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Ostwald, Madeleine
Thrift, Charles
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Phenotypic divergence in an island bee population: Applying geometric morphometrics to discriminate population-level variation in wing venation

Madeleine M. Ostwald ⬤ | Charles N. Thrift ⬤ | Katja C. Seltmann ⬤

Cheadle Center for Biodiversity and Ecological Restoration, University of California Santa Barbara, Santa Barbara, California, USA

Correspondence
Madeleine M. Ostwald, Cheadle Center for Biodiversity and Ecological Restoration, University of California Santa Barbara, Santa Barbara, CA, USA.
Email: ostwald.madeleine@gmail.com

Abstract

Phenotypic divergence is an important consequence of restricted gene flow in insular populations. This divergence can be challenging to detect when it occurs through subtle shifts in morphological traits, particularly in traits with complex geometries, like insect wing venation. Here, we employed geometric morphometrics to assess the extent of variation in wing venation patterns across reproductively isolated populations of the social sweat bee, *Halictus tripartitus*. We examined wing morphology of specimens sampled from a reproductively isolated population of *H. tripartitus* on Santa Cruz Island (Channel Islands, Southern California). Our analysis revealed significant differentiation in wing venation in this island population relative to conspecific mainland populations. We additionally found that this population-level variation was less pronounced than the species-level variation in wing venation among three sympatric congeners native to the region, *Halictus tripartitus*, *Halictus ligatus*, and *Halictus farinosus*. Together, these results provide evidence for subtle phenotypic divergence in an island bee population. More broadly, these results emphasize the utility and potential of wing morphometrics for large-scale assessment of insect population structure.

KEYWORDS
California Channel Islands, *Halictus tripartitus*, island-mainland variation, wing morphology

TAXONOMY CLASSIFICATION
Biodiversity ecology, Biogeography, Entomology, Evolutionary ecology, Population ecology, Zoology

1 | INTRODUCTION

Insular conditions are major drivers of population-level phenotypic differentiation (Meröndun et al., 2019; Phillimore et al., 2008; Runemark et al., 2014; Velo-Antón & Cordero-Rivera, 2017). In particular, island populations can experience rapid evolutionary changes in morphological traits due to founder effect and subsequent genetic drift (Alsos et al., 2015; Barton, 1996; Hedrick et al., 2001; Jordan & Snell, 2008; Santos de Souza et al., 2019; Sylvester et al., 1998; Velo-Antón et al., 2012). As such, islands have long been considered natural testbeds for evolutionary questions (MacArthur & Wilson, 1963; Warren et al., 2015), and have illuminated patterns

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of morphological variation, especially among birds and mammals (Cooper & Purvis, 2010; Grant, 1965; Millien, 2006). In contrast, trait variation in island populations of insects remains relatively unexplored, despite the prominence of these systems as ecologically important pollinators, pests, and invasive species (Fortuna et al., 2022; Hölldobler & Wilson, 1990; Klein et al., 2007; Traveset et al., 2013).

Beyond these taxonomic biases, our understanding of phenotypic variation across populations is biased toward traits that are easily distinguished or quantified by human observers, such as body size and coloration (Doucet et al., 2004; Kraemer et al., 2019; Lomolino, 1985; Palkovacs, 2003). In contrast, variation in traits that present measurement challenges, such as morphological traits with complex geometries, tends to be underexplored. One such trait is the pattern of venation in insect wings. Veins provide the primary structural support for wings, and while the functional significance of variation in venation patterns remains largely unclear (Combes & Daniel, 2003), they are highly conserved in insect lineages and thus are useful in phylogenetic reconstructions and taxonomic determinations (Comstock & Needham, 1898; Sharkey & Roy, 2002). Indeed, many identifying characteristics in bee taxonomy are found in wing venation patterns, with characteristic variation distinguishing genera and species (Michener, 1994).

Within a species, however, wing venation may present subtler patterns of variation that are undetectable via traditional observation methods. Geometric morphometrics, a set of methods that allows for spatial analysis of biological forms, has emerged as a promising approach to quantifying variation in complex morphological traits (Mitteroecker & Gunz, 2009; Rohlf & Marcus, 1993). This approach has been successfully implemented to discriminate patterns of insect wing venation among (Baylac et al., 2003; Deregnaucourt et al., 2021; Francoy et al., 2009, 2012; Kaba et al., 2017; Perrard et al., 2014; Rattanawannee et al., 2010, 2015; Santoso et al., 2018; Villemant et al., 2007) and even within species (Francisco et al., 2008; Francoy et al., 2011, 2016). Geometric morphometrics, therefore, has potential to assess the extent of phenotypic divergence among discrete insect populations by quantifying variation in this highly conserved trait.

We examined trait variation among island and mainland native bee populations in a Southern California coastal ecoregion. Santa Cruz Island is a 249 km^2 Pacific island located 32 km due south of mainland Santa Barbara, California. It is the largest of the California Channel Islands, an eight-island archipelago notable for its biodiversity and endemic species and which has served as a site for many microevolutionary studies of island-mainland variation (O'Reilly & Horn, 2004). Santa Cruz Island shares many of its bee fauna with mainland Santa Barbara (Seltmann, 2019), but the distance separating these locations generally precludes gene flow between populations. Bees typically forage within a few kilometers of their nesting sites, and dispersal distances are generally well under the 30 km water barrier separating Santa Cruz Island from the mainland (O'Reilly & Horn, 2004). Further, while stem- and wood-nesting bees have heightened island dispersal capabilities due to human transport

of wood materials (Poulsen & Rasmussen, 2020), ground-nesting bees have limited opportunities for human-mediated island dispersal. Honey bees (*Apis mellifera* Linnaeus, 1758) were eradicated from the island by 2004 and have not been observed there since (Naughton et al., 2014; Seltmann et al., 2019; Wenner et al., 2009), suggesting that the channel is not easily crossed even by medium-sized bees. As such, we are confident that gene flow between island and mainland bee populations in this context is minimal to nonexistent, increasing the likelihood of phenotypic divergence between populations.

In this study, we investigate variation in wing venation in island and mainland populations of the sweat bee, *Halictus tripartitus* Cockerell, 1985. *H. tripartitus* is a widespread, ground-nesting social bee native to western North America and locally abundant both in mainland Santa Barbara and on Santa Cruz Island. We analyze museum specimens using a geometric morphometrics framework to assess the extent of variation in wing venation patterns between these two reproductively isolated populations. To contextualize the degree of variation, we additionally characterize variation in wing venation between *H. tripartitus* and two sympatric congeners, *H. ligatus* Say, 1837 and *H. farinosus* Smith, 1853. In doing so, we assess the role of reproductive isolation on population differentiation of morphological traits.

2 | METHODS

2.1 | Specimens and wing imaging

To assess population-level variation in wing venation patterns, we imaged wings from three *Halictus* species: *H. tripartitus* ($n_{island}=149$; $n_{mainland}=149$), *H. ligatus* ($n_{mainland}=43$), and *H. farinosus* ($n_{island}=3$; $n_{mainland}=40$; Figure 1). To achieve even sampling across species, we randomly selected 43 specimens of each species to analyze in our species-level comparison. We obtained bee specimens from the University of California, Santa Barbara Invertebrate Zoology Collection housed by the Cheadle Center for Biodiversity and Ecological Restoration. All specimens were female and were collected between 1956 and 2020, with the majority of specimens collected recently (mean: 2018, median: 2019; Figure 2); (specimen catalog numbers available in Supplementary Materials). Species-level identifications were confirmed by California native bee taxonomist Jaime Pawelek.

We removed left forewings from all specimens and imaged them with a stereo microscope digital camera along with a 1 mm scale slide (Dino-Lite AM3111T; DinoXcope software 2.0.1). The basal tip of some forewings were removed if they were heavily sclerotized and prevented the wings from lying flat. We plotted 9 homologous wing venation landmarks (following Rattanawannee et al., 2015) onto each wing image using tpsDig software version 2.31 (Rohlf, 2015; Figure 3). All analysis was conducted in R version 4.2.2.
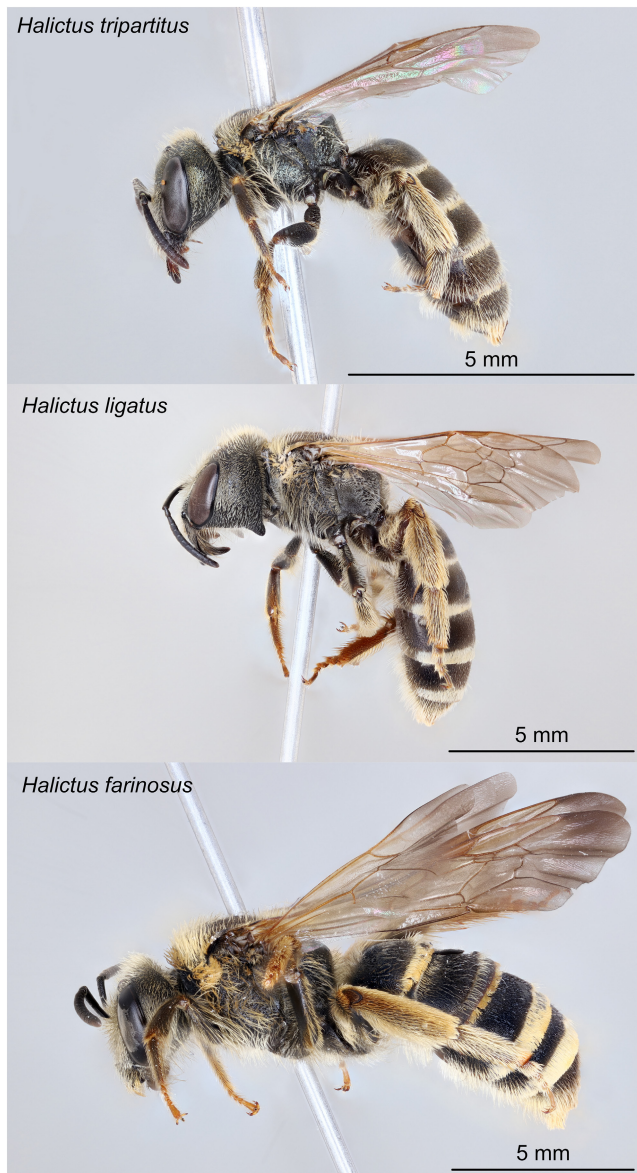
FIGURE 1 Lateral images of female *Halictus tripartitus* (top; UCSB-IZC00040597), *H. ligatus* (middle; UCSB-IZC00044094), and *H. farinosus* (bottom; UCSB-IZC00042935) specimens. Images produced by Luz Ceja.

2.2 | Data analysis

We Procrustes-aligned landmark coordinates using R package "geomorph" version 4.0.0 (Adams et al., 2022; Baken et al., 2021). To test for statistical differences between the two *H. tripartitus* populations and among the three species, we ran one-way multivariate analysis of variance (MANOVA) tests using R package "RRPP" version 1.3.1 (Collyer & Adams, 2018, 2019). To visualize separation among groups, we generated density plots with discriminant analysis of principal components (DAPC) using R package "adegenet" version 2.1.10 (Jombart, 2008; Jombart & Ahmed, 2011). To test the accuracy of using wing landmarks to predict an unknown bee's species or population, we utilized DAPC cross-validation. Cross-validation also

informed the number of principal components (PCs) retained in each analysis (Jombart & Collins, 2015).

3 | RESULTS

Our analysis of wing landmark coordinates successfully discriminated between wings of *H. tripartitus*, *H. ligatus*, and *H. farinosus* (MANOVA: *Pillai* = 1.817, *p* < .001); (full MANOVA tables in Table 1; landmark coordinates in Table S1). Based on cross-validation, 6 PCs were retained, and the density plot shows separation between species (Figure 4a). The cross-validation test assigned 100% of *Halictus* specimens to their correct species (Figure S1a).

Population-level discrimination was also successful. The two populations of *H. tripartitus* differed significantly in wing landmark coordinates (MANOVA: *Pillai* = 0.425, *p* < .001; Table 1). Based on cross-validation, 13 PCs were retained. The density plot shows some separation between populations, with overlap (Figure 4b). The cross-validation test assigned 80.7% of *H. tripartitus* specimens to their correct population (Figure S1b). This analysis accurately identified the two subgroups (populations) of *H. tripartitus*, although with a lower degree of separation (MANOVA, DAPC) and accuracy (cross-validation) than for the three congeneric *Halictus* species.

4 | DISCUSSION

We employed a geometric morphometrics framework to demonstrate strong differentiation in wing venation geometries across three sympatric species of *Halictus* bees, as well as significant (though less pronounced) differentiation across island and mainland populations of *H. tripartitus*. Species-level variation in wing venation is well established for many bee taxa, serving as useful characters for identification to family, genus, or species levels (Michener, 1994). Several studies have even successfully discriminated between distinct insect subspecies or genetic lineages within species using wing morphometrics (Akahira & Sakagami, 1959; Carneiro et al., 2019; Francoy et al., 2008, 2011). Fewer studies, however, have investigated population-level variation in wing venation (Francoy et al., 2011; Rossa et al., 2016). This variation in wing morphology can serve as a useful proxy for assessing the extent of divergence between closely related populations (Oleksa & Tofilski, 2015; Peil & Aranda, 2021). More broadly, these results provide robust evidence for microevolutionary change in wing morphology across reproductively isolated bee populations.

As for many island bee populations, it is unknown how and when this population of *H. tripartitus* colonized Santa Cruz Island. Island dispersal by bees is generally poorly understood, though phylogenetic analyses and behavioral studies can offer clues to potential avenues for colonization events. On Santa Cruz Island, colonization by many bee species could have occurred during the Last Glacial Maximum (17,000–18,000 years ago), when lowered sea levels reduced the distance from the mainland to about 6 km
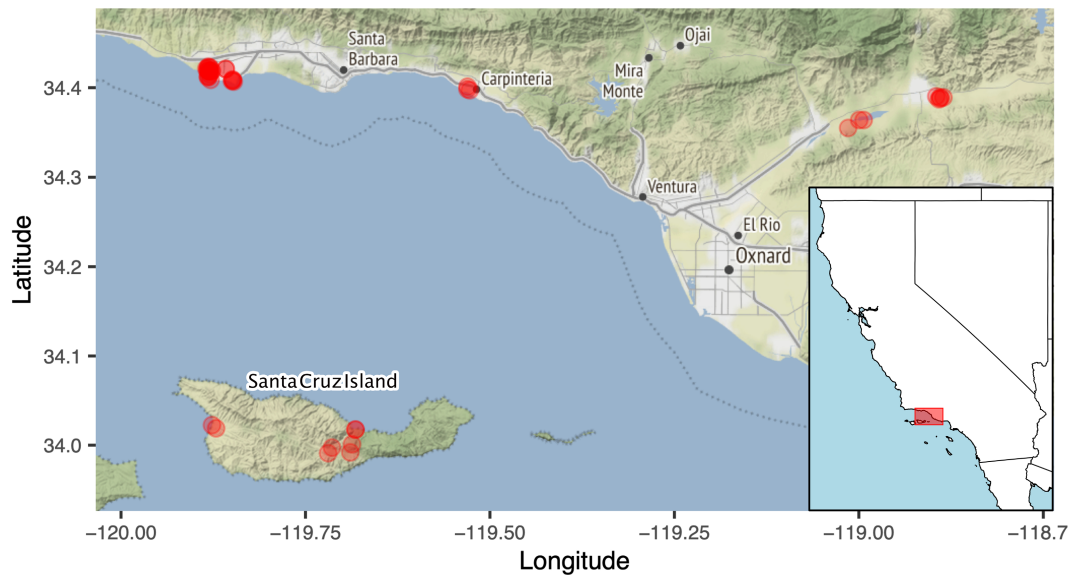
FIGURE 2 Map of sampling sites (red dots) on Santa Cruz Island and mainland Santa Barbara County and Ventura County. Inset map shows sampling region within California. *Halictus ligatus* was collected only on the mainland. *H. tripartitus* and *H. farinosus* were collected both on the mainland and on Santa Cruz Island.
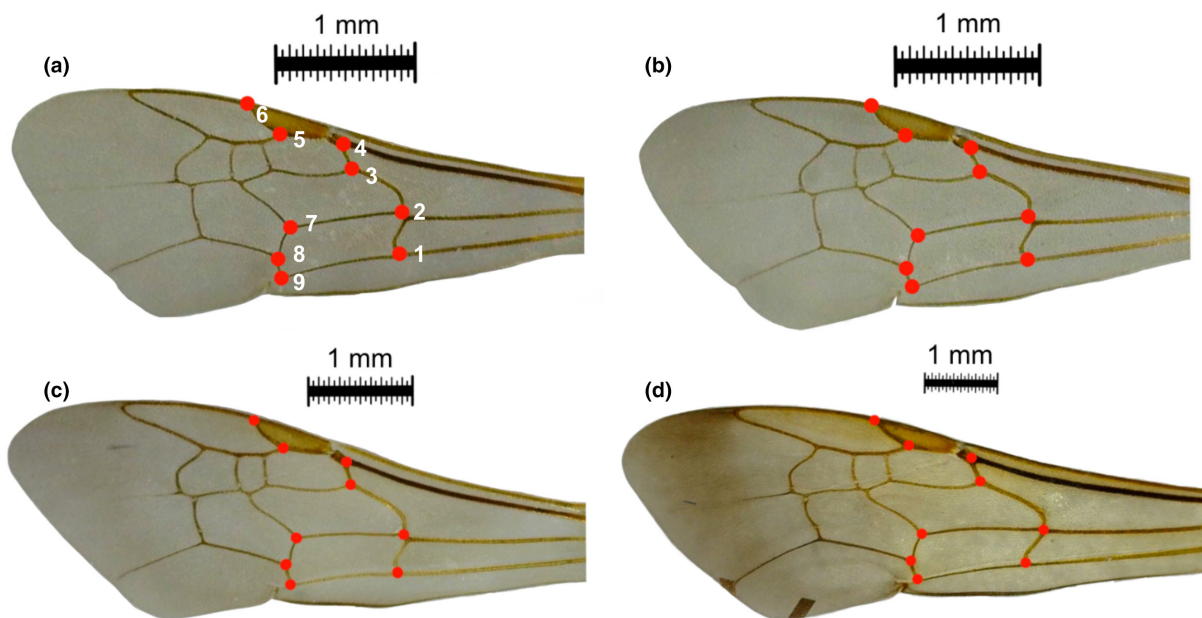
FIGURE 3 Landmarked images of *Halictus* forewings, including: (a) *Halictus tripartitus* from the mainland (UCSB-IZC00042220), (b) *H. tripartitus* from Santa Cruz Island (UCSB-IZC00040420), (c) *H. ligatus* (UCSB-IZC00039120; mainland), and (d) *H. farinosus* (UCSB-IZC00036955; Santa Cruz Island).

(Miller, 1985). Gene flow across the channel may have continued for an unknown period, depending primarily on the distance of the water barrier and the dispersal capabilities of *H. tripartitus*. Dispersal timing aside, it is evident from our results that the Santa Cruz Island population has diverged morphologically from the mainland population. The unique selective environment of the island (i.e., including climatic and ecological differences from the mainland) may contribute to this population divergence, in addition to founder effect and genetic drift. Future sampling across

the entire Channel Island archipelago could shed light on historical patterns of dispersal and population divergence, inferred from patterns of differentiation in wing landmark geometries. Finally, comparisons to other populations across the considerable geographic range of *H. tripartitus* would provide interesting context for assessing the relative magnitude of phenotypic divergence in the Santa Cruz Island population.

These results highlight the utility of geometric morphometrics for quantifying complex patterns of phenotypic variation that elude

TABLE 1 MANOVA tables showing results of the comparison between three congeneric species of *Halictus* and two populations (island vs. mainland) of *H. tripartitus*.

Level of comparison	df	Residuals	Pillai	Z	Pr(>Pillai)
Between Species (*H. tripartitus*, *H. ligatus*, *H. farinosus*)	2	126	1.817	12.925	<.001
Within Species (Island vs. Mainland *H. tripartitus*)	1	296	0.425	7.715	<.001
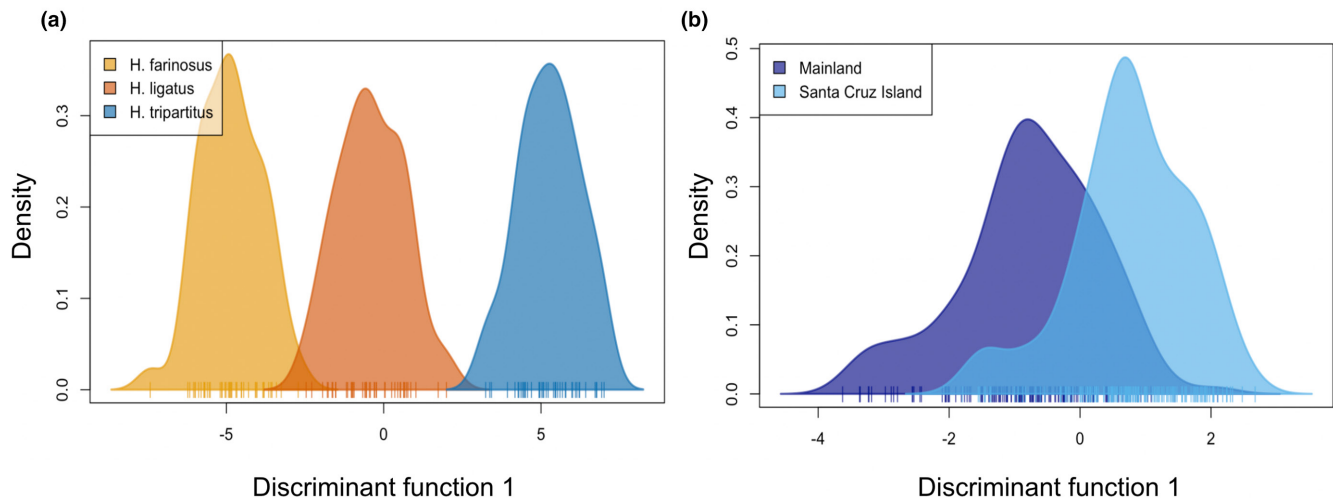
FIGURE 4 Density plots showing the most influential principal component for nine measured landmarks, comparing (a) three congeneric species of *Halictus* and (b) two separated populations of *H. tripartitus*.

observation via simple measurement techniques. The application of geometric morphometrics to insect wing venation patterns is still a relatively recent development, but already has shown promise for species identifications (Aytekin et al., 2007; Francoy et al., 2009; Rattanawannee et al., 2010). Our accurate discrimination between three *Halictus* species likewise supports a role for geometric morphometrics in taxonomic identification to the species level. Further, geometric wing morphometrics may be useful for distinguishing among populations (Henriques et al., 2020; Rossa et al., 2016) and between species within complexes (Francoy et al., 2011). Identifying features of wing venation have even been successfully integrated into computer-aided identification systems, which can accurately identify bee specimens to species and even subspecies from images of wings (Buschbacher et al., 2020; Rattanawannee et al., 2012). Our results indicate that population variation in wing venation can be successfully discriminated using geometric morphometrics, and suggest that these patterns could be usefully extended toward automated identification systems with the aim of further classifying specimens to the population level.

Beyond its use in population identification, wing morphometry holds valuable potential for large-scale population studies, by providing a tractable alternative to more costly and time-consuming molecular methods for analyzing population structure. Unlike some morphological traits that can degrade over time, wing venation is strongly preserved in museum specimens, presenting opportunities for sampling of existing specimens in place of conducting new surveys. Wings represent powerful candidates for geometric morphometric analysis because their two-dimensional surfaces

lend themselves to straightforward imaging, in contrast to three-dimensional traits that require additional protocols to standardize orientation within images. Future studies seeking to identify bees to species or population level may find this methodology viable and potentially more adaptable than traditional taxonomic identifications using dichotomous keys. In particular, we envision that wing morphometrics could increase the feasibility of large-scale monitoring projects by reducing taxonomic labor (Engel et al., 2021).

In conclusion, we demonstrated species- and population-level variation in *Halictus* wing venation. Our results provide evidence for the divergence of wing venation patterns in isolated island and mainland populations of *H. tripartitus*. Our study emphasizes that wing venation patterns can act as quantifiable indicators of phenotypic differentiation within species, and may be useful for inferring the extent of variation among reproductively isolated populations. Morphological population signatures such as these hold enormous potential for enabling broader assessments of evolutionary change across insect populations over time, over geographic space, or with climatic variables.

AUTHOR CONTRIBUTIONS

DATA AVAILABILITY STATEMENT

All data associated with this study are publicly available in Zenodo.

ORCID

Madeleine M. Ostwald https://orcid.org/0000-0002-9869-8835
Charles N. Thrift https://orcid.org/0000-0002-4257-6951
Katja C. Seltmann https://orcid.org/0000-0001-5354-6048

REFERENCES

Adams, D., Collyer, M., Kaliontzopoulou, A., & Baken, E. (2022). _geomorph: Geometric morphometric analyses of 2D/3D Landmark data_ (4.0.4). https://CRAN.R-project.org/package=geomorph

Akahira, Y., & Sakagami, S. F. (1959). _Observations on the Variability of Wing Venation in the Honeybees (With 4 Text-figures and 2 Tables)._ 14(2), 21.

Alsos, I. G., Ehrich, D., Eidesen, P. B., Solstad, H., Westergaard, K. B., Schönswetter, P., Tribsch, A., Birkeland, S., Elven, R., & Brochmann, C. (2015). Long-distance plant dispersal to North Atlantic islands: Colonization routes and founder effect. _AoB Plants_, 7, plv036. https://doi.org/10.1093/aobpla/plv036

Aytekin, M. A., Terzo, M., Rasmont, P., & Çağatay, N. (2007). Landmark based geometric morphometric analysis of wing shape in _Sibiricobombus_ Vogt (hymenoptera: Apidae: _Bombus_ Latreille). _Annales de La Société Entomologique de France (N.S.)_, 43(1), 95–102. https://doi.org/10.1080/00379271.2007.10697499

Baken, E. K., Collyer, M. L., Kaliontzopoulou, A., & Adams, D. C. (2021). Geomorph v4.0 and gmShiny: Enhanced analytics and a new graphical interface for a comprehensive morphometric experience. _Methods in Ecology and Evolution_, 12(12), 2355–2363. https://doi.org/10.1111/2041-210X.13723

Barton, N. H. (1996). _Natural selection and random genetic drift as causes of evolution on Islands._ https://royalsocietypublishing.org/

Baylac, M., Villemant, C., & Simbolotti, G. (2003). Combining geometric morphometrics with pattern recognition for the investigation of species complexes. _Biological Journal of the Linnean Society_, 80(1), 89–98. https://doi.org/10.1046/j.1095-8312.2003.00221.x

Buschbacher, K., Ahrens, D., Espeland, M., & Steinhage, V. (2020). Image-based species identification of wild bees using convolutional neural networks. _Ecological Informatics_, 55, 101017. https://doi.org/10.1016/j.ecoinf.2019.101017

Carneiro, L., Aguiar, C. M. L., Aguiar, W. M., Aniceto, E. S., Nunes, L. A., & Ferreira, V. S. (2019). Morphometric variability among populations of _Euglossa cordata_ (hymenoptera: Apidae: Euglossini) from different phytophysiognomies. _Sociobiology_, 66(4), 575–581.

Collyer, M. L., & Adams, D. C. (2018). RRPP: RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. _Methods in Ecology and Evolution_, 9(2), 1772–1779. https://doi.org/10.1111/2041-210X.13029

Collyer, M. L., & Adams, D. C. (2019). RRPP: Linear Model Evaluation with Randomized Residuals in a Permutation Procedure. https://CRAN.R-project.org/package=RRPP

Combes, S. A., & Daniel, T. L. (2003). Flexural stiffness in insect wings I. scaling and the influence of wing venation. _Journal of Experimental Biology_, 206(17), 2979–2987. https://doi.org/10.1242/jeb.00523

Comstock, J. H., & Needham, J. G. (1898). The wings of insects. Chapter III. The specialization of wings by reduction. _The American Naturalist_, 32(376), 231–257. https://doi.org/10.1086/276835

Cooper, N., & Purvis, A. (2010). Body size evolution in mammals: Complexity in tempo and mode. _The American Naturalist_, 175(6), 727–738. https://doi.org/10.1086/652466

Deregnaucourt, I., Bardin, J., Anderson, J. M., & Béthoux, O. (2021). The wing venation of a new fossil species, reconstructed using geometric morphometrics, adds to the rare fossil record of Triassic Gondwanian Odonata. _Arthropod Structure & Development_, 63, 101056. https://doi.org/10.1016/j.asd.2021.101056

Doucet, S. M., Shawkey, M. D., Rathburn, M. K., Mays, H. L., & Montgomerie, R. (2004). Concordant evolution of plumage colour, feather microstructure and a melanocortin receptor gene between mainland and Island populations of a fairy–wren. _Proceedings of the Royal Society of London. Series B: Biological Sciences_, 271(1549), 1663–1670. https://doi.org/10.1098/rspb.2004.2779

Engel, M. S., Ceríaco, L. M. P., Daniel, G. M., Dellapé, P. M., Löbl, I., Marinov, M., Reis, R. E., Young, M. T., Dubois, A., Agarwal, I., Lehmann, A. P., Alvarado, M., Alvarez, N., Andreone, F., Araujo-Vieira, K., Ascher, J. S., Baêta, D., Baldo, D., Bandeira, S. A., … Zacharie, C. K. (2021). The taxonomic impediment: A shortage of taxonomists, not the lack of technical approaches. _Zoological Journal of the Linnean Society_, 193(2), 381–387. https://doi.org/10.1093/zoolinnean/zlab072

Fortuna, T. M., Le Gall, P., Mezdour, S., & Calatayud, P.-A. (2022). Impact of invasive insects on native insect communities. _Current Opinion in Insect Science_, 51, 100904. https://doi.org/10.1016/j.cois.2022.100904

Francisco, F. O., Nunes-Silva, P., Francoy, T. M., Wittmann, D., Imperatriz-Fonseca, V. L., Arias, M. C., & Morgan, E. D. (2008). Morphometrical, biochemical and molecular tools for assessing biodiversity. An example in _Plebeia remota_ (Holmberg, 1903) (Apidae, Meliponini). _Insectes Sociaux_, 55, 231–237. https://doi.org/10.1007/s00040-008-0992-7

Francoy, T. M., Bonatti, V., Viraktamath, S., & Rajankar, B. R. (2016). Wing morphometrics indicates the existence of two distinct phenotypic clusters within population of _Tetragonula iridipennis_ (Apidae: Meliponini) from India. _Insectes Sociaux_, 63(1), 109–115. https://doi.org/10.1007/s00040-015-0442-2

Francoy, T. M., de Faria Franco, F., & Roubik, D. W. (2012). Integrated landmark and outline-based morphometric methods efficiently distinguish species of _Euglossa_ (hymenoptera, Apidae, Euglossini). _Apidologie_, 43(6), 609–617. https://doi.org/10.1007/s13592-012-0132-2

Francoy, T. M., Grassi, M. L., Imperatriz-Fonseca, V. L., de Jesús May-Itzá, W., & Quezada-Euán, J. J. G. (2011). Geometric morphometrics of the wing as a tool for assigning genetic lineages and geographic origin to _Melipona beecheii_ (hymenoptera: Meliponini). _Apidologie_, 42(4), 499–507. https://doi.org/10.1007/s13592-011-0013-0

Francoy, T. M., Silva, R., Nunes-Silva, P., Menezes, C., & Imperatriz-Fonseca, V. L. (2009). Gender identification of five genera of stingless bees (Apidae, Meliponini) based on wing morphology. *Genetics and Molecular Research*, *8*(1), 207–214.

Francoy, T. M., Wittmann, D., Drauschke, M., Muller, S., Steinhage, V., Bezerra-Laure, M. A. F., de Jong, D., & Goncalves, L. S. (2008). Identification of Africanized honey bees through wing morphometrics: Two fast and efficient procedures. *Apidologie*, *39*, 488–494. https://doi.org/10.1051/apido:2008028

Grant, P. R. (1965). The adaptive significance of some size trends in Island birds. *Evolution*, *19*(3), 355–367. https://doi.org/10.2307/2406446

Hedrick, P. W., Gutierrez-Espeleta, G. A., & Lee, R. N. (2001). Founder effect in an Island population of bighorn sheep. *Molecular Ecology*, *10*(4), 851–857. https://doi.org/10.1046/j.1365-294X.2001.01243.x

Henriques, D., Chávez-Galarza, J., Teixeira, J, S. G., Ferreira, H. J., Neves, C., Francoy, T. M., & Pinto, M. A. (2020). Wing geometric morphometrics of workers and drones and single nucleotide polymorphisms provide similar genetic structure in the Iberian honey bee (*Apis mellifera iberiensis*). *Insects*, *11*(2), 89.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press.

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521

Jombart, T., & Collins, C. (2015). *A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0.0*. Imperial College London, MRC Centre for Outbreak Analysis and Modelling.

Jordan, M. A., & Snell, H. L. (2008). Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex). *Molecular Ecology*, *17*(5), 1224–1237. https://doi.org/10.1111/j.1365-294X.2007.03658.x

Kaba, D., Berté, D., Ta, B. T. D., Tellería, J., Solano, P., & Dujardin, J.-P. (2017). The wing venation patterns to identify single tsetse flies. *Infection, Genetics and Evolution*, *47*, 132–139. https://doi.org/10.1016/j.meegid.2016.10.008

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Kraemer, A. C., Philip, C. W., Rankin, A. M., & Parent, C. E. (2019). Trade-offs direct the evolution of coloration in Galápagos land snails. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1894), 20182278. https://doi.org/10.1098/rspb.2018.2278

Lomolino, M. V. (1985). Body size of mammals on islands: The Island rule reexamined. *The American Naturalist*, *125*(2), 310–316.

MacArthur, R. H., & Wilson, E. O. (1963). An equilibrium theory of insular zoogeography. *Evolution*, *17*(4), 373–387. https://doi.org/10.2307/2407089

Meröndun, J., Murray, D. L., & Shafer, A. B. A. (2019). Genome-scale sampling suggests cryptic epigenetic structuring and insular divergence in Canada lynx. *Molecular Ecology*, *28*(13), 3186–3196. https://doi.org/10.1111/mec.15131

Michener, C. D. (1994). *The Bee Genera of North and Central America (Hymenoptera: Apoidea)*.

Miller, S. E. (1985). The California Channel Islands—Past, present, and future: An entomological perspective. In *Entomology of the California Channel Islands: Proceedings of the first symposium*. Santa Barbara Museum of Natural History.

Millien, V. (2006). Morphological evolution is accelerated among Island mammals. *PLoS Biology*, *4*(10), e321. https://doi.org/10.1371/journal.pbio.0040321

Mitteroecker, P., & Gunz, P. (2009). Advances in geometric Morphometrics. *Evolutionary Biology*, *36*(2), 235–247. https://doi.org/10.1007/s11692-009-9055-x

Naughton, I., Caterino, M. S., Hanna, C., & Holway, D. (2014). Contributions to an arthropod inventory of Santa Cruz Island, California. *Monographs of the Western North American Naturalist*, *7*(1), 297–305. https://doi.org/10.3398/042.007.0122

Oleksa, A., & Tofilski, A. (2015). Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. *Apidologie*, *46*, 49–60.

O'Reilly, K. M., & Horn, M. H. (2004). Phenotypic variation among populations of *Atherinops affinis* (Atherinopsidae) with insights from a geometric morphometric analysis. *Journal of Fish Biology*, *64*(4), 1117–1135. https://doi.org/10.1111/j.1095-8649.2004.00379.x

Palkovacs, E. P. (2003). Explaining adaptive shifts in body size on islands: A life history approach. *Oikos*, *103*(1), 37–44. https://doi.org/10.1034/j.1600-0706.2003.12502.x

Peil, A. C., & Aranda, R. (2021). Potential niche modeling distribution and wing geometric morphometrics of *Apis mellifera* in the Brazilian Pantanal. *Sociobiology*, *68*(2), e5629.

Perrard, A., Baylac, M., Carpenter, J. M., & Villemant, C. (2014). Evolution of wing shape in hornets: Why is the wing venation efficient for species identification? *Journal of Evolutionary Biology*, *27*(12), 2665–2675. https://doi.org/10.1111/jeb.12523

Phillimore, A. B., Owens, I. P. F., Black, R. A., Chittock, J., Burke, T., & Clegg, S. M. (2008). Complex patterns of genetic and phenotypic divergence in an Island bird and the consequences for delimiting conservation units. *Molecular Ecology*, *17*(12), 2839–2853. https://doi.org/10.1111/j.1365-294X.2008.03794.x

Poulsen, N. R., & Rasmussen, C. (2020). Island bees: Do wood nesting bees have better Island dispersal abilities? *Apidologie*, *51*(6), 1006–1017. https://doi.org/10.1007/s13592-020-00778-x

Rattanawannee, A., Chanchao, C., & Wongsiri, S. (2010). Gender and species identification of four native honey bees (Apidae: *Apis*) in Thailand based on wing Morphometic analysis. *Annals of the Entomological Society of America*, *103*(6), 965–970. https://doi.org/10.1603/AN10070

Rattanawannee, A., Chanchao, C., & Wongsiri, S. (2012). Geometric morphometric analysis of giant honeybee (*Apis dorsata* Fabricius, 1793) populations in Thailand. *Journal of Asia-Pacific Entomology*, *15*(4), 611–618.

Rattanawannee, A., Duangpakdee, O., Rod-Im, P., & Hepburn, R. (2015). Discrimination of two Tetragonula (Apidae: Meliponini) species in Thailand using geometric morphometric analysis of wing venation. *Kasetsart Journal – Natural Science*, *49*(5), 700–710.

Rohlf, F. J. (2015). The tps series of software. *Hystrix*, *26*(1), 1–4. https://doi.org/10.4404/hystrix-26.1-11264

Rohlf, J. F., & Marcus, L. F. (1993). A revolution morphometrics. *Trends in Ecology & Evolution*, *8*(4), 129–132. https://doi.org/10.1016/0169-5347(93)90024-J

Rossa, R., Goczał, J., & Tofilski, A. (2016). Within- and between-species variation of wing venation in genus *Monochamus* (Coleoptera: Cerambycidae). *Journal of Insect Science*, *16*(1), 5. https://doi.org/10.1093/jisesa/iev153

Runemark, A., Brydegaard, M., & Svensson, E. I. (2014). Does relaxed predation drive phenotypic divergence among insular populations? *Journal of Evolutionary Biology*, *27*(8), 1676–1690. https://doi.org/10.1111/jeb.12421

Santos de Souza, F., Nunes, L. A., Oliveira, E. J. F., de Costa, M. A. P. d. C., & de Carvalho, C. A. L. (2019). Population variation and Island effect in *Melipona subnitida* (hymenoptera: Apidae). *Journal of Apicultural Research*, *58*(1), 67–74. https://doi.org/10.1080/00218839.2018.1494920

Santoso, M. A. D., Juliandi, B., & Raffiudin, R. (2018). Honey bees species differentiation using geometric morphometric on wing venations.

IOP Conference Series: Earth and Environmental Science, *197*, 12015. https://doi.org/10.1088/1755-1315/197/1/012015

Seltmann, K. C., Dewey, D., Hobson, E., McLaren, L., Thrift, C. N., & Wood, Z. (2019). Native and non-native bees (Anthophila) of Santa Cruz Island: An annotated checklist (0.02). *Zenodo.* https://doi.org/10.5281/zenodo.3831088

Sharkey, M. J., & Roy, A. (2002). Phylogeny of the Hymenoptera: A reanalysis of the Ronquist et al. (1999) reanalysis, emphasizing wing venation and apocritan relationships. *Zoologica Scripta*, *31*(1), 57–66.

Sylvester, H. A., Limbipichai, K., Wongsiri, S., Rinderer, T. E., & Mardan, M. (1998). Morphometric studies of *Apis cerana* in Thailand and the Malaysian peninsula. *Journal of Apicultural Research*, *37*(3), 137–145. https://doi.org/10.1080/00218839.1998.11100965

Traveset, A., Heleno, R., Chamorro, S., Vargas, P., McMullen, C. K., Castro-Urgal, R., Nogales, M., Herrera, H. W., & Olesen, J. M. (2013). Invaders of pollination networks in the Galápagos Islands: Emergence of novel communities. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1758), 20123040. https://doi.org/10.1098/rspb.2012.3040

Velo-Antón, G., & Cordero-Rivera, A. (2017). Ethological and phenotypic divergence in insular fire salamanders: Diurnal activity mediated by predation? *Acta Ethologica*, *20*(3), 243–253. https://doi.org/10.1007/s10211-017-0267-2

Velo-Antón, G., Zamudio, K. R., & Cordero-Rivera, A. (2012). Genetic drift and rapid evolution of viviparity in insular fire salamanders (*Salamandra salamandra*). *Heredity*, *108*(4), 410–418. https://doi.org/10.1038/hdy.2011.91

Villemant, C., Simbolotti, G., & Kenis, M. (2007). Discrimination of *Eubazus* (hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. *Systematic Entomology*, *32*(4), 625–634. https://doi.org/10.1111/j.1365-3113.2007.00389.x

Warren, B. H., Simberloff, D., Ricklefs, R. E., Aguilée, R., Condamine, F. L., Gravel, D., Morlon, H., Mouquet, N., Rosindell, J., Casquet, J., Conti, E., Cornuault, J., Fernández-Palacios, J. M., Hengl, T., Norder, S. J., Rijsdijk, K. F., Sanmartín, I., Strasberg, D., Triantis, K. A., ... Thébaud, C. (2015). Islands as model systems in ecology and evolution: Prospects fifty years after MacArthur-Wilson. *Ecology Letters*, *18*(2), 200–217. https://doi.org/10.1111/ele.12398

Wenner, A. M., Thorp, R. W., & Barthell, J. F. (2009). Biological control and eradication of feral honey bee colonies on Santa Cruz Island, California: A summary. In *Proceedings of 327 the 7th California Islands Symposium. Institute for Wildlife Studies* (pp. 327–335). Institute for Wildlife Studies.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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