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Introduction

Phytophagous insects make up more than a third of all described organisms on Earth (Strong et al., 1984; Wilson, 1992). Although many attempts to explain this extraordinary diversification focus on patterns of host use (Ehrlich & Raven, 1964; Mitter et al., 1991; Novotny et al., 2006; Nyman et al., 2006), recent evidence suggests that the most rapid rates of evolution are caused by sexual selection (Arnqvist et al., 2000; Mendelson & Shaw, 2005). In many insects, mate choice follows elaborate sexually-selected courtship displays (Thornhill & Alcock, 1983; Choe & Crespi, 1997). Among insects that court and mate exclusively on their hosts, both shifts in host use and sexual selection on courtship displays can promote divergence of host-specific populations (Faust & Brown, 1998; Rodriguez et al., 2004; Cocroft & Rodriguez, 2005). Courtship displays can be directly affected by host plants: vibrational courtship signals can be transmitted differently by the same tissues of different plants, or by different tissues of the same plant (Sattman & Cocroft, 2003; Cocroft et al., 2006). Thus, courtship behaviours could serve as important reproductive isolating mechanisms. Two sets of hypotheses were tested: (i) species differ in wing shape and wing size; and (ii) species are sexually dimorphic in wing size and wing shape. Wing size differed among a few species, but wing shape differed significantly among all six species. Sexual dimorphism in wing size was found in only one species, but sexual dimorphism in wing shape was found in two of the three species known to court on the same host plant. In the two sexually dimorphic species, wing shape differed among males, but not among females. This suggests that selection for reproductive character displacement might accelerate divergence in wing shape.

Key words. Courtship, Diptera, flies, host specificity, neotropics, sexual selection, speciation, Tephritidae, tropical diversity, wing shape.

Six cryptic species on a single species of host plant: morphometric evidence for possible reproductive character displacement

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Abstract. 1. Diversification of some highly host-specific herbivorous insects may occur in allopatry, without shifts in host use. Such allopatric divergence may be accelerated by sexual selection operating on courtship displays. Wing size and shape may affect visual and vibrational courtship displays in tephritid fruit flies. Geometric morphometric methods were used to examine wings of six sympatric cryptic species in the neotropical genus Blepharoneura. All six species feed on flowers of the same species of host (Gurania spinulosa), a neotropical vine in the Cucurbitaceae. Three of the fly species court and mate in close proximity on the host. Thus, courtship behaviours could serve as important reproductive isolating mechanisms. Two sets of hypotheses were tested: (i) species differ in wing shape and wing size; and (ii) species are sexually dimorphic in wing size and wing shape. Wing size differed among a few species, but wing shape differed significantly among all six species. Sexual dimorphism in wing size was found in only one species, but sexual dimorphism in wing shape was found in two of the three species known to court on the same host plant. In the two sexually dimorphic species, wing shape differed among males, but not among females. This suggests that selection for reproductive character displacement might accelerate divergence in wing shape.

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as sites for egg laying and courtship and mating. In another te-
phritid genus, *Blepharoneura*, diversification may involve shifts
in use of both host taxa and host parts (Condon & Steck, 1997).
However, much diversification in *Blepharoneura* may occur in
allopatri without any host shifts, perhaps accelerated by sexual
selection (Condon et al., 2008a, b).

Courtship displays of *Blepharoneura* are elaborate and often
occur on the surfaces of host plants (Condon & Norrbom, 1999;
Condon et al., 2008a). Some displays of *Blepharoneura* involve
exremely rapid wing motions that could cause vibrations that
could be transmitted by plant tissue. Other displays involve
slower wing motions during which wings of males are rotated
and outstretched while males face females. In some species,
males display in leks on the surfaces of leaves of the host plants
where adult females often graze (Condon & Norrbom, 1994,
1999). In these situations, sexual selection could cause rapid
evolution of male display characters and accelerate allopatric
speciation (Mendelson & Shaw, 2005). In species that occur
sympatrically on the same host, selection for reproductive char-
acter displacement may cause further divergence of courtship
displays.

Multiple sympatric cryptic species of *Blepharoneura* often
infest single species of sexually dimorphic and functionally dio-
ecious host plants (Condon & Norrbom, 1994; Condon & Steck,
1997). In eastern Ecuador, six sympatric cryptic species of
*Blepharoneura* infest calyces of flowers of a single species of
host plant – *Gurania spinulosa* Cogn. (Condon et al., 2008a, b).
Two out of the six species lay eggs exclusively on female flow-
ers, a third lays eggs on both male and female flowers, and the
remaining three species are specialists on male flowers.

Although courtship of species feeding (as larvae) on female
flowers has not been observed in the field, the courtship of all
three male-flower specific species has been observed in the field
on a single individual host plant bearing male flowers (Condon
et al., 2008a, b). Species infesting *G. spinulosa* Flowers are almost
indistinguishable morphologically; however, substantial in-
ter-specific differences (7–10%) in nucleotide sequence in the
mitochondrial gene cytochrome oxidase subunit I clearly
distinguish species (Condon et al., 2008a, b). If species that
court and mate on the same host surfaces use similar cues during
courtship, inter-specific matings – even among distantly related
species – could occur. If fitness of hybrids is low (or if post-mating
isolation is complete), there should be strong selection for
reproductive character displacement (Coyne & Orr, 2004), par-
ticularly in species encountering similar species on the same
host plants. Indeed, courtship displays of these sympatric species
of *Blepharoneura* differ noticeably in speed, form, and tempo
(Condon et al., 2008a).

Courtship displays involving elaborate movements of wings
bearing intricate pigmentation patterns, could involve visual
cues (Edwards et al., 2007), which may be affected by wing
shape or size. Displays involving vibrational signals may also
be affected by wing size and shape (Sivinski & Dodson, 1992).
If wing shape affects displays, shape should differ among these
sympatric species, especially among species that court and mate
on the same surfaces. Sexual selection can accelerate evolution
and is associated with speciation in diverse groups (Andersson,
1994; Mendelson & Shaw, 2005) including remarkably species-
rich groups such as the Hawaiian *Drosophila* (Kaneshiro, 1988),
which – like *Blepharoneura* – engage in elaborate courtship dis-
plays and have elaborately patterned wings.

Significant sexual dimorphism is often a signature of sexual
selection (Andersson, 1994). If selection for reproductive char-
acter displacement also occurs, selection could favour diver-
gence of sexually selected characters in species that court and
mate in the same locations. If wing shape affects male displays
in *Blepharoneura*, species that court and mate in the same loca-
tions should differ in wing shape. To determine whether symp-
atic cryptic species of *Blepharoneura* in Ecuador differ in
wing shape or size, landmark-based geometric morphometric
methods (Rohlf & Marcus, 1993; Adams et al., 2004) were used
to test two sets of hypotheses: (i) species differ in wing shape
and wing size, and (ii) species are sexually dimorphic in wing
size and wing shape.

**Methods**

Wings of voucher specimens of six species of *Blepharoneura*
identified through phylogenetic analysis of the sequence of cy-
tochrome oxidase subunit I (COI), a mitochondrial gene (Condon
et al., 2008a, b) were examined. Species status of these
lineages has been corroborated through analysis of two nuclear
genes – EF1-α and CAD (Condon et al., 2008b). All specimens
were collected within a radius of 8 km from the Jatun Sacha
Biological Station, which is located near Misahuallí in the Napo
province of eastern Ecuador: 01°03.941S, 77°36.998W (Condon
et al., 2008a, b). For wing shape analyses, only specimens
reared from mature flowers were included, because flies com-
plete their development in mature flowers (not immature flow-
ers) in nature. All of these are newly discovered and undescribed
species (without scientific names). Thus, these lineages have ei-
ther been given monikers related to the species’ sites and styles
of courtship display (e.g. clapleaf, shiverer) or have been la-
belled with the specific epithets of closely-related species (e.g.
amatonia, perkinsi) (Table 1).

To prepare slide-mounted wings, the right wing of each fly
was removed. Wings were boiled in a dilute potassium hydrox-
ide (KOH) solution, rinsed, and mounted in Euparal on a glass
slide. As Euparal-mounted wings are permanently mounted, we
could not take multiple repeated measurements to estimate the
error associated with variation in compression and flattening of
the wings. If the right wing was damaged, the left wing was
used: preliminary analyses showed no significant difference in
shape between left and right wings (D. C. Adams, unpubl. data).
Images were captured with a FUJI FinePix S2 Pro digital cam-
era (resolution 4256 × 2848) mounted on a WILD3Z dissecting
microscope.

Wing shape was quantified using landmark-based geometric
morphometric methods (Rohlf & Marcus, 1993; Adams et al.,
2004). These methods allow a rigorous quantification of wing
shape after the effects of non-shape variation have been mathe-
matically held constant. For this approach, a series of biological
landmarks are identified on each wing mount image, and are
used for the subsequent quantification of shape (Fig. 1). First,
the x, y coordinates of 14 landmarks were digitised on wing

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mount images, using tpsDIG (Rohlf, 2004a). Next, the landmark coordinates for all specimens were aligned using a generalised Procrustes analysis (GPA; Rohlf & Slice, 1990), which scales, centres, and rotates landmark configurations using a least squares criterion. After GPA, the resulting configurations are invariant with respect to size, position, and orientation (i.e. they only vary by shape). From the aligned specimens, shape variables were then generated as partial warp scores from the thin-plate spline (Bookstein, 1991) and the standard uniform components (Rohlf & Bookstein, 2003). These variables can be used in analyses of shape variation or covariation with other variables (e.g. Adams, 2004; Adams et al., 2007). Principal component analysis (PCA) was used to visualise shape variation among groups. GPA, TPS, and PCA were performed with tpsRELW (Rohlf, 2004b).

Using the above procedure, wing shapes of 104 individual specimens were quantified. A permutation method was used to test for differences among species-sex groups for both wing size and shape. Wing size was measured as centroid size (CS), which is calculated as the square root of summed squared distances between landmarks of a specimen and its centroid (Bookstein, 1991). Shape was defined by the 2k – 4 shape variables possible from k landmarks (in this case, 14 landmarks leads to 24 shape variables; see Adams et al., 2004). Average CS and shape were calculated for the 12 observed species-sex groups. Individual values were subsequently randomly assigned to the 12 possible groups and average values were calculated for 9999 random permutations. In every random permutation, plus the observed case, the Euclidean distances (D) among means (for CS, this is equivalent to the absolute differences between means) were calculated. The significance of observed values was ascertained as the empirical probability of finding an equal or greater value from distributions of 10000 random values. Any observed value greater than the value of the 95th percentile (i.e. P < 0.05) from the random distribution was considered to be indicative of a significant difference. Finally, to assess specific differences in sexual dimorphism, the shape differences between males and females were expressed as a vector, and the angular difference in orientation between sexual dimorphism vectors for pairs of species was calculated (for details see Collyer & Adams, 2007).

Results

Wing size revealed a few inter-specific differences and only one instance of sexual dimorphism. Significant differences were found between two of the four species that infest male flowers: wings of clapleaf females were significantly larger than shiverer females (CS\text{clapleaf} = 6.49; CS\text{shiverer} = 5.73; P = 0.0017), and males of these two species showed a similar pattern (CS\text{clapleaf} = 6.24; CS\text{shiverer} = 5.48; P = 0.0048). Wings of males of male-flower-specific clapleaf were also significantly larger than wings of males of female-flower-specific perkinsi (CS\text{perkinsi} = 5.43; P = 0.0010). Among the three species infesting female flowers, flatclap males had significantly larger wings than males of either of the other two species (shiverer or perkinsi) (CS\text{flatclap} = 6.42; P = 0.0016 and P = 0.0006 respectively). When sexual dimorphism within species was examined, only one species, perkinsi, exhibited significant wing size dimorphism, with females having larger wings than males

Table 1. Six sympatric species of Blepharoneura feed as larvae on flowers of Gurania spinulosa in eastern Ecuador. Courtship display location determined through observation of a single individual male Gurania spinulosa (Condon et al., 2008a). Clades are identified by letters used to label clades revealed through phylogenetic analysis of mtDNA COI sequence (Condon et al., 2008a) and by numbers denoting the same clades revealed through phylogenetic analysis of mtDNA COI and nuclear genes EF1-α and CAD (Condon et al., 2008b).

<table>
<thead>
<tr>
<th>Moniker</th>
<th>Larval host part</th>
<th>Courtship display location (on male G. spinulosa)</th>
<th>Clade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomaria</td>
<td>Male flowers</td>
<td>Male inflorescence and leaves</td>
<td>A, sp4</td>
</tr>
<tr>
<td>Clapleaf</td>
<td>Male flowers</td>
<td>Leaf-distinctive display</td>
<td>B, sp8</td>
</tr>
<tr>
<td>Flatclap</td>
<td>Female flowers</td>
<td>Unknown (not on male plant)</td>
<td>E, sp11</td>
</tr>
<tr>
<td>Perkinsi</td>
<td>Female flowers</td>
<td>Unknown (not on male plant)</td>
<td>F, sp10</td>
</tr>
<tr>
<td>Shimmyleaf</td>
<td>Male flowers</td>
<td>Leaf-distinctive display</td>
<td>D, sp12</td>
</tr>
<tr>
<td>Shiverer</td>
<td>Male and female flowers</td>
<td>Unknown (reared from plant where behaviors observed, but no courting individuals discovered)</td>
<td>C, sp30</td>
</tr>
</tbody>
</table>

Fig. 1. Image of wing used for collection of morphometric data. The 14 landmarks used in this study are shown as open dots. Relevant wing cells are labelled (in boxes), and wing veins are labelled with arrows. Wing spots useful in identifying these species are also labelled (numbers).
Morphometric analysis of wing shape

When wing shape was examined, even more interesting patterns were exposed. For instance, between-species comparisons revealed considerable species-specific wing shape differences. For males, all 15 pairwise comparisons between species were significant, implying that each species is characterised by a distinctive male wing shape (Table 2). In addition, 13 out of 15 comparisons between females were significant (Table 2). The only inter-specific comparisons of female wing shape that were not significant were atomaria versus clapleaf (both of which infest male flowers), and flatclap versus perkinsi (both of which infest female flowers).

When patterns of wing shape variation within species were examined, most species did not exhibit significant sexual dimorphism. However, significant sexual dimorphism in wing shape was revealed for two species: clapleaf ($D_{M,F} = 0.0217; P = 0.0029$) andatomaria ($D_{M,F} = 0.0151; P = 0.0372$), neither of which was sexually dimorphic in wing size. Interestingly, females of these species were not different in wing shape, but males did differ in wing shape (Table 2). Therefore, not only did these species exhibit significant sexual dimorphism, but they also had differing patterns of sexual dimorphism, where males became more divergent relative to females.

A PCA on all specimens was performed to visualise patterns of shape variation (i.e. relative warps analysis). When species × sex wing shape means were plotted along the first two principal components of shape, statistical patterns were visually confirmed (Fig. 2). When viewed along PC1, wing shapes varied from relatively elongated wings towards the positive side of PC1, to relatively compressed wings towards the negative side of PC1. In addition, changes along PC1 corresponded to changes in the shape of wing cell $r_{4+5}^2$ (Fig. 2). Species-specific differences aligned mostly along PC1 (Fig. 2), where perkinsi and flatclap exhibited relatively longer wings than the remaining four species. The exception to this pattern was shimmyleaf, which was differentiated from the remaining species along PC2. Wing shapes of this species appeared relatively shorter in the basal radial (br) wing cell. Shimmyleaf is one of the three species that have been observed courting and mating on their larval host, and the sixth species (shiverer) feeds on both male and female flowers. All three of the species specific to male flowers court and mate on their larval host, and have even been seen courting and mating on the same individual host plant as shimmyleaf (Condon et al., 2008a).

To quantify the differences in sexual dimorphism between atomaria and clapleaf, the angle between sexual dimorphism vectors of atomaria and clapleaf was calculated. Their orientation differed by 60.5°, conforming the divergent patterns of sexual dimorphism in these species (atomaria versus clapleaf). When viewed as deformation grids (Fig. 3), the difference between males in these sexually dimorphic species was most evident in the central region of the wing, where the distance between $r$–$m$ and $Rs$ to $bm$–$cu$ is longer in clapleaf relative to atomaria. The two also differ noticeably in the relative lengths of $r_{4+5}$ (atomaria is longer) and in the shape of $r_{3+4}$. In clapleaf, $r_{3+4}$ is distinctly triangular and broader at the distal edge of the wing than atomaria, which has a bend in the proximal portion of the intersection with $r$–$m$.

**Discussion**

Significant inter-specific differences in wing shape of males were found among all six sympatric cryptic species of *Blepharoneura* that infest flowers of the same host species in eastern Ecuador. Three of the species feed exclusively on male flowers (atomaria, clapleaf, shimmyleaf), two feed exclusively on female flowers (perkinsi, flatclap), and the sixth (shiverer) feeds on both male and female flowers. All three of the species specific to male flowers court and mate on their larval host, and have even been seen courting and mating on the same individual host (Condon et al., 2008a). Given such extreme spatial and temporal overlap in the locations of courtship arenas, it is likely that courtship displays function as important reproductive isolating mechanisms, and that wing shape may evolve in response to selection on courtship displays.

<table>
<thead>
<tr>
<th></th>
<th>Atomaria</th>
<th>Clapleaf</th>
<th>Flatclap</th>
<th>Perkinsi</th>
<th>Shimmyleaf</th>
<th>Shiverer</th>
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<td>Atomaria</td>
<td>--------</td>
<td>0.0245</td>
<td>0.0217</td>
<td>0.0270</td>
<td>0.0263</td>
<td>0.0199</td>
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<tr>
<td>Clapleaf</td>
<td><strong>0.0135</strong></td>
<td>--------</td>
<td>0.0277</td>
<td>0.0312</td>
<td>0.0358</td>
<td>0.0256</td>
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<tr>
<td>Flatclap</td>
<td>0.0281</td>
<td>0.0290</td>
<td>--------</td>
<td>0.0210</td>
<td>0.0317</td>
<td>0.0254</td>
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<tr>
<td>Perkinsi</td>
<td>0.0243</td>
<td>0.0271</td>
<td><strong>0.0218</strong></td>
<td>--------</td>
<td>0.0413</td>
<td>0.0294</td>
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<tr>
<td>Shimmyleaf</td>
<td>0.0297</td>
<td>0.0350</td>
<td>0.0428</td>
<td>0.0427</td>
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<td>0.0385</td>
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<tr>
<td>Shiverer</td>
<td>0.0208</td>
<td>0.0187</td>
<td>0.0268</td>
<td>0.0327</td>
<td>0.0415</td>
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<tr>
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<td>0.0428</td>
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</tr>
<tr>
<td>Shiverer</td>
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<td>0.0187</td>
<td>0.0268</td>
<td>0.0327</td>
<td>0.0415</td>
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Courtship behaviours of many tephritids involve either elaborate wing displays (Headrick & Goeden, 1994), acoustic vibrational signals (Webb et al., 1983, 1984; Sivinski et al., 1984; Sivinski & Webb, 1985; Alonso-Pimentel et al., 2000) or both (Sivinski et al., 1999). Courtship displays of these species of Blepharoneura involve elaborate wing motions (Condon & Norrbom, 1999; Condon et al., 2008a). Most species incorporate a type of semaphoring, during which the wing is rotated and held outstretched (with the plane of the wing perpendicular to the substrate) while the male stands or paces in front of the female. Such displays may provide visual cues (e.g. wing spot patterns; Condon & Norrbom, 1994; Condon et al., 2008a) that could be affected by wing shape.

Other wing movements repeated during courtship displays of Blepharoneura are extremely rapid. To freeze wing motion, high-speed video requires a frame rate of 1000 fps with 1/4000 s shutter speed (M. Condon, unpubl. data). In all species that have been observed, these displays are performed while flies are standing on the surfaces of the plants. In some of the species (e.g. shiveringer), rapid wing movements during courtship are also accompanied by rhythmic contact between the abdomen and the wings. All these movements would generate vibrations that could travel through the plant tissue and be detected by potential mates (Cocroft & Rodriguez, 2005; Cocroft et al., 2006). Wing shape could affect the frequency and form of the signals used during courtship.

The finding that wing shape differs among males of all species, supports our hypothesis that wings – either through their involvement in visual displays or acoustic displays – are used in courtship, which functions as an important pre-mating reproductive isolating mechanism. As all the species in our study are sympatric, and oviposit in the same species of host, they may share a history of selection on characters affecting reproductive isolation. For most species, patterns of divergence in wing shape appear to be similar for both males and females: evidence of sexual dimorphism was not found within most species. Only one species (perkinsi) was sexually dimorphic in wing size: males are smaller than females, as in many other species of tephritids (Sivinski & Dodson, 1992). As courtship displays of perkinsi and the other two species infesting female flowers have not yet been observed in the field, the relationship between sexual dimorphism and selection for reproductive character displacement in these female-flower infesting species cannot currently be assessed.

Three of the species that infest male flowers are known to court and mate in the same location (Condon et al., 2008a). Sexual dimorphism in wing shape (but not wing size) was discovered in two of the three species that have been observed courting on the surface of the same individual host plant. Both atomaria and clapleaf are sexually dimorphic: shapes of males’ wings differ significantly, but shapes of females’ wings do not

Fig. 2. Principal components (PC) analysis of all specimens (i.e. relative warps). In plots, group means and convex hulls are displayed. In each plot, the first two PCs (accounting for 39.9% of the overall shape variation) are shown: PC1 = 21.16%, PC2 = 18.75%. Solid lines (a, b) and vectors (c) indicate species exhibiting significant sexual dimorphism. a and b) male and female means and convex hulls are illustrated, respectively. c) male (square) and female (circle) means are displayed connected by vectors representing sexual dimorphism. Sample sizes: atomaria (12♂, 9♀), clapleaf (9♂, 11♀), flatclap (6♂, 4♀), shiverer (9♂, 11♀), shimmyleaf (3♂, 2♀). Thin-plate spline deformation grids, shown to indicate patterns of variation in shape space, have been accentuated by a factor of three to enhance visual interpretation.
Morphometric analysis of wing shape

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differ. Molecular phylogenetic analyses show that atomaria and clapleaf are more closely related to each other than to the other species (shimmyleaf) that courts on male plants (Condon et al., 2008b). Furthermore, our morphometric analyses show that wing shapes of both males and females of shimmyleaf differ significantly from all other species. If wing shape affects courtship displays and mate choice, one would expect that selection for reproductive character displacement would be strongest between species with the most similar wing shapes, especially if those species display in courtship arenas that overlap in space (i.e. occur on leaves on the same individual plants).

Although these species are recently discovered and morphologically similar, available molecular evidence suggests that these six species diverged at least 3 million years ago, probably in allopatry, not in sympatry (Condon et al., 2008b). In isolated populations, sexual selection can lead to rapid evolution of distinctive courtship displays (Mendelson & Shaw, 2005). Thus, sexual selection can lead to rapid divergence of allopatric populations, without any changes in patterns of host use. Indeed, in Blepharoneura, patterns of host use appear to be highly conserved within lineages. If divergent populations rejoined (perhaps as a consequence of habitat expansion), two species that overlap in patterns of host use (and courtship arenas) would come into sympatry. Selection for reproductive character displacement could follow, and might be most intense on male characters affecting female choice. In such circumstances, sexual dimorphism would result if there is no strong genetic correlation between the sexes for the trait under selection.

Selection for reproductive character displacement is expected to be most intense on closely related species (such as atomaria and clapleaf) that court and mate in the same locations (e.g. in the same places on the same individual plants). Further, sexual dimorphism should be most pronounced where the two species are found in sympathy, and less pronounced in regions where the species do not overlap. These predictions suggest a direct link between behavioural displays exhibited by males of different species, the degree of wing shape dimorphism, and the extent of reproductive isolation. If these predictions are correct, they suggest an explicit model for Blepharoneura diversification. Sexual selection acts on differences in behavioural courtship signals, generates differential selective pressures on morphological characteristics of males, and results in morphological divergence in wing characteristics affecting courtship displays. Sexual selection on courtship displays would therefore accelerate lineage diversification and radiation. Thus, similar to models proposed to explain the stages of vertebrate adaptive radiations (e.g. Streelman & Danley, 2003), wing shape in Blepharoneura may serve as a template for understanding the complex interplay between courtship, sexual selection, and speciation in host-specific tropical insects.

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References


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