A Database of Wing Diversity in the Hawaiian Drosophila

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Background. Within genus Drosophila, the endemic Hawaiian species offer some of the most dramatic examples of morphological and behavioral evolution. The advent of the Drosophila grimshawi genome sequence permits genes of interest to be readily cloned from any of the hundreds of species of Hawaiian Drosophila, offering a powerful comparative approach to defining molecular mechanisms of species evolution. A key step in this process is to survey the Hawaiian flies for characters whose variation can be associated with specific candidate genes. The wings provide an attractive target for such studies: Wings are essentially two dimensional, and genes controlling wing shape, vein specification, pigment production, and pigment pattern evolution have all been identified in Drosophila. Methodology/Principal Findings. We present a photographic database of over 180 mounted, adult wings from 73 species of Hawaiian Drosophila. The image collection, available at FlyBase.org, includes 53 of the 112 known species of “picture wing” Drosophila, and several species from each of the other major Hawaiian groups, including the modified mouthparts, modified tarsus, antopocerus, and haleakalae (fungus feeder) groups. Direct image comparisons show that major wing shape changes can occur even between closely related species, and that pigment pattern elements can vary independently of each other. Among the 30 species closest to grimshawi, diverse visual effects are achieved by altering a basic pattern of seven wing spots. Finally, we document major pattern variations within species, which appear to result from reduced diffusion of pigment precursors through the wing blade. Conclusions/Significance. The database highlights the striking variation in size, shape, venation, and pigmentation in Hawaiian Drosophila, despite their generally low levels of DNA sequence divergence. In several independent lineages, highly complex patterns are derived from simple processes. These lineages offer a promising model system to study the evolution of complexity.

INTRODUCTION

Nearly 1000 species of Drosophila are endemic to Hawaii, yet current evidence suggests they arose from a single introduction to the Hawaiian Island chain roughly 26 million years ago [1–6]. The “picture wing” group consists of 112 known species, most of which are quite distinct from each other in morphology, pigmentation, and behavior, even when they are separated by ~0.5 million years of divergence (the age of the island of Hawaii [3,7]). This explosive adaptive radiation is now known to have occurred with relatively little change in DNA sequence [8–10]. These factors make the Hawaiian Drosophila an important model system for analysis of evolutionary processes at the species level.

The Drosophila grimshawi genome has been sequenced [11,12], providing a major new entry point into genomic and molecular genetic analyses of the Hawaiian flies. High levels of similarity to the grimshawi sequence should permit the amplification of nearly any gene of interest from a range Hawaiian species. Identified sequence differences can then be correlated with phenotypic variations among the species, providing insights into molecular mechanisms of evolution. To make the most of this opportunity, it is important for researchers to have access to uniformly collected phenotypic data from numerous species. The data can be used to identify characters that show interesting patterns of variation, and for which candidate genes can be hypothesized. The Drosophila wing is an attractive target for such candidate-based studies, since wing development has been analyzed in great detail in D. melanogaster [13–16], and genes controlling wing shape [17–19] and pigmentation [20–23] have been identified.

Wing pigment spots occur in highly reproducible, species-specific, two-dimensional patterns, and their genetics and development are beginning to be understood. True et al. [21] found that wing spot patterns have two main components: a vein-independent “prepattern” formed during wing development prior to eclosion, and vein-dependent melanization that forms after eclosion. In species such as grimshawi, the prepattern is faintly visible upon eclosion, marked by an arrangement of dark versus light wing hairs. In the first day or two after eclosion, pigment precursors travel through the wing veins and diffuse into the intervein regions, allowing further darkening of the cuticle into clearly visible spots. In this model, the spots must contain localized enzymes that are waiting to convert the precursors to melanins. This enzyme prepattern is most likely formed by localized expression of pigmentation genes in response to the wing’s basic patterning machinery. Wing spot evolution would then involve changes in either the upstream patterning genes, or the downstream pigmentation genes. Changes in patterning genes would tend to be pleiotropic, altering other features of the wing, so this explanation is unlikely when only pigment changes are observed. Thus, the favored explanation is that mutations occur in the cis-regulatory regions of the pigmentation genes, bringing them under control of existing, region-specific activators or repressors [23]. Such mutations could be very selective, altering only portions of the
original expression pattern. A related possibility is that a “dedicated” transcription factor controls one or more pigmentation genes, and this transcription factor is the target of regulatory mutations [24].

Studies of the yellow locus have provided multiple examples of regulatory mutations controlling the evolution of wing spots. The Yellow protein is required to pigment the cuticle, and ectopic Yellow causes dark pigmentation in a wild type background. This Yellow-dependent pigmentation is strongly enhanced by removal of Ebony protein (beta-alanyl-dopamine synthase) [22]. Yellow and Ebony promote separate branches of the pathway from dopa to variously colored pigments [23]. The yellow and ebony genes have been co-opted during evolution to produce wing spots: a male-specific wing spot in D. biarmipes is presaged by increased Yellow and decreased Ebony protein levels, and the extent of the spot is controlled in part by engrailed regulation of yellow via a novel cis-regulatory element [25]. The expression of Yellow protein in presumptive wing spots has been gained and lost multiple times in the evolution of genus Drosophila, and yellow has at least two distinct regulatory elements that can be co-opted to produce spots [26].

These studies provide the framework required to understand the evolution of complex pigment patterns in the Hawaiian Drosophila. Unfortunately, these pigment patterns have not been photographically documented in the literature, apart from a few sporadic examples (e.g., True et al. [21]). Intact flies have been photographed [27], but those pictures cannot be used for quantitative analysis or direct comparisons of wings between species. The original species descriptions (e.g. [28–32]) employ hand drawings, which are inherently limited in their ability to capture subtle variations in pigment color and density. These publications can also be difficult to obtain (though scanned versions can be found at the Japan Drosophila Database on Taxonomy, www.dgrc.kit.jp/~jdd). Here we present a photo database documenting the wings of 73 Hawaiian Drosophila species. Mounted wings were digitally photographed under uniform conditions to allow for comparisons between specimens, and the photos have been made available for download at FlyBase [33]. This collection highlights the astonishing diversity of the Hawaiian flies, first noted by Grimshaw in 1901 [34], and we hope it will inspire the fly community to leverage the grimshawi genome to gain further molecular insights into morphological evolution.

RESULTS AND DISCUSSION

The endemic Hawaiian Drosophila arose from an introduction of a continental species to an island (now subsided) that predates Kauai, the oldest of the current high islands [2]. These flies diversified into several major species groups; Fig. 1 provides an overview of the relationships among the groups discussed here.

Over 180 different wings were photographed from 73 species of field-caught or lab-reared Hawaiian Drosophila. The original photographs and the montages are available in the Hawaiian Drosophila Wing Database at FlyBase [33]. Table 1 lists all the species available in the image database. In many cases, the database includes multiple wings per species; in this paper, the single most intact wing from each species is shown (Figs. 2–7). When both male and female wings are available, and sexual dimorphism is apparent, both sexes are shown; the most dramatic cases of dimorphism occur in the adiastola subgroup of picture wing species (Fig. 2). We have attempted to organize the figures based on established species groupings: the photos tend to be arranged phylogenetically and thus are not alphabetical. The full species descriptions, phylogenies, and behavioral and ecological data have been previously reported and are beyond the scope of this paper [1,7,8,10,28–32,35–38].

The picture wing flies

We obtained specimens of 53 of the 112 species in the picture wing group, including representatives of all major lineages. Notably, the
US Federal endangered species list includes a total of just 51 insect species, and 11 of these are Hawaiian picture wing Drosophila. Five of these endangered species are included here: heteroneura, differens, and hemipeza (Fig. 3); aglais and montgomeryi (Fig. 4). All were collected prior to the endangered species designation.

The picture wing group is divided into four major subgroups named for representative species: adiastola (Fig. 2), planitibia (Fig. 3), glabriapex (Fig. 4) and grimshawi (Fig. 5). A nearly complete lineage of the picture wing flies was determined by H.L. Carson, who used polytene chromosome banding patterns to map chromosomal inversions in each species [1,39–41]. Carson’s inversion tree is highly congruent with molecular phylogenies of the picture wings [42]. In order to provide some phylogenetic context for comparing the wings, we have reproduced the chromosomal lineages of the species that are shown in each of the picture wing figures (insets in Figs. 2–5). D. grimshawi is the arbitrarily chosen chromosomal standard (+). Each box represents a unique inversion genotype or karyotype present in the designated species (abbreviated to 3 letters). Circles represent inversion genotypes that do not match any species in the database; these are only included when they constitute nodes in the tree. The actual inversion names have been omitted for simplicity; see Carson [40] for complete genotypes.

The chromosome map for a given species can be derived by adding all the inversions along the path to the standard, “+”. The ovals indicate three key sets of inversions, designated Xo 2c; Xik; and 4b; that uniquely define each picture wing subgroup. Specifically, the grimshawi subgroup lacks these inversions (since D. grimshawi is the standard); the glabriapex subgroup has 4b, the planitibia subgroup has Xik and 4b, and the adiastola and primavea subgroups have Xo 2c, Xik, and 4b. Relationships among the four subgroups can be obtained by connecting the trees at these points, as summarized in Fig. 1. Note that branch lengths are arbitrary, since the number of inversions is not necessarily proportional to the time since divergence. Chromosomal trees are also inherently
unrooted; this tree is rooted at *primaeva* based on DNA and biogeographic evidence [7,8,40].

Fig. 2 shows eight of the 16 members of the *adiastola* subgroup. These species are particularly notable for the intricate and subtly graded pigment patterns of the wings. In addition, much of this group shows pronounced sexual dimorphism, and so Fig. 2 includes male/female pairs for 7 species. The group’s wing patterns are quite diverse. A third crossvein appears in *clavisetae* (*neoclavisetae*, not shown), likely as an adaptation that provides mechanical support for larger wings. This adaptation arose independently in the *planitibia* subgroup [43]. In *spectabilis*, the pigment spots are expanded and fused, giving the appearance of a black wing with light spots. The most extreme wing shape change in this collection (and perhaps in the genus) is seen in *transpenna*, in which the male wings are blunted at the tips giving a nearly rectangular appearance. The female wing is slightly blunted as well, but the selection pressure on this phenotype appears to be focused on the males. The *hamifera* wing is perhaps the most divergent overall, with an exceptional combination of large size, distorted shape, and complex, dimorphic pigmentation. The males and females share a dark spot over the proximal part of longitudinal veins L2–4, but the rest of their patterns appear to be almost completely unrelated.

Fig. 2 includes a *primaeva*/*attigua* specimen; these two species are considered to form their own subgroup at the base of the picture wing clade (Fig. 1) [8,40,42]. The distinction between *primaeva* and *attigua* could not be made in this specimen since it was female.

Fig. 3 shows 13 of the 17 *planitibia* subgroup species (see recent phylogenetic analysis [7]). The group features the well-studied “hammerhead” species *heteroneura* and sister species *silvestris* (see Boake et al. [44] and refs therein). Flies of this group are
exceptionally large, and this size increase is correlated with the appearance of a third crossvein in most species. The extra crossvein is usually aligned with the standard posterior crossvein, but it is shifted proximally in the closely related species complex heteroneura, silvestris, planitibia and differens. The subgroup is also known for using wing vibrations to produce complex courtship songs, and this behavior may be related to the unusual wing shapes of some species (e.g., cyrtoloma [45]). The picticornis wing is quite divergent, being mostly pigmented with numerous light spots; this reflects an early division in the planitibia subgroup that separates picticornis and setosifrons from the rest of the species [7].

The remaining 31 picture wing species in the database are divided into the glabriapex and grimshawi subgroups (Figs. 4, 5) based on the presence or absence of the 4b chromosomal inversion [40]. Most of these species have patterns that are variations on a basic plan of 7 spots: one at the distal tip of each longitudinal vein L2–5, a central spot on L4 at the posterior crossvein, a central spot on L2, and a spot in the anterior/proximal region. This could be the ancestral pattern since it is found in the basal species, glabriapex. Most species also have an 8th spot located centrally on L3, but this character has been gained or lost multiple times (based on the chromosomal lineage [40]). This L3 spot was gained at least once en route from the glabriapex to the grimshawi subgroup, then lost in spratti and limisata, and either gained or lost within the orthofascia/orthofascia lineage. Fairly subtle variations in the intensity, extent, and position of these 7 or 8 spots can create very different visual effects: stripes in hawaiensis and orthofascia, a “T” formation in virgulata, discrete spots in discreta, etc. The basic spot arrangement has been elaborated into an ornate checkerboard pattern in grimshawi and relatives, primarily by adding a proximal stripe along L3–4, and extending and refining the spot on L5. In crucigera the pattern is further shaped to form distinct crosses (as noted by Grimshaw in 1901 [34]) as well as two bulls-eyes in the posterior half. Comparison to the more basal grimshawi suggests that these isolated bulls-eye spots appeared de novo in clear areas of the pattern.

Non-picture wing species groups

The antopocerus group species (Fig. 6, upper) are sexually dimorphic; males display long, specialized bristles on the foreleg, and extended aristae (visible on the cognata slide). The wings may be dimorphic in shape (longiseta) and pigmentation (stigma). The stigma wing pattern closely matches those of the Asian species D.
biarmipes and elegans, which have been recently analyzed by Gompel et al. [25] and Yeh et al. [46].

The fungus feeder/"haleakalae" group (Fig. 6, lower) is the most basal lineage of the endemic Hawaiian Drosophila and diverged from the picture wing group an estimated 20 million years ago [10]. Some of these species are large, with relatively slender bodies and elongated wings; for example, dolichotarsus displays sexual dimorphism in which the male wing is quite extended and slightly curved (Fig. 6, lower left).

Fig. 7 shows anomalipes and quasianomalipes, which comprise the anomalipes group; they are closely related to the picture wings [8,36]. The remaining samples represent the modified mouthpart [37] and modified tarsus [35] groups. These groups typically have plain wings, but exhibit remarkable male-specific specializations of the mouthparts or forelegs [47]. Light and SEM micrographs of some of these specializations will be presented elsewhere and added to the photo database.

Photographic comparison of wing patterns

To better assess the variation among the wings, we made direct comparisons by color-coding sets of wing images and overlaying them (Fig. 8). In Fig. 8A, three grimshawi subgroup wings are overlaid: engyochracea, orphnopeza, and sproati. Among these species, the spot that occurs medially along L2 (arrow) can “slide” to different positions along the proximodistal axis, generating a rainbow-like pattern in the overlay; the other spots remain largely fixed. The proximal and distal borders of this spot can vary independently, as shown by the aligned close-ups of L2 (Fig. 8A, right). This result suggests that wing
Figure 6. The antopocerus and haleakalae/fungus feeder groups. Upper six panels, antopocerus group species D. longiseta, stigma, adunca, and cognata. Sexual dimorphism is shown for longiseta and stigma. The extended male antennal structures, characteristic of the antopocerus group, can be seen co-mounted with the cognata wing. Lower six panels, haleakalae/fungus feeder group: dolichotarsus, nigra, cilifemorata, and fungiperda. Sexual dimorphism is shown for dolichotarsus and nigra. doi:10.1371/journal.pone.0000487.g006

Figure 7. Other non-picture wing species. The anomalipes group: anomalipes and quasianomalipes. "Modified mouthparts group": clydonia, aethostoma, mimica, kambysellisi, polliciforma, and diminuens. The ornate pattern of clydonia is rare among the small, non-picture wing species. The curved L3 vein in clydonia is a characteristic of the species [28]. "Modified tarsus group": basimacula, petalopeza, spiethi, and dasycnemia. doi:10.1371/journal.pone.0000487.g007
A table is shown with columns labeled "Species," "Island," "Group," and "Figure." The table lists various species with corresponding islands and groups. The "Figure" column contains numerical values indicating the figure number where the species is illustrated.

Additional text is present, discussing the pigmentation process in Drosophila wings, with a focus on how certain genes control the dimorphism and the importance of the veins in the pigmentation process.

A table labeled "Table 1. cont." follows, continuing the list of species with the same structure as the first table.

The text also mentions the use of cobalt-60 irradiation to obtain the genome sequence of the Hawaiian Drosophila species, and the database includes several informative examples of wings showing sexual dimorphism.

A section titled "Pigmentation in mutants and natural variants" discusses how the database includes species that deviate from the standard pattern, and highlights specific examples of mutations that affect pigmentation.
tation of the wing cuticle requires pigment precursors that are delivered through the veins to intervein regions. They also demonstrate that there is an inherent pattern of wing hair pigmentation that is not dependent on intact veins [21].

Natural variants are sometimes found in the field that also support this two-step model. Fig. 9C shows an unusual clavisetae wing in which the intervein spots did not become filled in; dark pigmentation is limited to narrow strips along the veins. The clavisetae prepattern is still apparent (grayish regions of the intervein clavisetae pigment pattern). The wing pattern in hamifera can become altered in an even more complex manner. Compared to the typical pattern (Fig. 9D), the male in Fig. 9E–F has “holes” in five of the spots and incomplete pigmentation around L5. This male’s two wings (panels E vs. F) differ in the extent to which the dark pigment has penetrated into the intervein regions, indicating the phenotype is sensitive to local conditions. One explanation is that this male did not produce enough pigment precursors in the body permit their transport/diffusion throughout the wing spots. This hypothesis is consistent with previous experimental findings of True et al. [21]: removing the wings from D. rajasekari upon eclosion prevents pigmentation from developing, but bathing these wings in the pigment precursor dopamine can restore the normal pigment pattern. Alternatively, in these clavisetae and hamifera examples, efficient transport could have been blocked by structural defects in the veins, extracellular matrix, etc.

Possible drivers of wing diversity

Wing pigment patterns may be employed for courtship, camouflage, or mimicry [23,26,46,48,49], although their functions are difficult to prove experimentally. Without a firm grasp on their functional relevance in the wild, it is difficult to assess why the patterns have diversified so extensively. We speculate that, in many of the examples shown here, the patterns strike a balance between the need to hide from predators and the need to attract mates. At rest, when the wings are folded back over the thorax and abdomen, the wing patterns of many species blend with their dorsal cuticle markings, producing a camouflage pattern that could protect the fly from bird or insect predation [1]. The level of protection afforded by any given pattern could depend on a wide variety of environmental parameters that are unique to each species (and each sex within a species). For example, females spend considerable time seeking favored substrates for oviposition; one species may need to blend in with bark, another with leaves, etc. [1,8].

During courtship, however, males of many picture wing species prominently display their wing markings to the female. The female
might use these markings for species recognition and even to assess the fitness of the male; we have noted that smaller flies tend to have noticeably reduced pigment spots. Sexual selection is known to be a key driver of morphological change in the Hawaiian flies [3,27,47]. Several species groups are characterized by extreme male-specific ornamentations used to stimulate the female during courtship, including major modifications of the forelegs, bristles, antennal structures, and mouthparts. Altering wing pigmentation would seem to have a lower fitness cost than these other options, and may be favored for that reason. On the other hand, female flies may not be as attuned to visual cues as to tactile ones. Finally, the Hawaiian species have generally been subject to small population sizes and frequent exposure to founder events and bottlenecks. Over time, a given lineage accumulates a unique set of random mutations in the pigmentation genes. Essentially, each species is dealt a different genetic hand that it can use to accommodate these diverse selection pressures, and this may also contribute to diversification.

Hawaiian Drosophila as a model for the evolution of complexity?

We show several examples in which, along a known lineage, species exhibit increasing pigment pattern complexity or gain/loss of discrete pattern elements. It will be extremely informative to sequence candidate loci such as yellow and ebony in these species. D. primaevae provides a convenient reference species since it has no spots on the longitudinal veins, and is the most basal picture wing species; presumably both the plain wing pattern and the sequence of the pigment genes are fair representations of the ancestral state of this group (Fig. 1). The complex including villosipes, grimshawi, and crucigera provides a clear example of increasing complexity (Fig. 1). These three species are similar or identical at the polytene chromosome level, and grimshawi and crucigera genes differ by just one base change or small indel every 55 bp (averaging over the 6 crucigera nuclear genes present in Genbank). Thus, comparisons among these species could provide insights into the evolution of complexity.

Another candidate for comparative study is the adiastola subgroup. Evidence suggests the basal primaevae wing gave rise to the simple, wave-like pattern of ornata, and the more derived species have extensively modified this pattern along different branches of the adiastola subgroup (Figs. 1, 2). We would expect to find shared mutations that are responsible for shared pattern elements, and additional mutations that differ in each branch of the lineage and account for novelties in the pattern [26]. Functional mutations identified in one subgroup can then be compared to other subgroups that have qualitatively different types of patterns; for example the grimshawi subgroup is characterized by distal spots, while the basal species of the adiastola subgroup lack distal spots. This approach would capitalize on a rare advantage of the Hawaiian Drosophila, that pattern evolution has been “replayed” multiple times in a shared genetic background.

Are the Hawaiian Drosophila tractable for developmental biology?

The utility of the Hawaiian flies for experimental studies varies substantially among species. We can consider several levels of experimental tractability relevant to the studies suggested above: (1) availability of genomic DNA for comparative sequence analysis; (2) ability to grow larvae for studies of gene expression and developmental biology; (3) ability to make transgenic flies; (4) ease of performing transmission and quantitative genetics (keeping multiple lines, generating markers, obtaining fertile hybrids, etc.)

Each of these milestones has been reached in the picture wing flies, albeit with more effort than required for D. melanogaster. DNA is available from most of the species pictured here, and cloning genes of interest will be greatly facilitated by the high sequence identity levels among the Hawaiian species. Carson’s chromosome phylogeny was derived by analysis of larval chromosomes, indicating that larvae can be cultured from nearly every picture wing species [43]. We have successfully performed immunostaining of picture wing larvae and pupae using several antibodies to D. melanogaster proteins (not shown). Transgenic Hawaiian Drosophila have been produced by injecting P element DNA into D. hawaiiensis embryos [50]. There were no visible markers available at that time, so transformants were identified by Southern blot analysis of the offspring of individual injected animals. Current availability of additional transposon vectors and transformation markers should simplify the transformation process [51]. It should be possible to transform flies with both plain (minicua) and ornate (grimshawi, crucigera) wing patterns, although grimshawi lay eggs at a greater rate than minicua. For optimal egg collection, specialized substrates are required (e.g., moistened corn flakes). Stocks of minicua, grimshawi, and several other endemic Hawaiian species are available at the Tucson Drosophila Species Stock Center. Genetic markers are not currently available, although we have demonstrated that visible mutations can be isolated and maintained in grimshawi [21]. The greatest limitations to genetic analysis are the space and labor required for stock keeping (see Materials and Methods), and the 2–3 month generation time. Finally, it should be possible to identify X chromosomal vs. autosomal contributions to patterning, and estimate the number of major loci involved, by hybridizing species with distinct wing patterns in the lab (as done for silvestris vs. heteroneura coloration and head shape [52]). D. grimshawi, for example, can hybridize with balioptera, bostrycha, crucigera, disjuncta, pilimana, and others [53].

MATERIALS AND METHODS

Field collections

Flies were collected from banana or mushroom baits, or by netting, at previously described locations on Kauai, Oahu, Molokai, Maui, and Hawaii (Big Island) [1,36,43,54]. Species identifications were made by K.Y.K.

Stock maintenance

For disjuncta, grimshawi, hemipeza, heteroneura, planitibia, and silvestris, specimens were taken from laboratory stocks maintained at the Univ. of Hawaii at Manoa instead of from the field. Picture wing stocks are cultured at 18°C. Oviposition occurs in vials of Wheeler-Clayton medium [55] supplemented with an aqueous extract of Clermontia (a natural host plant which helps to stimulate oviposition). Once larval activity is observed, cornmeal-molasses-agar medium is added to the vials. Vials with third instar larvae are placed in a gallon jar half filled with damp, coarse sand. Larvae tunnel into the sand to pupate, and adults crawl back out upon eclosion. Newly eclosed adults require 2–3 weeks to reach sexual maturity; females especially require 3–4 weeks before mating and egg laying begins. Temperature and humidity regulation, culture media specific to larval and adult nutritional requirements, sterile sand as the pupation medium, etc., make laboratory husbandry of the Hawaiian Drosophila species significantly more complex than D. melanogaster. However, a number of laboratories in the U.S. as well as internationally have been successful in maintaining laboratory stocks of Hawaiian Drosophila and have been able to conduct genetic and behavioral studies on these species.
Sample preparation and documentation

In most cases wings were removed from live flies; for *algaina, cyrtolona, clydonia, difforses, dimauens, hamisera female, hirtiplatus, truncipenna, and virgulata*, the flies were recently dead when the wings were removed. Older, pinned specimens were found to be rather unsuitable for the project since their pigmentation had faded. The wings were permanently mounted in Euparal (BioQuip Products, Gardena, CA) between a slide and coverslip, taking care to avoid damage and folding. Slides were incubated overnight at 37°C to allow bubbles to dissipate, and stored in the dark. The wings were all photographed in one session under uniform conditions, using a digital camera mounted on a dissecting scope and illuminated with an overhead ring light. Raw images were adjusted in Photoshop using the “Warming Filter 81” command to neutralize the background toward gray, and contrast was restored using “Curves”. All adjustments were performed to make the backgrounds uniform across images, so that the wings are as directly comparable as possible. In Figs. 2–7, debris was edited out of some photos using Photoshop, but the wings were not altered; the unedited photos are found in the database. In Fig. 2 and Fig. 5, the backgrounds (away from the wings) were blurred to facilitate file compression. All images in the database were taken at the same magnification. In Figs. 2–7, all wings in a given figure are on the same scale, so one scale bar is shown per figure. The sex is listed if known. Only in Fig. 8, some wings were distorted using the “scale” or “skew” commands where noted. See Stark et al. [13] for explanation of wing vein nomenclature; the *Drosophila* system is used here for simplicity and longitudinal veins L1–5 are defined in Fig. 0A.

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Dedication

This work is dedicated to Hampton L. Carson [56].

Author Contributions

Conceived and designed the experiments: KE DY KK. Performed the experiments: KE LD. Analyzed the data: KE. Wrote the paper: KE.

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