tpi*) markers with host strains has been sufficiently robust to demonstrate marker-defined differences in female pheromone constitution, mating behavior, and mating compatibility between strains (Schöfl et al. 2009, Schöfl et al. 2011, Unbehend et al. 2013, Kost et al. 2016).

Studies of fall armyworm in Colombia have reported the first indication of anatomical differences between the strains. Cañas-Hoyos et al. (2014, 2016) described strain differences in wing size and shape that were detected using geometric morphometric analysis (Cañas-Hoyos et al. 2014, 2016). In addition to potentially providing a nonmolecular means of strain identification, differences in wing morphology could impact flight performance and thereby migratory capability. If such differences are significant, then the strains would need to be analyzed differently when modeling migration and projecting the risk of fall armyworm infestations. However, such considerations first require establishing that strain differences in wing morphology are a general characteristic of the species rather than a regional variation specific to Colombian populations. Genetic variability between geographically distant populations has previously complicated identification of strain-specific traits (Unbehend et al. 2014). In addition, examining the degree to which the wing phenotypic differences are independent of strain biases in habitat choice has implications for the accuracy of strain identification by wing morphometrics and its biological relevance.

In the present study, pheromone trap collections were made from three habitats in Florida that differed in the predominant host plant present and in strain composition of the fall armyworm infestation. This made possible comparisons of wing morphology between strains collected from different habitats or within the same habitat. The methodology was designed for direct testing of molecular identification of strain for each individual wing against wings from field specimens without intervening artificial. The results were used to test whether the wing differences observed in Colombia are also present in Florida populations, and the degree to which the wing phenotype was dependent on habitat as opposed to strain identity.

Materials and Methods

Fall Armyworm Collection

Adult male fall armyworms were collected from pheromone traps at three locations representing distinct habitats based on the majority host plant species present (Fig. 1). Collections were made in May and June 2019 in cornfields in Manatee County, FL (29.3179° N, 82.8210° W), in pasture areas from December 2018 to June 2019 in Hardee County, FL (27.4820° N, 81.9190° W), and in peanut fields from August to October, 2009 in Levy County, FL (29.3875° N, 82.4468° W). The pasture and corn dominated areas were approximately 50 km apart, while the peanut site is located about 200 km to the north. These habitats should be considered complex as other plant species are present that could contribute as hosts, though our expectation is that most of the collection will have arisen from the indicated predominant plant type. Fall armyworms from all three locations were presumed to have annually migrated from the same overwintering population in southern Florida and are expected to be genetically similar (Nagoshi et al. 2008b).

Trap collections were performed using Universal moth traps (Great Lakes IPM, Vestaburg, MI) that were either a standard

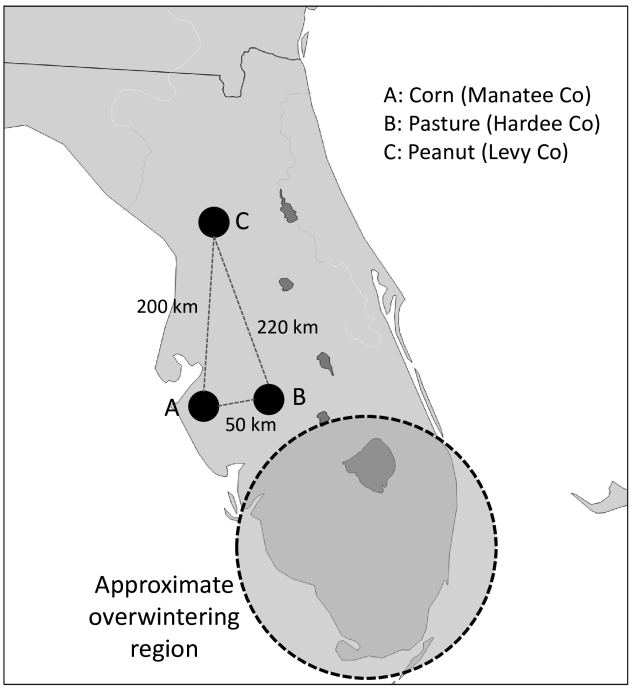

Fig. 1. Map of collection sites used in this study. Circled area approximates where fall armyworm populations are consistently found during the winter season.

configuration (green top, yellow funnel, white bucket) or entirely green (Meagher 2001). These were baited with a commercially available fall armyworm pheromone (Scentry Biologicals, Inc., Billings, MT and Trécé, Inc., Adair, OK). Each trap contained insecticide strips containing 10% 2,2-dichlorovinyl dimethyl phosphate (Hercon Environmental, Emigsville, PA) to kill moths. Collections were made using 3–4 traps per site. The collected specimens were identified as fall armyworm based on morphological criteria and stored at –20°C before subsequent analysis.

The collections were screened for specimens with intact left wings. These wings were detached and used for the morphometric analysis while the carcass was stored in 80% ethanol prior to DNA processing and strain identification. Specimens were labeled so that the wing and strain identification could be matched.

Wing Morphology Analysis

Left wings were obtained from 182 wild-caught specimens ($n = 60$ from cornfields, $n = 55$ from pasture areas, $n = 67$ from peanut fields). To remove both wing scales and pigment and make landmarks clear, wings were soaked in sodium hypochlorite (5.25%) and brushed with a camel hair brush. Wings were then rinsed in water and mounted in Mirsky's Fixative (National diagnostics, Atlanta, GA) on the slide with the cover slip sealed using clear fingernail polish. Wing digital images were recorded using a digital microscope (VHX-5000, Keyence, Osaka, Japan). For each wing, a set of 15 anatomical landmarks (Fig. 2) previously described by Cañas-Hoyos et al. (2016) were digitized as *x*- and *y*-coordinates in a Cartesian space using ImageJ software (NIH, Bethesda, MD) in conjunction with the plugin Point Picker (http://bigwww.epfl.ch/thevenaz/pointpicker/). Raw coordinates were converted to .txt file format and imported into MorphoJ v1.07a (Klingenberg 2011), which was used for all subsequent analyses. A full generalized Procrustes fit, or superimposition, was performed on the raw coordinates, which eliminated nonshape variables from the dataset (Rohlf and Slice 1990).

Wing size comparisons were made using centroid size, which is a unit-less metric defined as the square root of the sum of squares of the distances of each landmark to the centroid/center of all landmarks (Zelditch et al. 2012). Centroid size was calculated for each wing and groups were compared by ANOVA and Tukey tests or *t*-tests. Variations in wing shape were compared by linear discriminant function analysis performed on subsets of the Procrustes-transformed coordinates. The degree of dissimilarity was estimated by Mahalanobis distances through a permutation test with 10,000 randomizations. Pairwise shape comparisons were tested for significance using Hotelling's T^2 distribution, which characterizes the differences between multivariate means of the two groups. The results were visualized through histograms showing the number and type of individuals relative to the Procrustes-derived shape classes (x-axis). To assess allometry, multivariate regression analysis was performed using the Procrustes shape coordinates as the dependent variables and centroid size as the independent variables, followed by permutation tests (10,000 randomizations) against the null hypothesis of independence between wing shape and size to determine statistical significance.

DNA Preparation and PCR Amplification

DNA was prepared using a simplified modification of previous methods (Nagoshi et al. 2008a). Individual carcasses were homogenized in a 5-ml Potter Homogenizer (Bellco Glass, Inc, Vineland, NJ) in 800 µl cell lysis buffer (0.2 M sucrose, 0.1 M Tris–HCl at pH 8.0, 0.05 M EDTA, and 0.5% sodium dodecyl sulfate), transferred to a 2.0-ml microcentrifuge tube and incubated at 55°C for 1 h. Debris was pelleted by centrifugation at 14,000 rpm (about 9,000 × *g*) for 5 min. at room temperature. The supernatant was transferred to a Zymo-Spin III column (Zymo Research, Orange, CA) and processed according to manufacturer's instructions. The DNA preparation was increased to a final volume of 200 µl with distilled water. Genomic DNA preparations of fall armyworm samples were stored at –20°C and analyzed as needed.

PCR amplification of the mitochondrial *COI* gene was performed in a 30 µl reaction mix containing 3 µl 10X manufacturer's reaction buffer, 0.5 µl 10mM dNTP, 0.5 µl 20 µM primer mix, 1–2 µl DNA template (between 0.05 and 0.5 µg), 0.5 unit Taq DNA polymerase (New England Biolabs, Beverly, MA). The thermocycling program was 94°C (1 min), followed by 30 cycles of 92°C (30 s), 56°C (45 s), 72°C (45 s), and a final segment of 72°C for 3 min. Typically 96 PCR amplifications were performed at the same time using either 0.2-ml tube strips or 96-well microtiter plates. Amplification of the *COI* region used the primer pair *COI-891F* (5′-TACACGAG CATATTTTACATC-3′) and *COI-1303R* (5′-CAGGATAGTCAG AATATCGACG-3′) to produce a 410-bp fragment. Amplification of the *Tpi* exon–intron segment used the primers *Tpi412F* (5′-CCGGACTGAAGGTTATCGCTTG -3′) and *Tpi1140R* (5′-GCGGAAGCATTCGCTGACAACC-3′) to produce a variable length fragment due to insertion and deletion mutations. It was sometimes necessary to use nested PCR to obtain the *Tpi* amplified fragment. In

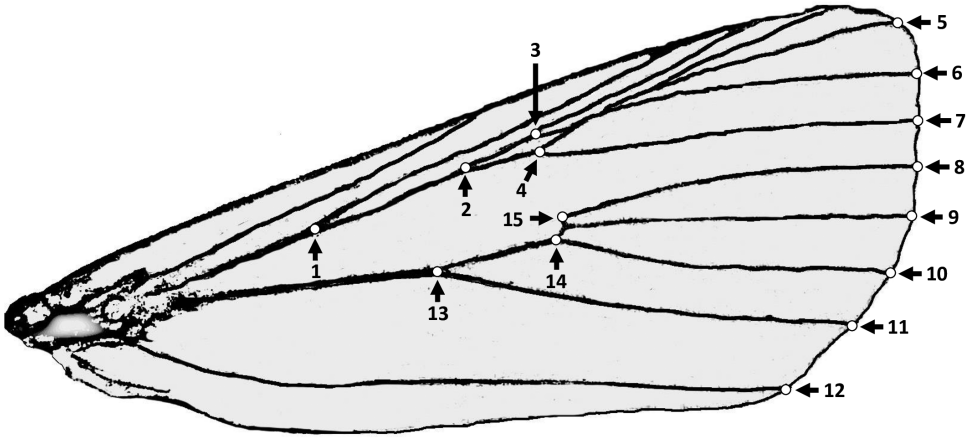

Fig. 2. Diagram of wing landmarks used for the geometric morphometric analysis. The landmarks are as described in Cañas-Hoyos et al. (2014, 2016).

this case, the first PCR was performed with the primers *Tpi634F* and Tpi780R. The reaction mix was then diluted with the addition of 100 µl of water and 1 µl of this mix was used in the second PCR with primers *Tpi412F* and *Tpi1140R*. Primers were synthesized commercially (Integrated DNA Technologies, Coralville, IA).

Strain Identification and DNA Sequence Analysis

A total of 192 specimens from the field collections, including all 182 specimens examined for wing morphology, were analyzed for strain identity by restriction fragment length polymorphism (RFLP) analysis. Within the 434-bp *COI* segment amplified by *COI-891F*/*COI-1303R* is an *Eco*RV site present only in the *COI*-RS group that produces two fragments of 290 bp and 144 bp (Nagoshi et al. 2008b). Five microliters of the *COI* PCR reaction was digested with 5 units of the restriction enzyme *Eco*RV (New England Biolabs, Beverly, MA) in a 20 µl of 1X manufacturer recommended restriction enzyme buffer. For each reaction, 5 µl of 6X gel loading buffer was added and the entire sample run on a 1.8% agarose horizontal gel containing GelGreen (at one-third the concentration recommended by manufacturer's instructions, Biotium, Hayward, CA) in 0.5× Tris–borate buffer (TBE, 45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). Fragments were visualized on a blue-light box.

A subset of 160 specimens was further analyzed by DNA sequencing of the COIB segment to confirm species and strain identity. In these cases, the PCR fragments visualized during the RFLP analysis were cut out of the gel and DNA isolation was performed using Zymo-Spin I columns (Zymo Research, Orange, CA) according to manufacturer's instructions. The isolated fragments were analyzed by DNA Sanger sequencing (Genewiz, South Plainfield, NJ). Strain identification was confirmed by identifying strain-diagnostic polymorphisms in the amplified region (Nagoshi et al. 2006b, Nagoshi et al. 2007a).

The field collections were also tested for strain identification using the *Tpi* markers as described previously (Nagoshi et al. 2019). Polymorphisms in the fourth exon of the *Tpi* protein-coding region identify host strain with results generally comparable with the *COI* marker (Nagoshi 2010). Site gTpi183Y located 183 bp from the 5′ splice site of the fourth exon is diagnostic, with the C-strain allele (*Tpi*C) indicated by a C and the R-strain (*Tpi*R) by a T (Nagoshi 2010). The *Tpi* gene is located on the *Z* sex chromosome that is present in one copy in females and two copies in males. Since males can be heterozygous for *Tpi*, there is the potential for the simultaneous display of both alternative nucleotides at gTpi183Y (denoted as *Tpi*H), which would be indicated by an overlapping C and T DNA sequence chromatograph (Nagoshi et al. 2017a). These were found in about 10% of the specimens and were not included in the *Tpi* strain data. Useable *Tpi* sequence was obtained from 159 specimens.

DNA alignments and consensus building were performed using MUSCLE (multiple sequence comparison by log-expectation), a public domain multiple alignment software incorporated into the Geneious Pro 10.1.2 program (Biomatters, New Zealand, http://www.geneious.com) (Kearse et al. 2012). Generation of graphs was done using Excel and PowerPoint (Microsoft, Redmond, WA). Other statistical analyses including *t*-tests and χ^2 were performed using GraphPad Prism version 6.00 for Mac (GraphPad Software, La Jolla, CA).

Results

Distribution of *COI* and *Tpi* Strain Diagnostic Markers in Field Collections

In total, 182 specimens were characterized for strain identity using the mitochondrial *COI* marker diagnostic of strain identity (Fig. 3A). The expected biased distribution associated with the host strains was observed for these collections with *COI*-CS (the C-strain marker) predominating in the corn habitat while *COI*-RS (R-strain) was the majority haplotype in the pasture collections. Both strains were present in near equal proportions in the peanut habitat. As an additional test, we examined a subset of the specimens with the *Tpi* strain marker. The results were similar to that found with *COI* as the C-strain marker (TpiC) predominated in corn, the R-strain marker (TpiR) was the majority in pasture, and both markers were equally present in peanut (Fig. 3B). Overall, these observations demonstrate that the two strains as defined by *COI* markers are present in the collections and were behaving as expected. Furthermore, there is no evidence that either strain exhibited preference for peanuts and the substantial presence of both strains in this collection provides an opportunity to test for phenotypic differences between strains captured in the same habitat (peanut), thereby minimizing geographical and host plant effects.

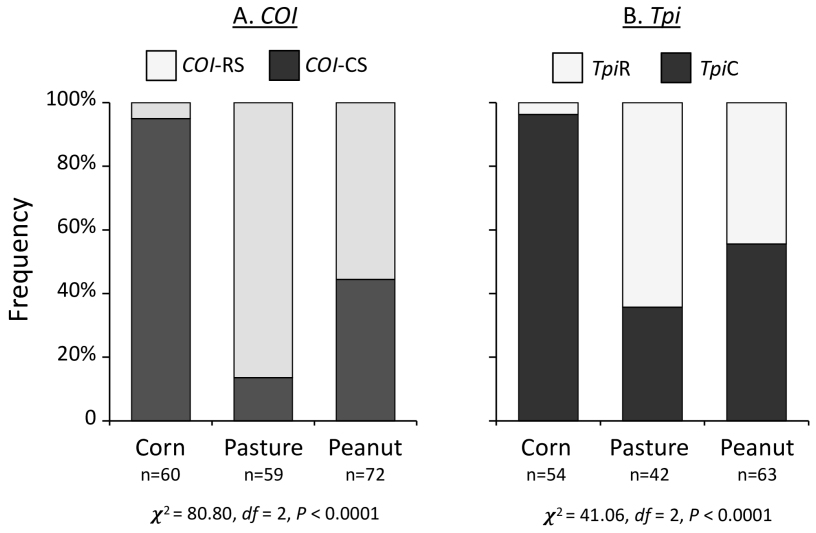

Fig. 3. Bar graphs showing frequency of *COI* and *Tpi* strain diagnostic haplotypes in different habitats. (A) COI analysis. (B) A subset of specimens analyzed for *COI* were retested with *Tpi*. Comparisons were tested by χ^2 analysis.

Comparisons of Wing Size by Habitat and Strain

Centroid size (Klingenberg 2016) for each specimen was calculated based on the same 15 forewing landmarks previously used to demonstrate strain differences in wing morphology (Cañas-Hoyos et al. 2014, 2016) (Fig. 4). Statistically significant differences were found in comparisons between collections from the corn and pasture sites and as well as between the pasture and peanut collections, but not between corn and peanut (Fig. 4A). Using the same data set we also compared the centroid sizes between *COI* groups from all habitats. The mean for the *COI*-CS group was significantly higher than that of *COI*-RS specimens (Fig. 4B).

To assess the relative importance of habitat and strain, comparisons were made using the same data set but now grouped by both factors. There were four groups with sufficient sample size ($n > 30$) for comparisons, *COI*-CS from corn, *COI*-RS from pasture, and both *COI* types from peanuts, with the latter providing an opportunity to compare strains arising from the same habitat. The *COI*-RS from pasture group had the lowest mean centroid size, which was significantly different from the same strain *COI*-RS specimens from peanut, as well as the different strain *COI*-CS from corn and peanut groups (Fig. 4C). Further evidence for the influence of habitat was the observation that the *COI*-RS from peanut group was not significantly different in wing size from the *COI*-CS specimens from either peanut or corn despite the strain differences. These results indicate that it is habitat, or more specifically the host plant, rather than strain that is the primary determinant of wing size differences in the field collections.

Comparisons of Wing Shape by Habitat and Strain

Pairwise comparisons of wing shape between the various groups performed by linear discriminant analysis of shape variables obtained from the full Procrustes fit algorithm detected significant differences (Fig. 5). The Hotelling's T^2 test based on Mahalanobis distances revealed statistically significant differences between all pairwise shape comparisons between habitats (Fig. 5A–C), including corn versus peanut where no significant differences were found between their mean centroid sizes (Fig. 4A). When specimens from all three habitats were pooled a significant difference was found between strains, *COI*-CS versus COI-RS (Fig. 5D). However, when the *COI* comparison was limited to the peanut collection, the shape difference between strains was not statistically significant (Fig. 5E). Multivariate regression analysis revealed that wing size explained 1.9% of wing shape variation with a *P*-value of 0.002, indicating a statistically significant but relatively minor dependence of wing shape on size.

Discussion

Studies of fall armyworm populations from central Colombia reported evidence from wing morphometric studies of strain differences in both wing size and shape that were of sufficient magnitude that they could potentially substitute for the genetic markers currently used to identify strains (Cañas-Hoyos et al. 2014, 2016). A method of strain identification that did not require molecular analysis could provide more timely and affordable characterization of fall armyworm infestations, a particular benefit in the Eastern Hemisphere where fall armyworm is newly arrived and a target of enhanced monitoring.

The present study demonstrated that wing differences in size and shape can be found between populations of wild fall armyworm in Florida, which are almost certainly isolated from those in Colombia (Nagoshi et al. 2017b), indicating this is likely to be a general characteristic of the species. However, our data indicate substantial overlap in the wing phenotypes between strains that was much larger than that observed in the Colombia studies, suggesting that in Florida populations the strain differences in wing morphology are a secondary consequence of host plant preference combined with differences between fall armyworm growth patterns on different hosts. When both habitat and strain are taken into account, significant differences between C-strain wings were only found in comparisons with R-strain from the pasture collection, while no significant differences were found in strain comparisons between the corn and peanut collections or between the two strains in the peanut collection. This suggests that pasture grasses give rise to fall armyworms with altered wing size relative to those that developed on corn and peanut hosts regardless of strain identity. It, therefore, appears that host plant species, rather than strain identity, is strongly associated with wing size differences in the Florida field collections.

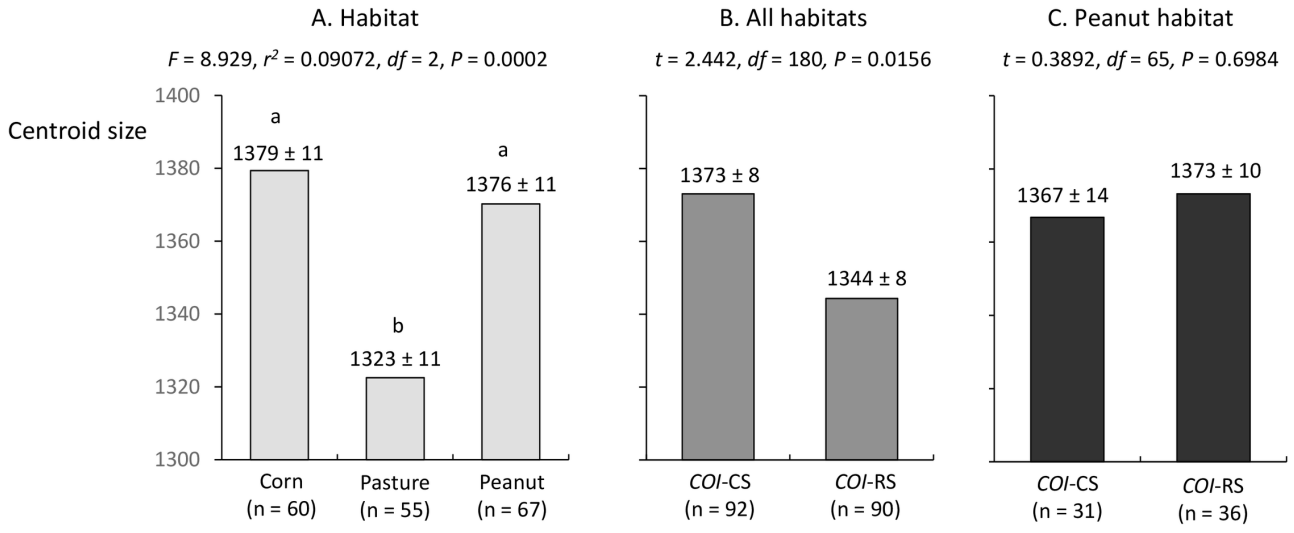

Fig. 4. Bar graphs of mean centroid sizes for groups defined by *COI* strain, habitat, or both. The mean ± standard error of the mean is shown above each bar. For each figure means with the same letter are not significantly different. Analysis for (A) and (C) was done by one-way ANOVA and Tukey's multiple comparison test, while (B) used a two-tailed *t*-test.

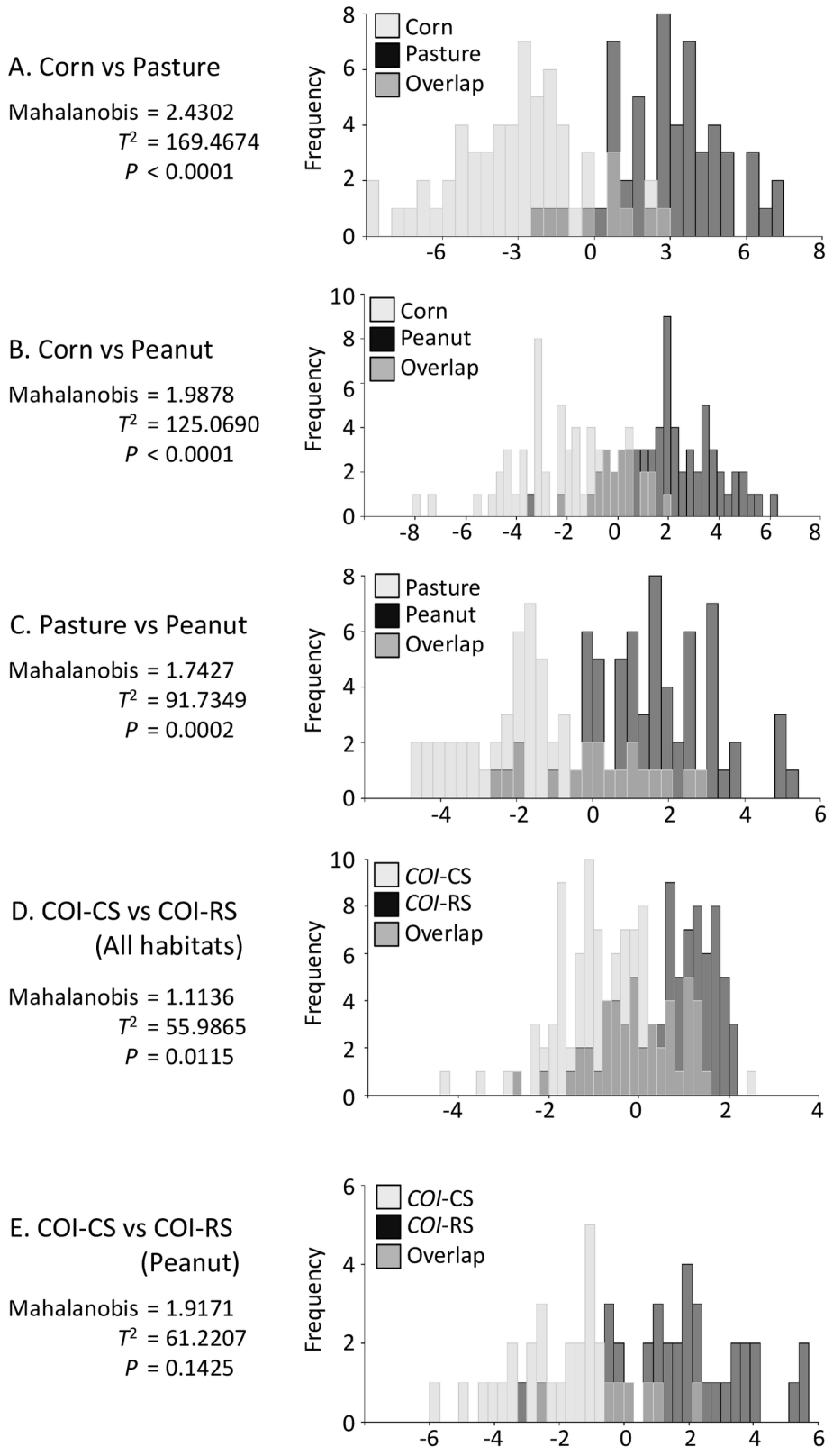

Fig. 5. Results of linear discriminant analysis displayed as histograms. The x-axis denotes the vectors created by linear discriminant analysis based on the variables used and their importance in defining category differences. The y-axis represents the proportion of each vector in a given category.

Comparisons of wing morphology by linear discriminant analysis supported this conclusion. Statistically significant differences in wing shape were found in all comparisons of moths collected from different plant hosts. However, because the collections also differ in strain composition, these comparisons cannot differentiate between host plant and strain identity as the primary determinant of the

wing morphology difference. The collection from peanuts consisted of nearly equal numbers of the two strains based on the *COI* or *Tpi* markers allowing a comparison between strains from the same habitat. In this instance, the strain difference in wing morphology is not statistically significant, consistent with plant host being the most important factor in explaining the observed wing shape differences. However, we note that the *COI*-CS and *COI*-RS in the peanut collection do not completely overlap, suggesting that other factors, such as strain identity, might also have influence.

That the type and quality of larval diet can influence later development is not surprising, as there have been several studies with fall armyworm demonstrating that corn-raised larvae of both strains produced heavier pupae compared to those using diets based on R-strain preferred plants (Pashley 1988, Pashley et al. 1995, Meagher et al. 2004, Meagher and Nagoshi 2012). There is also evidence from other insects demonstrating that the type of host plant can significantly influence adult wing shape and size (Mozaffarian et al. 2007, Soto et al. 2008). Given the entirety of the data to date, we conclude that most, if not all, of the strain differences in wing morphology are an incidental effect of strain-biased habitat choice, probably reflecting differences in the pasture grass diet relative to other host plants. Host plant choice remains the most likely driver of strain divergence and may be related to such factors in difference in cyanide metabolism observed between the two strains (Hay-Roe et al. 2011). Furthermore, given fall armyworm's large host range, the dependence of wing morphology on habitat and host plant suggests substantial variations in the wing phenotype found in wild populations. Therefore, the use of wing morphology to identify strains as previously suggested (Cañas-Hoyos et al. 2014, 2016) seems problematic in environments where other host plants are present besides corn and pasture. As just one example, in our study the similarity in wing morphology between the corn and peanut collections would identify peanut as a C-strain preferred plant even though both the *COI* and *Tpi* markers suggest a lack of strain preference.

Our finding of no evidence of strain specificity to peanuts should be considered as preliminary and followed by additional surveys for moths from peanut field in other locations and also by collections of larvae which would confirm peanuts as a host plant. The peanut habitat included other grass and weed species that could potentially have served as a fall armyworm host nor can we preclude the possibility of contributions from nonlocal migrants.

In summary, this paper demonstrates that the morphological differences between fall armyworm strains first reported in Colombia are also present in field populations in Florida, indicating that it is likely to be a general characteristic of the species. However, this phenotype is most likely a consequence of strain-specific habitat preference, in particular environmental factors like host plant type and quality. As such, the use of wing morphology as a substitute for molecular methods of strain identification seems limited. The very different strain composition found in the peanut-dominated habitat emphasizes the need for future work on describing the strain-specificity of the more than 80 plant species that fall armyworm is reported to be capable of using (Luginbill 1928). This is particularly relevant given the recent invasion of fall armyworm into the Eastern Hemisphere and attempts to assess what agricultural products there are now at risk.

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