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Stronger interspecific sexual differences may be favored when females search for mates in the presence of congeners

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ABSTRACT

Why are some species sexually dimorphic while other closely related species are not? While all females in genus *Strauzia* share a multiply-banded wing pattern typical of many other true fruit flies, males of four species have noticeably elongated wings with banding patterns "coalesced" into a continuous dark streak across much of the wing. We take an integrative phylogenetic approach to explore the evolution of this dimorphism and develop general hypotheses underlying the evolution of wing dimorphism in flies. We find that the origin of coalesced and other darkened male wing patterns correlate with the inferred origin of host plant sharing in *Strauzia*. While wing shape among non-host-sharing species tended to be conserved across the phylogeny, shapes of male wings for *Strauzia* species sharing the same host plant were more different from one another than expected under Brownian models of evolution and overall rates of wing shape change differed between non-host-sharing species and host-sharing species. A survey of North American Tephritidae finds just three other genera with specialist species that share host plants. Host-sharing species in these genera also have wing patterns unusual for each genus. Only genus *Eutreta* is like *Strauzia* in having the unusual wing patterns only in males, and of genera that have multiple species sharing hosts, only in *Eutreta* and *Strauzia* do males hold territories while females search for mates. We hypothesize that in species that share host plants, those where females actively search for males in the presence of congeners may be more likely to evolve sexually dimorphic wing patterns.

Introduction

Sexually dimorphic traits – characters that differ between biological sexes – have long been a focus of biologists studying how and why selection acts differently on individuals of the same species. Often dimorphism in one sex results from direct interactions between sexes (Slatkin 1984). Sexual dimorphism can play a role in mating behavior with differences emerging as a result of sexual selection. Specific examples include when dimorphic traits emerge due to sexual signaling mechanisms, including both mate attraction (Lande 1981; Lande and Arnold 1985; Allen et al., 2011) and the evaluation of mate quality (Funk et al. 2000; Bonduriansky 2001). In some other cases, sexual dimorphism can be the result of ecological factors unrelated to intersexual interactions, such as when different sexes have different ecological roles, and those roles favor divergent morphologies (Slatkin 1984). Alternatively, the emergence of sexually dimorphic traits can result from interspecific interactions. For instance, reproductive character

displacement can occur when congeners are found in close contact, and this is usually ascribed to selection against interspecific hybridization (Noor 1999). One pattern resulting from reproductive character displacement is a higher prevalence of sexual dimorphism when species are in sympatry with close relatives than when they are not (Price 1998; Figuerola and Green 2000). Discriminating among the many possible hypotheses to explain the evolution of sexual dimorphism in any given species or genus can be challenging because objectively evaluating all potential explanations may often require a complete accounting of the biology, ecology, behavior, and evolutionary history of the focal group. However, when much of this information is known, and when the presence or degree of sexual dimorphism can be measured across a single genus, it is possible to develop an integrative phylogenetic understanding of the evolution of dimorphic traits (e.g., Baker and Wilkins 2001; Emlen et al. 2005).

Flies in the genus *Strauzia* Robineau-Desvoidy (Diptera: Tephritidae) provide an opportunity to integrate morphology, phylogeny, behavior,

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and ecology towards understanding the evolution of sexual dimorphism. *Strauzia* have a long history of taxonomic uncertainty, with a considerable degree of apparent intraspecific variation in several putative species (Foote et al. 1993). Recent phylogenetic work has clarified that some of this variation is interspecific: while the majority of *Strauzia* species are the lone fly from their genus feeding on any given plant host species, in each of two instances three *Strauzia* species were discovered to share the same plant host (Hippee et al. 2021). Though this work has improved the taxonomy of the genus, it has also confirmed some intraspecific trait variation, and at least two traits – wing shape and pattern – are strongly sexually dimorphic in some *Strauzia* species but less variable in others.

Like most true fruit flies (Foote et al. 1993; Sivinski and Pereira 2005; Norrbom et al. 2010), all *Strauzia* have distinctive darkened patterns on their wings, in most species comprised by orange to moderately brown bands. The most common pattern, shared across females and males of most *Strauzia* species, is the "F-pattern" (Axen et al. 2010) where the bands on the distal third or more of the wing form an "F" (Fig. 1a-c). The "F" is conserved in all female *Strauzia*, except for one species (*S. arculata* (Loew)), which has a slightly modified pattern with most of the elements of the F present, although some species may also have anterior, medial or posterior connections of the F to other wing bands. Conservation of the F-pattern may confer a fitness benefit: similar banding patterns in other Tephritidae mimic the appearance of jumping spiders and offer protection from predation by those same spiders (Greene et al. 1987; Mather et al. 1987; Whitman et al. 1988). Though this putatively beneficial trait is otherwise strongly conserved across the genus (and indeed in many other tephritid genera), the males of four *Strauzia* species instead have a "coalesced" wing pattern, wherein the wing is predominantly occupied by a broad dark brown marking running longitudinally down the wing, with the bands that would otherwise constitute the apical "F" fused, sometimes shortened posteriorly, and not or at most partially recognizable (Figs. 1d, 3). This coalesced patterning is also often a noticeably darker brown than the wing pattern of the conspecific female. Such wing dimorphism is not only unusual in *Strauzia*, but among Tephritidae generally.

Wings of some *Strauzia* males are also noticeably different in size and shape compared with those of the conspecific females (Stoltzfus 1988) but why some species have this shape dimorphism while others do not is not immediately evident. Wing shape in some tephritids influences mating success: in courtship rituals, wings can generate visual, acoustic (wing vibration), and chemical (pheromone wafting) signals (Briceño

et al. 1996; Souza et al. 2015). Differences in wing size may alter acoustic signaling by changing the frequency and duration of wing vibrations, which influence mate preference and copulation success (Souza et al. 2015; Benelli et al. 2016). Wing shape may also be phenotypically plastic, with some previous work reporting differences in wing shape that may correspond to differences in environmental variables among populations (Lemic et al. 2020).

Strauzia are also unusual among tephritid flies in that some species specialize on the same host plants. All *Strauzia* species have univoltine life cycles intimately tied to their plant host: males stake out territories on plant leaves, females search among plants to find males, eggs are laid in the apical meristem of the plant, larvae feed on the pith, and pupariation occurs either in the lower stem, root, or soil directly around the plant. In two cases, three species of *Strauzia* specialize on the same host plant. Jerusalem artichoke (*Helianthus tuberosus* L.) is host to *Strauzia longipennis* (Wiedemann), *Strauzia vittigera* (Loew), and *Strauzia longitudinalis* (Loew), while *Strauzia arculata* Steyskal, *Strauzia noctipennis* Stoltzfus, and "Bush's Fly" (a species not yet formally named and described) all share the sawtooth sunflower (*Helianthus grosseserratus* Martens) (Hippee et al. 2016; Hippee et al. 2021). As predicted for closely related species that largely overlap in the same habitat (Lack 1947; Brown and Wilson 1956; Schluter 2000; Pfennig and Pfennig 2010), previous work has identified evidence of apparent character displacement among the three species of *Strauzia* that share the *H. tuberosus* host, most notably in the form of differences in adult emergence timing (Hippee et al. 2016). New resolution of *Strauzia* species limits also demonstrates that three of the four *Strauzia* species with coalesced male wing patterns are among the fly species that share plant hosts with congeners (Hippee et al. 2016; Hippee et al. 2021).

Because *Strauzia* are unusual both in their having sexually dimorphic wings and in sharing hosts with congeners, we ask whether the two traits might be connected in *Strauzia* and across Tephritidae. We leverage the *Strauzia* phylogeny alongside new morphometric data and previous work detailing their respective host associations, mate choice behaviors, and phenology, to characterize the evolution of sexually dimorphic wing pattern and shape. We also review host association, behavior, and wing dimorphism across other North American Tephritidae to assess whether patterns found in *Strauzia* are representative of a larger theme across the fruit flies. Using our findings in *Strauzia* alongside the records in other Tephritidae, we propose a hypothesis that host plant sharing coupled with particular mate-finding behaviors may promote the emergence and maintenance of sexually dimorphic character traits.
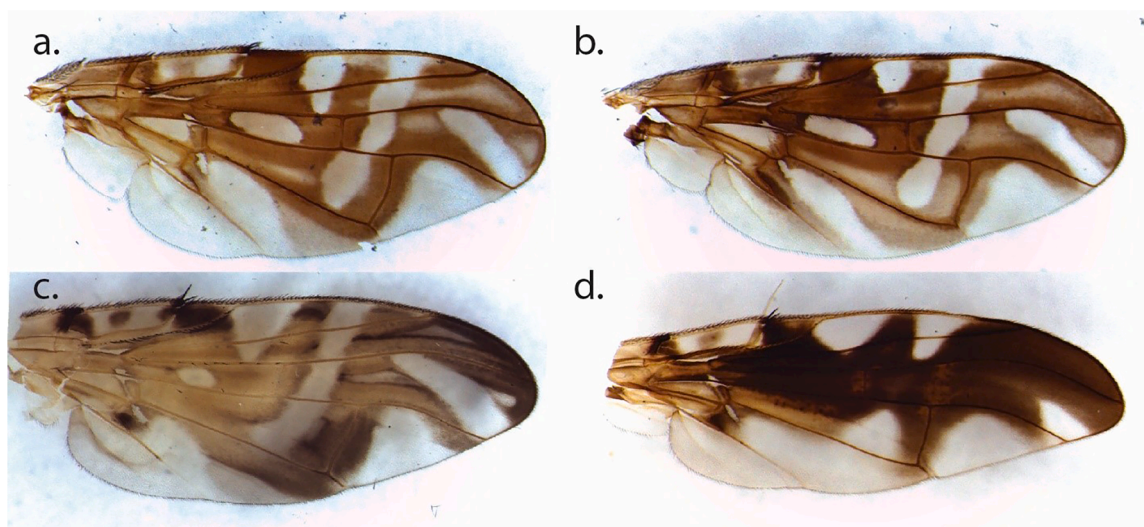

Fig. 1. Female and male wings from two representative *Strauzia* species. Wing patterns are generally similar in most species. In Strauzia intermedia, for example, females (a) and males (b) both have the typical "F" banding pattern. In other species, like Strauzia noctipennis, females (c) have the F pattern, while male wings (d) have a "coalesced" pattern that is darker and more continuous across the center of the wing.

Methods

Adult fly collections and wing mounting

From 2011–2021, we collected adult *Strauzia* representing 11 of the 12 named species, plus the undescribed "Bush's Fly" and another undescribed species that is sister to *S. vittigera* reared from *Helianthus strumosus* ("*strumosus* Fly"). Host-sharing species are broadly sympatric and for this study were often collected from the same individual patches of plants on the same days (Supplemental Table 1). We captured adult flies individually in plastic cups while they rested on host plants. Some flies were reared from pupae that we had dissected from plant stems and artificially overwintered for 4 months in a refrigerator at 4–8 °C. We removed pupae from the refrigerator after 4 months, held them at 18 °C for 1 week, and then moved them to a light- and temperature-controlled incubator (16:8 photoperiod; 25 °C) to encourage eclosion of adults. All flies were preserved in 95 % ethanol and stored at −80 °C until use. Only adult flies with wings that were intact or nearly intact were included in the dataset (Supplemental Table 1 – flies included in study).

We removed both fly wings using fine point tweezers and preferentially selected the most intact wing for analysis. We mounted wings on glass slides by soaking each wing in a NaOH solution at 100 °C for 1 min, followed by a 1 min soak in 95 % ethanol - a modified version of the protocol described in Steyskal et al. (1986). Using featherweight tweezers, we gently placed the wing on a glass slide, allowed the remaining ethanol to evaporate, and mounted the wing with several drops of warmed Euparal (BioQuip Products Inc, Rancho Dominguez, California, USA) and a glass coverslip. We allowed the slides to dry on a slide warmer for approximately one week before taking pictures of the slides. Due to changes in product availability, 66 wings were mounted using Permount (Thermo Fisher Scientific, Waltham, MA, USA) instead of Euparal and then were allowed to dry for 48 h at room temperature prior to wing photography. In total, we analyzed 254 wing slides including 211 slides that we mounted and 43 additional slides that were provided by Dr. Marty Condon (Cornell College, Mt. Vernon, IA).

Wing morphometrics and centroid analysis

We photographed all *Strauzia* wings using a Leica IC80 HD camera linked to a Leica M125 microscope (Leica Microsystems, Wetzlar, Germany) set to 2X magnification. We opened each wing image in ImageJ v1.52a (Abramoff et al. 2004), then converted images to grayscale, adjusted the orientation so each wing was facing the same direction, and checked to make sure the number of pixels was identical across all images. Then, using the landmark tool in ImageJ, we laid eight single point landmarks that represented the most consistent vein intersections across

all *Strauzia* wings (Fig. 2). These landmarks were based on a previous set of fourteen landmarks used in analyses of other tephritid fly wings (Marsteller et al. 2009), but we eliminated six landmarks because we failed to find consistent vein intersections across all *Strauzia* species. To avoid variation introduced by different researchers laying the landmarks, one person (ACH) completed all wing landmark analyses. To further eliminate variation introduced by the landmarking process, each wing was landmarked twice, on two separate occasions and in a random order. Then, we compared both sets of landmarks, and the wing sample was eliminated from the analysis if the landmark coordinates differed by more than 1 % across the two sets of landmarks for each individual fly. If the sample passed this accuracy threshold, the two sets of landmark coordinates were averaged together to generate a single set of eight coordinates for each wing sample. To test for differences between left and right wings, we mounted both wings from the same male *S. vittigera* ($n = 6$) and *S. longitudinalis* ($n = 5$) flies and compared landmarks using the MANOVA statistical procedures described below. We found no difference (P[*vittigera*] = 0.95; P[*longitudinalis*] = 0.99), providing justification for using either wing in subsequent tests, particularly when one wing had been damaged before capture in the wild or during occasional failed slide mounting.

We imported our landmark coordinates into geomorph v4.0.1 (Adams and Otárola-Castillo 2013) implemented in R to complete a series of wing morphometric analyses. First, we used a generalized Procrustes analysis (GPA (Rohlf 1990; Slice 1996)) using *gpagen* to align the coordinates of all samples using a least squares criterion and projected the resulting coordinates on a linear tangent space (Abramoff et al. 2004; Adams et al. 2004). Completing the GPA eliminates existing variation due to size, position, and orientation in the landmarks, allowing all remaining variation in landmarks to describe shape differences (Adams et al. 2004). The resulting landmarks can be used for multivariate statistical comparisons of shape. We used Principal Component Analyses (PCA) to visualize shape variation between males and females of each *Strauzia* species. All six *Strauzia* species that share plant hosts and four species that do not share hosts were analyzed. For three additional species (*S. rugosum*, *S. uvedaliae*, and *S. verbesinae*), fewer than three male or female wings were available, which was too few for statistical comparison. We repeated this procedure using the program PAST v4.04 (Hammer et al. 2001) to verify that different morphometrics programs produce similar results.

To determine if wing shape was significantly different between males and females of the same species, we generated a multivariate analysis of variance (MANOVA) of principal components generated during the PCA analysis. We used the broken-stick model (Frontier 1976; Jackson 1993; Peres-Neto et al. 2003) on the scree plot generated in PAST v4.04 to select only principal components that account for the majority of the
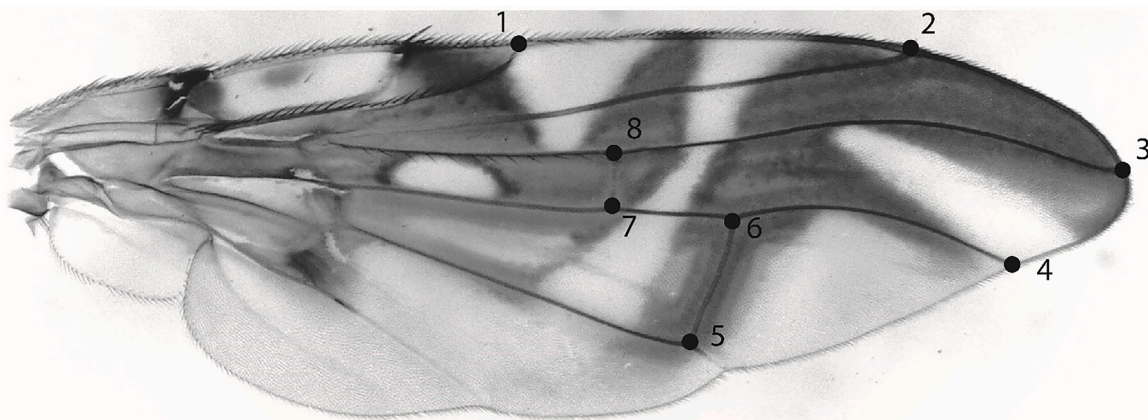

Fig. 2. Example *Strauzia* wing with landmarks. Black circles indicate vein intersections used as landmarks for wing morphometric analysis. Numbers next to each circle indicate the landmark number. Landmark locations are based on those described in Marsteller et al. (2009).

variance for our analyses. In most cases, this was between 2 and 4 principal components in each analysis. For some comparisons, only one principal component was selected from the broken-stick model. For those cases, a *t*-test was used to compare the principal components, and we also did a MANOVA by including a second principal component despite it not meeting the broken-stick model criteria. To visualize the magnitude and direction of wing shape change, we used *mshape* in geomorph v4.0.1 to calculate the mean male and female wing shape for each species. Then, using *plotRefToTarget*, we generated points and vectors showing how each wing landmark differs between females and males for each species.

We calculated centroid size for males and females of each species using PAST v4.04 and geomorph v4.0.1. We tested for differences in centroid size between males and females of the same species using *t*-tests and calculated the average centroid size for males and females of each species. To determine if centroid size differed between *Strauzia* species independently of body size, for all male *S. longipennis, S. longitudinalis, S. perfecta, S. intermedia, S. vittigera, S. noctipennis*, "Bush's Fly", and *S. arculata* flies we scaled fly wing centroid sizes by the average male fore femur length of each species, as fore femur length has been shown to correlate to body size in Tephritid fruit flies (Sivinski and Pereira 2005). Indeed, we tested fore femur length and body length size correlation in three species, *S. longipennis, S. longitudinalis*, and *S. vittigera*, and found a strong positive correlation for each species individually (Pearson's $r =$ 0.89, 0.92, and 0.97 respectively) and combined (Pearson's $r = 0.92$). We then tested for a correlation between fore femur length and wing centroid size using log-transformed values for *S. longipennis, S. noctipennis, S. arculata, S. perfecta, S. intermedia, S. vittigera*, and "Bush's Fly" males.

We also compared wing shape variation among male and female *Strauzia* that share the same host plants. Following the same procedures for principal component analyses and statistical comparison, we compared all males and females that utilize *H. tuberosus* (*S. longipennis, S. vittigera*, and *S. longitudinalis*) and all males and females that share *H. grosseserratus* (*S. arculata, S. noctipennis*, and "Bush's Fly") in four separate PCAs and MANOVA procedures. In all analyses, statistical significance was evaluated following a Bonferroni correction for multiple comparisons.

Phylogenetic analyses of wing pattern and shape

To contextualize patterns in *Strauzia* wing variation alongside their evolutionary histories, we mapped representative images of male and female wings, PCA plots, and the shape change landmarks on a previously published phylogeny of *Strauzia* (Hippee et al. 2021). Generated with SNP data from reduced-representation genomics sequencing (3RAD), this phylogeny included 127 *Strauzia* specimens representing 11 of the 12 known *Strauzia* species as well as at least two currently undescribed species.

To explore the potential impact of host plant sharing on wing shape variation, we used geomorph v4.0.1 to determine how wing shape evolved across the *Strauzia* phylogeny. We generated a time-calibrated phylogeny of *Strauzia* using 3RAD sequencing data from 70 individuals that represented all known *Strauzia* species. Using ipyrad v0.9.84 (Eaton and Overcast 2020), we compiled a concatenated alignment of 163 loci and using RAxML (Kozlov et al. 2019), we produced a maximum-likelihood phylogeny for the genus. Then, we used the program BEAUTI2 v2.6.2 (Bouckaert et al. 2019) to prepare input files for the program BEAST2 v2.5.1 (Bouckaert et al. 2019), that we used to create our time calibrated phylogeny. We ran the BEAST2 analysis with the maximum likelihood phylogeny generated in RAxML as a fixed topology using a lognormal relaxed molecular clock with a GTR + Γ substitution model and a Yule process tree prior with a fixed topology throughout the run, allowing only adjustments in node heights. Because we only required relative and not absolute time-calibration, we arbitrarily selected the molecular clock rate of Papadopoulou et al.

(2010). We applied this as a mean rate across all loci as it was not computationally feasible to assign individual molecular clocks to each of the 163 loci and this rate has been used previously to make relative comparisons in *Strauzia* in the absence of a known mutation rate in the genus (Hippee et al. 2021). We generated our final tree using TreeAnnotator v2.6.2 (Bouckaert et al. 2019).

We estimated the degree of phylogenetic signal in wing shape (compared to a Brownian model) using the function *physignal* implemented in geomorph. *Physignal* estimates K_{mult}, a multivariate version of the K-statistic (Adams 2014) that compares the phylogenetic signal of a dataset (e.g., wing shapes) to an expectation based on a Brownian motion model of evolution. We pruned the *Strauzia* phylogeny down to one individual per species using phytools (Revell, 2012). We first ran *physignal* inclusive of all *Strauzia* species. Then, to determine the relative phylogenetic signal of host-sharing and non-host-sharing species, we ran *physignal* with only non-host plant sharing *Strauzia* (*S. perfecta, S. intermedia, S. gigantei, S. verbesinae*, "strumosus Fly", and *S. uvedaliae*) and then only host-plant sharing *Strauzia* (*S. vittigera, S. longitudinalis, S. longipennis, S. arculata, S. noctipennis*, and "Bush's Fly"). We also used the *compare.evol.rates* function in geomorph to compare the relative evolutionary rates of wing shape change over time between non-host plant sharing species, *Strauzia* sharing *H. tuberosus*, and *Strauzia* sharing *H. grosseserratus*. The statistical significance of pairwise evolutionary rate comparisons was evaluated by comparing observed rates to a rate matrix calculated in 999 iterations of a phylogenetic simulation under Brownian motion.

Host sharing and sexual dimorphism in other tephritid flies

We reviewed the literature pertaining to the biology and morphology of the Tephritidae of the USA and Canada to investigate whether there are common patterns of sexual dimorphism in wing pattern correlated with ecology. We searched the *Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico* (Foote et al. 1993) for all instances where two or more species of the same genus specialized on the same plant host. We narrowly defined a "specialist" fly species as one for which all or most records were from a single plant species. However, we recognize that natural history records might omit geographically restricted or otherwise understudied plant hosts (which would result in flies appearing more specialized than they actually are), or records might include incorrect insect-plant associations, which could result in flies looking more generalist than they actually are. We included instances wherein ≥2 specialist species co-occurred on the same host plant with other, more generalist, congeners but did not include situations where only one specialist species used a plant also used by a congeneric generalist species. Our reasoning in being so restrictive was that we wanted to avoid systems where flies had the option of moving to alternative host plants when congeners were locally present.

For each genus identified as having ≥2 specialist species using the same host plant, we then performed a qualitative analysis of its respective natural history literature. Specifically, for each genus we determined 1) whether any of the host-sharing species showed wing patterns unusual for that genus, 2) whether any species were noted as being sexually dimorphic in wing patterns or shape, and 3) whether both sexes actively searched for mates or if only one sex searched while the other held territories, as is the case in *Strauzia*.

Results

Wing morphometrics and centroid analysis

Centroid size measurements showed that wing size is variable among *Strauzia* species and by sex, with *S. intermedia* females having the smallest wings (mean = 874.7 ± 70.26, *n* = 5) and *S. uvedaliae* females having the largest (mean = 1207.2 ± 20.322, *n* = 2). Among comparisons of males and females of the same species, only *S. arculata* (*t*-test; P-

value = 0.0005) and *S. longitudinalis* (*t*-test; P-value = 0.004) wings had significantly different centroid sizes (Supplemental Table 2). Pairwise comparisons of male and female centroids compared to other species show that the majority (82 %) of male and female wing centroids do not differ significantly from each other, with some exceptions in male wings and even fewer among the female comparisons (Supplemental Table 3). Our tests for correlation between fore femur length and centroid size found an overall positive correlation among all species (0.58). Only *S. arculata* had a strong negative correlation between fore femur length and wing centroid size (−0.84). If *S. arculata* is excluded from the pooled analysis, the remaining species have a correlation of 0.62. After scaling male wing centroid size by fore femur length as a proxy for body size, we found even fewer significant differences in centroid size among all possible pairwise comparisons across the genus when fore femur measurements were available (Supplemental Table 4). The comparisons that were significantly different all included comparisons with *S. noctipennis* males, which had a significantly different centroid size than males of *S. arculata*, "Bush's Fly", *S. intermedia*, *S. perfecta*, and *S. longipennis*.

Principal Component Analyses (PCAs) of wing shape (with size variation excluded from the comparison) between males and females of the same species identified variable degrees of wing shape dimorphism across *Strauzia*. Using MANOVAs, we statistically compared the differences between the principal components for each species to determine if wing shape differed significantly among males and females of the same

species and among males or females of different species. Three species - *S. intermedia*, *S. gigantei*, and *S. arculata* - had male and female wings that were not significantly different in shape (Fig. 3; Table 1). The remaining *Strauzia* species for which male and female comparisons were possible – *S. noctipennis*, "Bush's Fly", *S. longipennis*, *S. longitudinalis*, *S. perfecta*, "strumosus Fly", and *S. vittigera* had wing shapes that were significantly different between sexes (Fig. 3; Table 1). We were not able to make wing comparisons of *S. rugosum*, *S. uvedaliae*, and *S. verbesinae* due to low sample sizes. We also generated vectors showing the direction and magnitude of shape change between the mean female and mean male wing shape of each *Strauzia* species. Across all *Strauzia* wings, male wings were generally narrower and longer than female wings, with *S. noctipennis*, "Bush's Fly", *S. longipennis*, *S. longitudinalis*, "strumosus Fly", and *S. vittigera* showing extreme examples manifested in changes in wing landmarks 1–5 (Fig. 3, Supplemental Fig. 1).

MANOVAs of wing shape of all *Strauzia* males showed that the majority of *Strauzia* species have significantly different male wing shapes, with *S. noctipennis* and "Bush's Fly" males differing significantly from those of all other *Strauzia* species included in the analysis (Supplemental Table 5; Supplemental Fig. 2). *Strauzia intermedia* males were also significantly different in nine of the eleven species comparisons (Supplemental Table 5). In the *Strauzia* female analysis, the majority (82 %) of comparisons did not show a significant difference in shape (Supplemental Table 6; Supplemental Fig. 3). *Strauzia longitudinalis* females
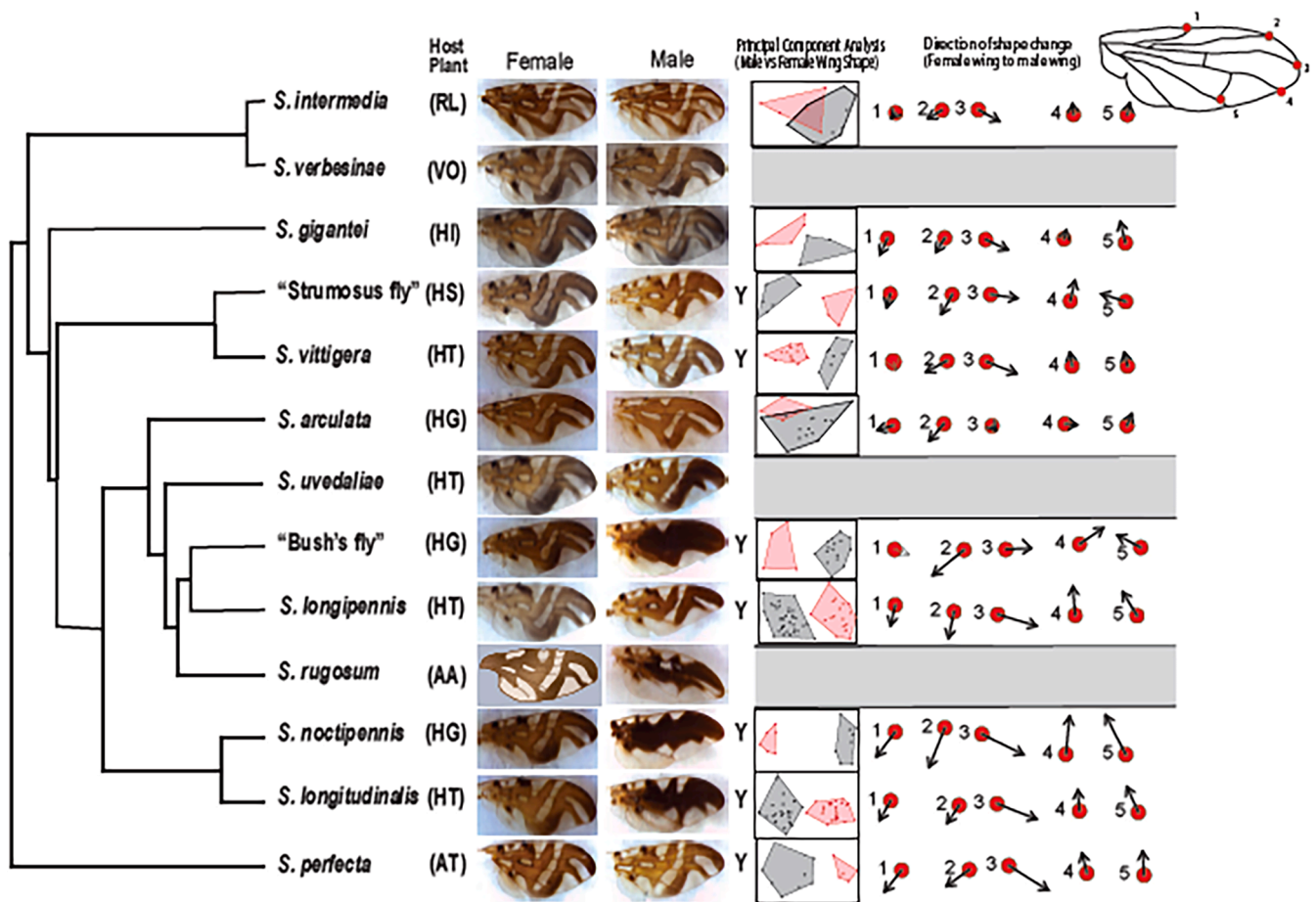

Fig. 3. Phylogeny of *Strauzia* with male and female wings. Maximum likelihood phylogeny of *Strauzia* from Hippee et al. (2021) with female (left column) and male (right column) wings next to each *Strauzia* species and the results of the PCA analyses of males (black) and females (red) for each species. Points and arrows on the far right represent the direction and magnitude of shape change between an average female wing (red point) and an average male wing (black arrow). A diagram on the top right shows the location of landmarks 1–5 included in the figure on a standard fly wing. A "Y" next to a PCA plot indicate significantly different male vs. female wing shapes (Table 1). Rows with grey shading and no PCA plots were those that did not have adequate sample sizes for analysis. The *S. rugosum* female wing picture is an illustration based on images and descriptions from Stoltzfus (1988). Host plants: RL = *Rudbeckia lacianata*; VO = *Verbesina occidentalis*; HI = *Helianthus giganteus*; HS = *Helianthus strumosus*; HT = *Helianthus tuberosus*; HG = *Helianthus grosseserratus*; AA = *Ageratina altissima*; AT = *Ambrosia trifida*.

Table 1

MANOVA comparisons of male and female wing shape. The Wilks' lambda statistic, F value, and P-value are listed for each comparison. The number of principal components included in the analysis based on the results from the broken stick model and the sample size (N) are also included. Bolded rows indicate male and female wings that were significantly different in shape after a correction for multiple comparisons.

Species	Wilks' lambda	F	P-value	# of PCs included	Males (N)	Females (N)
S. intermedia	0.5248	2.49	0.1042	4	11	5
S. gigantei	0.4364	3.875	0.0831	2	4	5
S. vittigera	**0.1781**	**30.77**	**>0.0001**	**3**	**27**	**25**
"strumosus Fly"	**0.1135**	**35.15**	**>0.0001**	**2**	**35**	**18**
S. arculata	0.5604	3.922	0.0299	3	15	4
S. longitudinalis	**0.1348**	**157.3**	**>0.0001**	**2**	**9**	**15**
"Bush's Fly"	**0.1626**	**43.76**	**>0.0001**	**2**	**8**	**4**
S. noctipennis	**0.02645**	**202.4**	**>0.0001**	**2**	**16**	**4**
S. longipennis	**0.1377**	**156.6**	**>0.0001**	**2**	**8**	**6**
S. perfecta	**0.2697**	**14.89**	**0.0007**	**2**	**9**	**5**

were the most different from other species, with their shape differing significantly from five of the ten species included in the analysis (Supplemental Table 6).

In another set of MANOVAs we also compared the wing shapes of males and females of *Strauzia* species that share the same host plant species. Three *Strauzia* species – *S. longipennis, S. longitudinalis*, and *S. vittigera* – share the *H. tuberosus* host plant. Three other *Strauzia* species – *S. arculata, S. noctipennis*, and "Bush's Fly" – also share their host plant, *H. grosseserratus*. All males on *H. tuberosus* and *H. grosseserratus*, respectively, were significantly different from each other (Supplemental Table 7; Fig. 4). Among the females on *H. tuberosus, S. longipennis* and *S. longitudinalis* were significantly different from each other (P-value =

0.003), but *S. vittigera* was not significantly different from *S. longipennis* or *S. longitudinalis* (P-value > 0.05) (Supplemental Table 7; Fig. 4). On *H. grosseserratus, S. arculata* females were significantly different from females of both "Bush's Fly" (P-value = 0.01) and *S. noctipennis* (P-value = 0.004), but "Bush's Fly" and *S. noctipennis* were not significantly different from each other (P-value > 0.05) (Supplemental Table 7, Fig. 4). Wing shape variation was primarily in landmarks 1 through 5, with landmarks 6 through 8 showing little to no variation between males and females of any *Strauzia* species.
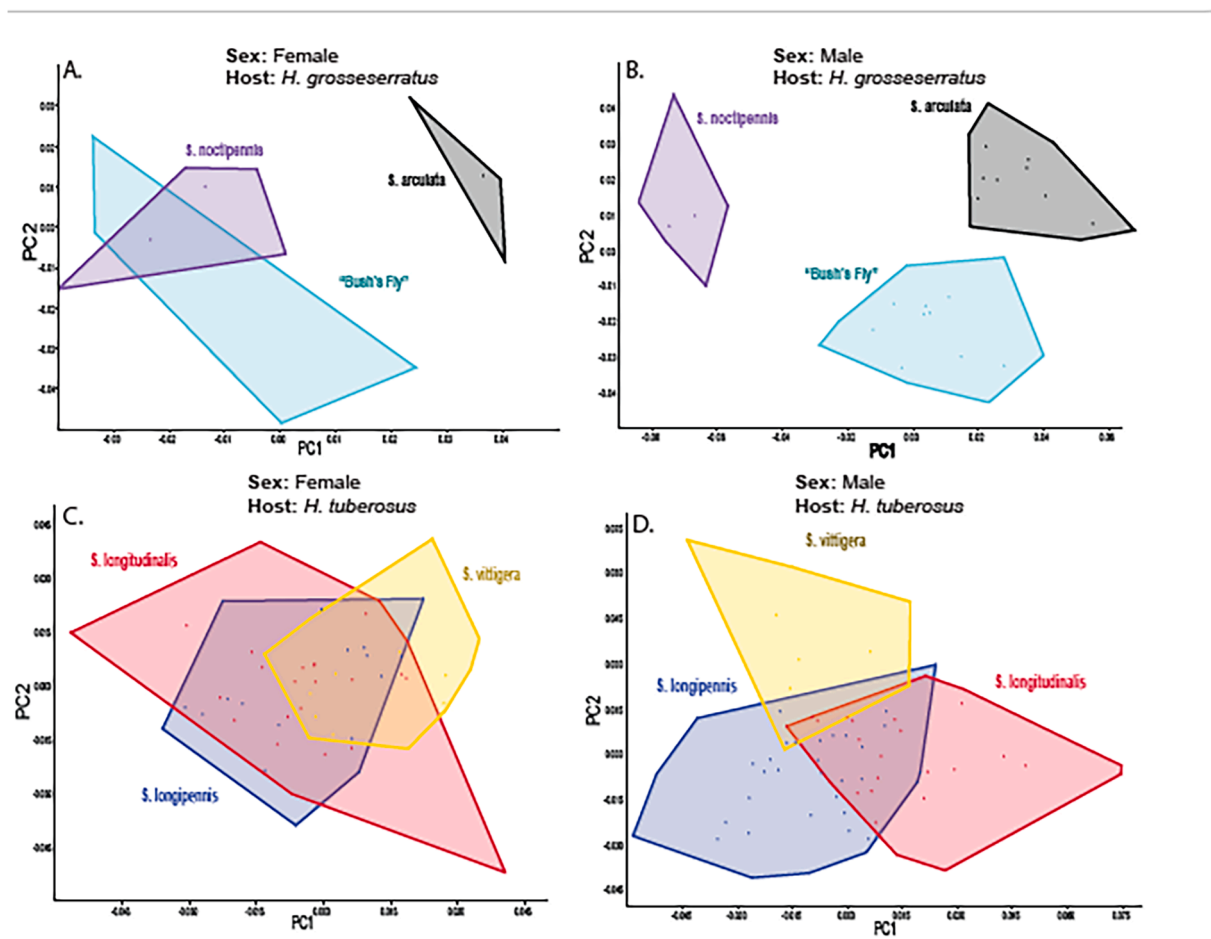

Fig. 4. Principal component analyses of wing shape differences in *Strauzia* females (A and C) and males (B and D) that share *H. grosseserratus* (A and B) and *H. tuberosus* (C and D). In all panels, axes represent PC1 and PC2. Points, lines encompassing points, and species labels are color coded to indicate which points are associated with each species. Fly sex and host plant association is listed above each panel.

Phylogenetic analyses of wing pattern and shape

We summarized the *Strauzia* phylogeny (Hippee et al. 2021) into a species tree representing all valid *Strauzia* species, with the exception of *S. stoltzfusi* Steyskal whose host is unknown, plus "Bush's Fly" and the currently undescribed "*strumosus* Fly" (included in the Hippee et al., 2021 phylogeny as *S. vittigera* from host *H. strumosus*) (Fig. 3). Female wings showed no major pattern variation across the phylogeny with the exception of those of *S. arculata* and "Bush's Fly", which both have anterior and posterior connections between the "F" and the more basal wing markings and a near to total loss of connection between the anterior and posterior parts of the "F", such that it instead takes the form of two chevron shapes at the distal end of the wing (Fig. 3).

The male wings of many species show more extreme variation in wing pattern across the genus. Males of "Bush's Fly", *S. noctipennis, S. rugosum*, and *S. longitudinalis* all have a fully coalesced wing pattern (Fig. 3). Males of two other species, *S. uvedaliae* and *S. longipennis*, also had noticeably darker banding patterns on the apical part of the wing than their respective conspecific females, but their basal bands were distinct and were not coalesced into a single broad marking. All six

species with some obvious difference between male and female wing patterns were in the same clade, joined by only *S. arculata* as the exception in having no apparent wing pattern dimorphism. The remaining *Strauzia* species (*S. perfecta, S. intermedia, S. verbesinae, S. gigantei, S. vittigera,* and the undescribed "*strumosus* Fly" collected from *H. strumosus*) lacked obvious sexual dimorphism in wing pattern.

Wing shape also generally differs between male and female flies, with male wings across all species relatively elongate compared with female wings. However, among measured species that do not share hosts with other *Strauzia*, only *S. perfecta* and the "*strumosus* fly" showed significant male-female wing shape differences. Meanwhile, five of six host-sharing *Strauzia* species showed significant male-female wing shape differences and had no overlap in PCA space between male and female wings. Only *S. arculata* co-occurs on a plant with other *Strauzia* species and had overlap in PCA space between males and females (Table 1, Fig. 3).

Male wing shapes across the entire *Strauzia* phylogeny did not have evidence of significant phylogenetic signal ($K_{mult} = 0.863$; P-value = 0.151) (Supplemental Fig. 4). When we applied this analysis to include only *Strauzia* that share plant hosts, we again found no significant

Table 2

Table summarizing wing pattern and mate finding behavior traits for congeneric Tephritidae that share a single plant host, with specialists (S) and generalists (G) indicated next to species names. *Aciurina* species marked with * are putative specialists – they have occasionally been noted as having other hosts, but these records are unconfirmed, and we consider them questionable. Species with uncertain host ranges do not affect overall trends.

Genus	Host plant	Relevant species S = specialist G = generalist	Species with particularly divergent wing patterns	Is the divergent wing pattern found in only one sex?	Notes on wings	Biology/Life history
Aciurina	*Ericameria nauseosa* (Rubber rabbitbrush)[Ψ]	*A. bigeloviae* (S)* *A. maculata* (S)* *A. notata* (S) *A. opaca* (S) *A. trilitura* (S) *A. trixa* (S)*	*A. notata* *A. bigeloviae*	No	All six species can be distinguished from one another based on wing pattern, with *A. notata* wings being most different from others (hyaline with a few thin dark patches along veins). *Aciurina bigeloviae* wings have a high degree of intraspecific variation (Steyskal 1984).	Males and females both walk on stems and leaves of host plant. When males see a female, they pursue (Dodson 1987).
Aciurina	*Chrysothamnus viscidiflorus* (Yellow rabbitbrush)	*A. ferruginea* (S)* *A. idahoensis* (S) *A. lutea* (S)* *A. michaeli* (S) *A. semilucida* (S)	*A. idahoensis* *A. semilucida*	No, but see notes next column	Both *A. idahoensis* and *A. semilucida* are unusual among *Aciurina* in having most of the wing surface hyaline with a few dark transverse bands (Goeden and Teerink 1996a,b). Though all five species on this host have some sexual dimorphism in wing patterns, both sexes of *A. idahoensis* and *A. semilucida* have wings that differ from the more common *Aciurina* wing pattern.[§]	Males and females both walk on stems and leaves of host plant. When males see a female, they pursue (Dodson 1987).
Eutreta	*Artemisia tridentata* (big sagebrush)	*E. divisa* (S) *E. oregona* (S) *E. diana* (G)	*E. divisa* (males)	Yes	While most male and female *Eutreta* have dark wings with small to tiny hyaline spots, male *E. divisa* wings have two diagonal hyaline stripes not seen on the wings of any other *Eutreta* flies, including conspecific females.	Males hold territories and "fight" with wing displays. Females enter territories for mating (Benbow 1978).
Strauzia	*Helianthus tuberosus* (Jerusalem artichoke)	*S. longipennis* (G) *S. longitudinalis* (S) *S. vittigera* (S)	*S. longitudinalis* (males)	Yes	Male *S. longitudinalis* wings have "coalesced" bands of dark color across the length of the wing (Hippee et al. 2021; Fig. 3 this paper).	Males hold territories on leaves. "*Wing and body movements may be involved in attracting the female.*" (Stoltzfus 1988)
Strauzia	*Helianthus grosseserratus* (sawtooth sunflower)	*S. arculata* (S) "Bush's Fly" (S) *S. noctipennis* (S)	"Bush's Fly" (males) *S. noctipennis* (males)	Yes	Wings of male of "Bush's Fly" and *S. noctipennis* both have "coalesced" wing patterns (Hippee et al. 2021; Fig. 3 this paper).	Males hold territories on leaves. "*Wing and body movements may be involved in attracting the female.*" (Stoltzfus 1988)
Valentibulla	*Ericameria nauseosa* (Rubber rabbitbrush)[Ψ]	*V. californica* (S) *V. dodsoni* (S) *V. steyskali* (S)	*V. dodsoni*	No	*Valentibulla dodsoni* wings are "*the most distinctive in the genus*" (Foote et al. 1993), with the more usual hyaline spots coalesced into a large hyaline patch across much of the posterobasal quadrant.	Males and females both walk on stems and leaves of host plant looking for mates (Wangberg 1978).

[Ψ] We note that the taxonomy of Ericameria nauseosa is uncertain, and that it may be a complex of different host plants rather than a single species, which could mean that some of these congeneric fly species do not directly interact on the same plant.

[§] There appears to be some geographic variation in the degree of sexual dimorphism in these flies, though wings of both sexes generally differ from the "usual" Aciurina wing. Differences between male and female wing patterns in both A. idahoensis and A. semilucida are more pronounced in California populations than in Idaho populations (Goeden and Teerink 1996a, b). Only S. semilucida females from California approach the groundplan for genus Aciurina (Goeden and Teerink 1996a).

evidence of a phylogenetic signal (K_{mult} = 0.783; P-value=0.302) (Supplemental Fig. 5). However, male wing shapes among the *Strauzia* that do not share host plants had significant evidence of phylogenetic signal and wing shape was more similar than expected when compared to a simulated Brownian motion model of evolution (K_{mult} = 1.218; P-value = 0.024) (Supplemental Fig. 6). Rates of wing shape change differed significantly between non-sharing species (1.11×10^{-3}) and *H. grosseserratus* sharing species (3.65×10^{-3}; P-value = 0.021) but did not differ significantly between non-sharing species and *H. tuberosus* sharing species (8.28×10^{-4}; P-value > 0.05).

Host sharing and sexual dimorphism in other tephritid flies

In our review of the US and Canadian tephritid genera, we found only three additional genera (*Aciurina, Eutreta*, and *Valentibulla*) with two or more specialists listed as sharing the same host plant. *Aciurina* and *Valentibulla* are closely related genera (Foote et al. 1993; Korneyev et al. 2005) and thus could be treated as one clade for comparison with *Strauzia*. In *Aciurina*, as in *Strauzia*, two sets of congeners shared two different host plants, such that we identify just six cases of a plant species with multiple specialist congeneric tephritid fly associates (Table 2). In all six cases, one or more of the specialist fly species had wing patterns described by other authors as being unusual for that fly genus, either in both sexes, or only in the males (and see below).

Across these four genera, flies differed in their reported mate-finding behavior and in whether or not divergent / unusual wing patterns occurred in one or both sexes. In *Eutreta*, as in *Strauzia*, males stake out territories on leaves while females fly or walk about the plant in search of males (Benbow 1978). And as in *Strauzia, Eutreta* that co-occurred on the same plants alongside congeners had a species (*Eutreta divisa*) with sexually dimorphic wings, with males having the wing pattern unusual for that genus – males, and not females, of *E. divisa* have two diagonal hyaline stripes interrupting the otherwise primarily dark wing (Table 2). In *Aciurina* and *Valentibulla*, both male and female flies are described as walking along stems and leaves, with mating occurring when they encounter one another (Wangberg 1978; Dodson 1987). Some host-sharing congeners in these two genera also had wings that differed from each genus' wing groundplan, but these divergent patterns occurred in both sexes, even though sexual dimorphism within the divergent pattern was sometimes evident (Goeden and Teerink 1996a, b).

Discussion

Our collective results refine our understanding of the evolution of wing shape and pattern. They underscore the importance of species interactions in morphological evolution, but also how differences in mating behavior may change the selective landscape and result in different outcomes for males versus females. We discuss our findings first in the context of *Strauzia* alone and then approach a synthesis by incorporating our review of other tephritids.

Major sexual dimorphism in wing patterns occurred in one internal clade of *Strauzia* that included five of the six species that share host plants. Only *S. vittigera* share a host with other *Strauzia* and are outside of this clade. Four of the six species with strongly coalesced or darkened male wing patterns occur on either *H. tuberosus* (*S. longitudinalis, S. longipennis*) or *H. grosseserratus* (*S. noctipennis*, "Bush's Fly") alongside other specialist *Strauzia*. The other two species with darkened or coalecesed male wings (*S. rugosum and S. uvedaliae*) did not share hosts but were also in this clade suggesting common ancestry of male wing pattern dimorphism. If we infer an ancestral state in *Strauzia* of males having standard "F" patterned wings, then coalesced/darkened male wing patterns either evolved once and were lost in *S. arculata* and *S. longipennis* males, or evolved two or more times independently. In either case, a consequence of the evolution of wing pattern dimorphism is that male flies that share hosts differ from one another in their respective wing patterns.

Sexual dimorphism in wing shape was found across the phylogeny but was most pronounced in host plant-sharing species. All host-sharing males differed from each other in wing shape (Supplemental Table 7), and male wing shape in host-sharing males did not have a significant phylogenetic signal, which may indicate that alternative factors, including ecological interactions, are driving differences in morphology among host-sharing species (Kerschbaumer and Pfingstl 2021). At the same time, wing shape among non-host-sharing males was more similar than expected, indicating that wing shape is more conserved in the absence of host plant sharing (Supplemental Fig. 5). Taken alongside the evolution of wing patterns, wings of male *Strauzia* that share hosts differ from one another in *both* shape and pattern, and male wing shapes appear to be diverging faster than expected when they share *H. grosseserratus* than when they do not share plant hosts. Further, changes in wing shape and pattern may interact in *Strauzia* wings, as the wing cells that experienced the greatest change in shape were also those that had increased pigmentation.

Reproductive character displacement in the context of shared plant hosts could explain the evolution of novel male wing patterns and shapes in *Strauzia*. Two specific causes might drive such character displacement: a) avoidance of combat between interspecific males and/or b) avoidance of costly interspecific mating attempts. In many tephritids, males congregate at lekking sites (here, leaves) and engage in wing waving and head butting, resulting in one or more interacting males being driven away (Benelli et al. 2014). Male *Strauzia* have been observed to engage in these male-male battles and the elongated setae on heads of males may be related to male-male aggression (Wangberg 1978; Moulds 1977). Wing markings may also directly convey visual signals to rivals, and interspecific differences may help flies avoid conspecific battles, which may be beneficial despite the loss of potential fitness benefits associated with predation avoidance for tephritids with an "F" pattern wing (Greene et al. 1987; Mather et al. 1987; Whitman et al. 1988).

Alternatively, because female *Strauzia* search for territorial males waiting on plant leaves (Stoltzfus 1988), female choice is an important component of mating success and could drive character displacement. It can be costly to attempt mating or successfully hybridize with a different species due to time and energy wasted (Gröning and Hochkirch 2008; Kyogoku 2015; Greenway et al. 2021), the risk of physical damage or mortality during mating (Sota and Kubota 1998; Hosken and Stockley 2004), or the wasting of reproductive investment if hybrids are less fit (Coyne and Orr 2004; Egan and Funk 2009; Matsubayashi 2010). Though pheromone signals are important for finding mates in many tephritids (Benelli et al. 2014), wing markings and wing movements are known to be important at close range (Sivinski et al. 1999; Benelli et al. 2014), and visual signals are the primary long-range attractant in some genera (Prokopy et al. 1971). Our data alone do not favor one hypothesis over the other for *Strauzia* (though see discussion regarding the broader patterns in the North American Tephritidae below).

While reproductive character displacement seems to best fit patterns in *Strauzia*, the lack of phylogenetic independence among species and the potential for morphological variation to be influenced by multiple evolutionary forces, such as sexual selection (Pfennig 1998; Pfennig and Pfennig 2009) and genetic drift (Lande 1976) over the course of evolutionary history, allows for alternative explanations for the correlation between wing patterns and host sharing. And indeed, perhaps belying the idea that wing pattern dimorphism is a result of reproductive character displacement, *Strauzia rugosum* (which has a fully coalesced male wing pattern) and *S. uvedaliae* (which has a darkened apical "F" in its male wing pattern) both have modified male wings but do not share hosts with other *Strauzia*. However, the *Strauzia* phylogeny makes clear that sexually dimorphic wing patterns are not phylogenetically independent (Fig. 3). The evolution of differences in wing pattern appears to have occurred either once, with one subsequent loss in the branch leading to *S. arculata*, or twice (or more), with potential origins in the

respective ancestors of the *S. noctipennis/S. longitudinalis* and the *S. uvedaliae/"Bush's Fly"/S. longipennis/S. rugosum* clades. Whichever the case, *S. rugosum* and *S. uvedaliae* are embedded in a clade for which the common ancestor probably had a sexually dimorphic wing pattern. Thus, these species may not be exceptions to a rule, but instead represent lineages that moved to new host plants after wing pattern dimorphism had already evolved.

When considered alongside our survey of wing patterning and mating behavior for the North American Tephritidae, the reproductive character displacement hypothesis is hard to replace with another. First, the rarity of host sharing among specialist tephritids – we find this in only three other genera (twice in genus *Aciurina*, just as in *Strauzia*) – suggests that use of the same hosts may be usually disfavored. Second, in all six cases where two or more specialist congeners do share the same host plant, at least one species on the shared host plant has wing pattern differences in one or both sexes that is unusual for the genus (Table 2). For instance, Foote et al. (1993) describe *Valentibulla dodsoni* as having wings that are "the most distinctive in the genus", while in Steyskal's (1984) description of *Aciurina idahoensis* he notes the "…very characteristic pattern of the wing… readily distinguishes this species from any other." Wing shape follows a similar pattern where *Strauzia* that do not share hosts tend to have wings that are phenotypically more similar to each other than expected, indicating that the wing shape is strongly conserved unless *Strauzia* are sharing hosts and wing shape becomes more different than expected by their evolutionary history. Host sharing being consistently correlated with wing morphologies that diverge from a presumed original state supports a general hypothesis for reproductive character displacement driving changes in tephritid wing patterns.

Further, mate-finding behaviors across our (admittedly small) sample of tephritids correlate with sexually dimorphic versus monomorphic wing morphologies. One of the non-*Strauzia* genera in Table 2 – *Eutreta* – includes a species with sexually dimorphic wing patterns like we see in *Strauzia*. While females of *Eutreta divisa* have wings much like other flies in the genus, male *E. divisa* flies have two diagonal white stripes not seen on the wings of any female *Eutreta* species (Foote et al. 1993). *Eutreta* also share a behavioral similarity with *Strauzia*: males hold territories on leaves while females fly to search for mates (Stoltzfus 1974). In *Valentibulla* and *Aciurina*, by contrast, both males and females walk around the surface of the host plants searching for mates (Goeden and Teerink 1997), and when host sharing flies in these genera have divergent wing patterns, those patterns are seen in both sexes (though some sexual dimorphism may still be present). Selection against mating with other congeneric species may therefore favor more extreme wing pattern changes in both sexes when males and females both search for mates, while the same dramatic wing pattern changes may occur just in males when only the female sex is actively searching. These broader patterns of wing pattern evolution in the Tephritidae place more weight on the idea that female choice, not male aggression, drives dimorphic patterns in *Strauzia*, as there are no records of male-male (or, importantly, female-female) aggressive behaviors in *Valentibulla* or *Aciurina*). One other species in this genus, *Eutreta fenestrata*, also shows striking male-female wing pattern dimorphism (female as *E. modocorum* and male as *Metatephritis fenestrata* in Foote et al. 1993), but we do not know if it shares hosts with congeners (or if its ancestors did); its only reported host, *Artemisia nova*, is shared with the non-specialist *Eutreta diana*. Finally, outside of North America in the *Bactrocera tau* complex in Thailand, host plant association and host plant sharing among two species in the *B. tau* complex was shown to be associated with male wing shape differences (Kitthawee and Dujardin 2010), highlighting the potential that (rare) host plant sharing in tephritids is broadly associated with the emergence of wing dimorphism.

A formal test of the specific hypothesis that morphological character displacement evolves when congeners share hosts requires directly measuring selection – possibly via experimental manipulation of fly wings (e.g., Sivinski and Pereira 2005; Menezes et al. 2013). There may also be a role for other forms of reproductive isolation to influence wing

pattern differences in concert with reproductive character displacement. Previous work on *H. tuberosus*-associated *Strauzia* found evidence of temporal isolation (Hippee et al. 2016), whereas the three *H. grosseserratus*-associated *Strauzia* flies appear to have greater overlap in phenology (ACH, personal observation). More temporal overlap in the *H. grosseserratus*-associated flies might select more strongly for character displacement, such as wing pattern differences, that reinforce species boundaries. Future work should also consider these and other reproductive barriers that may be present when multiple species are sharing the same host.

Finally, a chicken or egg problem: do major changes in fruit fly wing patterns result from host sharing, or do they facilitate shifts to already-occupied hosts? Our results suggest both may be true. While we argue that our findings suggest reproductive character displacement as a driver of changes in wing pattern for *Strauzia* and other true fruit flies, morphological differences could instead represent exaptations, facilitating host sharing after morphological differences had already evolved. In both *Aciurina* and *Strauzia*, two different plant species are host to more than one congener (Table 2). The scarcity of host sharing by specialists in most tephritid genera, juxtaposed against it occurring twice in both of these morphologically diverse genera, suggest that once new wing morphologies evolve in a fly lineage, it may be easier for those flies to shift to plant hosts that already host a congener. Further testing of this hypothesis in other groups of specialist insects that share hosts is necessary to evaluate the ways in which morphological evolution is impacted by interspecific interactions.

CRediT authorship contribution statement

Alaine C. Hippee: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Marc A. Beer:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Allen L. Norrbom:** Conceptualization, Writing – original draft, Writing – review & editing. **Andrew A. Forbes:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of competing interest

Data availability

All supporting data are available on Dryad: https://doi.org/10.5061/dryad.k6djh9w90 Explaining dimorphism polymorphism: stronger interspecific sexual differences may be favored when females search for mates in the presence of congeners (Original data) (Dryad).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2024.100084.

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