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The Interommatidial Bristle Variability of *Diptera*

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Thesis Advisor: Professor Markus Friedrich

ABSTRACT

Insects of the order *Diptera* are a popular biological model for understanding morphological trait evolution. One area of particular interest is the development of the compound eyes. While the development of ommatidia and photoreceptors has been thoroughly studied in this case, little attention has been paid to the interommatidial bristles (IOBs) present on the eyes of some dipteran families. A preliminary survey suggested that these bristles exhibit high variability among IOB families on multiple taxonomic levels and are not uniformly present or absent in any suborder of the *Diptera*. To confirm this observation, I conducted a literature search to quantify how many dipteran families uniformly possessed IOBs, lack IOBs, or include species with both trait states. This effort revealed a slight bias towards lack of IOBs in the *Diptera*. Parsimony and maximum likelihood ancestral state reconstructions showed that IOBs are likely to have been present on the eyes of early dipteran ancestors, despite the bias towards lack of IOBs in the extant families. The absence of IOBs is therefore speculated to be the result of frequent evolutionary losses. Finally, the comparison with the 371 other previously studied traits suggests that IOBs have experienced the highest number of loss events among known fly traits

INTRODUCTION

With 188 recognized families and around 10,000 genera (Yeats et al. 2007), the insect order *Diptera*, which represents all true flies, is one of the largest and most thoroughly studied insect orders in the world today. The focus placed on this order is partly due to the fact that it contains the species *Drosophila melanogaster*. As a model organism, *Drosophila melanogaster* is wildly popular in the scientific world to study development and molecular biology.

Discoveries made using this species have helped us make great leaps in understanding human disease states such as Alzheimer's disease and Parkinson's disease. It is also used to make advancement in other fields such as ecology, genetics, population biology, and systematics (Powell 1997). The sheer amount of useful information provided by *Drosophila melanogaster* is precisely why it has been necessary to study and analyse the rest of the order *Diptera*. As Dobzhansky famously quoted, "Nothing in biology makes sense except in the light of evolution." For every developmental process and biologically relevant piece of information provided by *Drosophila melanogaster* there is a rich and complete evolutionary history to back it up. Understanding how *Diptera* evolved is incredibly useful for understanding how one of the world's most popular model organisms works. Finally, even if *Diptera* did not include an important model organism, it would still be well studied because it is a massive order, rich in genetic and morphological variation that makes it an ideal animal group for evolutionary study. Evolutionary reconstructions have been created for many dipteran traits with particular emphasis on molecular evolution due to its usefulness in disease studies (Beverley and Allan 1984). But

there have also been studies into the evolution of external structures such as larval maxilla (Harbach and Peyton 1993) and genitalia (Arnqvist 1998). Surprisingly, one particularly interesting trait has received almost no attention at all: The interommatidial bristles (IOBs).

Like many insects, flies have compound eyes that consist of hundreds of identical subunits called ommatidia. Each ommatidium contains support cells, pigment cells, and a cluster of photoreceptor cells similar to those found in the human retina. Innervation of each ommatidium allows the brain to receive hundreds of picture elements which it can use to form a comprehensive image of the fly's surroundings (Melamed and Trujillo-Cenóz 1967). As the name suggests, IOBs are bristles found between the ommatidia of compound eyes (Fig. 1A). They appear similar to touch receptor bristles elsewhere on the fly body in terms of structure (Perry 1968). However, there is currently no evidence to suggest that they serve a similar function.

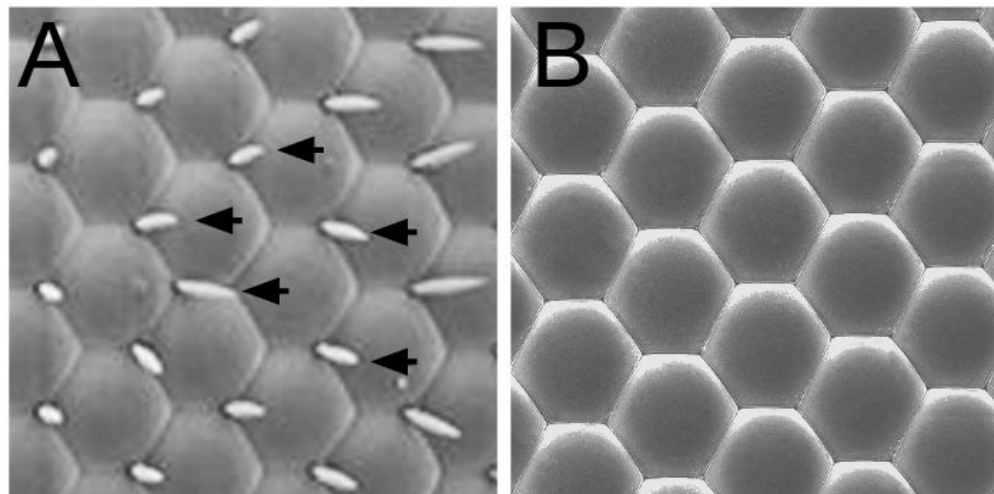


Figure 1: Examples of Fly Eyes with and without IOBs: These images show the difference between IOB presence and absence. **A)** Lens surface of *D. melanogaster* compound eye with regularly spaced IOBs a selection of which is highlighted by black arrows. **B)** Lens surface of the compound eye of the house fly *Musca domestica* with no IOBs.

The development of fly eyes has been the focus of many research efforts. Discoveries that have been made include the developmental process of ommatidia and the fact that common ancestors of insects and crustaceans were likely to have had ommatidia with focusing crystalline cones, and colour and/or polarization vision (Nilsson and Kelber 2007). The emphasis scientists have placed on eye evolution makes it curious that no one has paid particular attention to the IOBs. Further remarkable is that IOBs are present on the eyes of *Drosophila melanogaster*, but absent on the eyes of other *diptera* species. This raises the question: how did IOBs evolve and for what purpose? To explore this variation, I hypothesized that IOBs were a hypervariable trait that was repeatedly lost and regained throughout the evolution of *Diptera*. Since the presence or absence of IOBs does not currently appear to affect the rest of fly morphology, it is possible that the trait is controlled in such a way that it is highly susceptible to evolutionary pressures.

MATERIALS AND METHODS

Data acquisition and trait state definition

To compile IOB variability and quantify how many families possessed the trait for IOBs, I turned to the 107 families described in *The Manual of Nearctic Diptera* (McAlpine 1981) with its detailed scientific fly morphology drawings by a single illustrator: Ralph Idema. Surveying the documentation of IOB presence vs absence in this comprehensive source, I identified four possible character states relating to IOBs: IOB present, IOB absent, Irregular eyes that could not have IOBs, and IOB variable. Families classified as IOB variable have the IOBs present in some

genera and absent in others. A single genus that did not match the rest was considered the threshold for whether or not a family qualified as variable.

Confirmation of the character state analysis was done using the high resolution photographs available online at websites such as Bug Guide, a picture database hosted by The Iowa State University Department of Entomology (bugguide.net) and Bugwood Images, a grant-funded project that was started in 1994 by the University of Georgia's Center for Invasive Species and Ecosystem Health (images.bugwood.org). Literature searches were also done to confirm IOB expression with particular attention was paid to large IOB variable families. For many fly families there are still genera and species that have not been studied in full detail. Useful morphological and phylogenetic information was found regarding *Tachinidae* (Cerretti 2014), *Stratiomyidae* (Brammer 2007), and *Phoridae* (Cook et al. 2004).

Character state Reconstruction

Character state reconstructions were performed using the phylogenetic software program Mesquite (Version 3.10; Maddison and Maddison, 2016). The character state information on 107 fly families obtained from The *Manual of Nearctic Diptera* was entered into the program as a table. From there a phylogenetic tree was created based on (Wiegmann et al. (2011)). In order to compare IOB ancestral reconstructions to the reconstructions of other characters, it was necessary to reduce the number of families analysed to the families documented in Lambkin et al. (2011). These limitations lowered the number of families from 107 to 35. Reconstructions were performed using parsimony and likelihood models. Unordered parsimony was used with the cost of state change set to 1. The likelihood model used was *mk1(est)*, a one-parameter

Markov k-state model (Lewis, 2001). For the sake of simplicity, IOB ancestry was reconstructed through both of these methods under the assumptions that IOB variable families were either fully positive or fully negative. Outgroups were also defined to test the sensitivity of the reconstruction methods. A total of 8 reconstructions were conducted for the IOB trait with variations in reconstruction method used, how the IOB variable families were categorized, and positive or negative character of the outgroup.

Trait Comparisons.

Summary state changes were quantified in Mesquite using likelihood and parsimony reconstruction methods. The 371 morphological characteristics documented in Lambkin et al. (2011) were entered into the taxa vs. character table in Mesquite. Each character was then analyzed for its average estimate of trait gains and losses.

RESULTS

Overview of IOB variability.

Preliminary analysis of IOB presence and absence revealed that the trait is highly variable on multiple taxonomic levels. It varies not only between families, but also from genera to genera. According to data from *The Manual of Nearctic Diptera*, at least 24 *Diptera* families can be considered variable in this way. These include some large notable families such as scuttle flies (*Phoridae*), hoverflies (*Syrphidae*), and flesh flies (*Tachinidae*). However, despite the

surprisingly large number of families that were classified as IOB variable, the IOB negative character state is slightly more prevalent among *Diptera* families than either IOB positive or IOB variable states. Among the 107 families that were documented, 60.75% were characterized as being IOB negative (Fig. 2A). This is a significant majority when compared to either of the other states alone and it would still be the majority if IOB variable and positive families were considered one group.

Interestingly, far less dramatic results were found when the focus was narrowed to include only the *Diptera* families included in the comprehensive morphological studies conducted by Lambkin et al. (2011). Of those 35 families, only 42.86% were IOB negative, a smaller percentage than IOB positive and variable combined (Fig. 2B). This difference is likely due to the greatly reduced sample size, but it remained necessary to study only the families for which extensive morphological information was available.

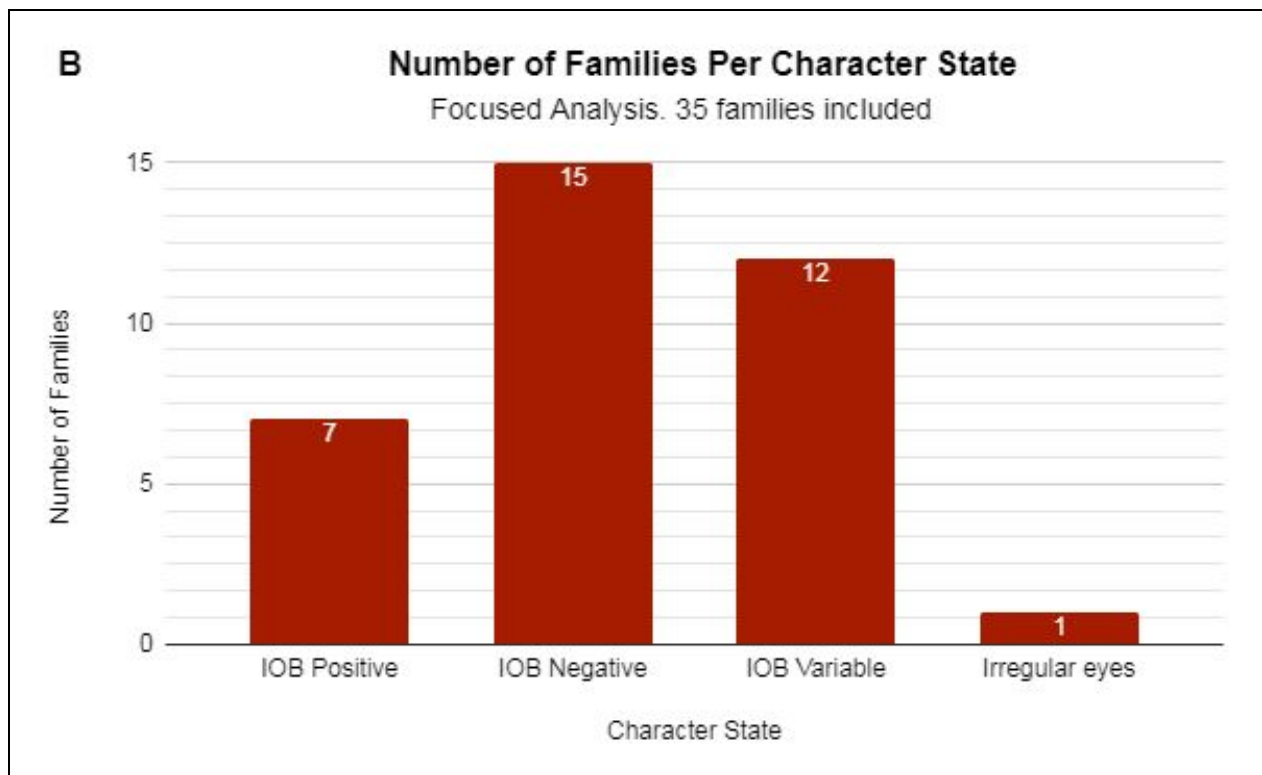
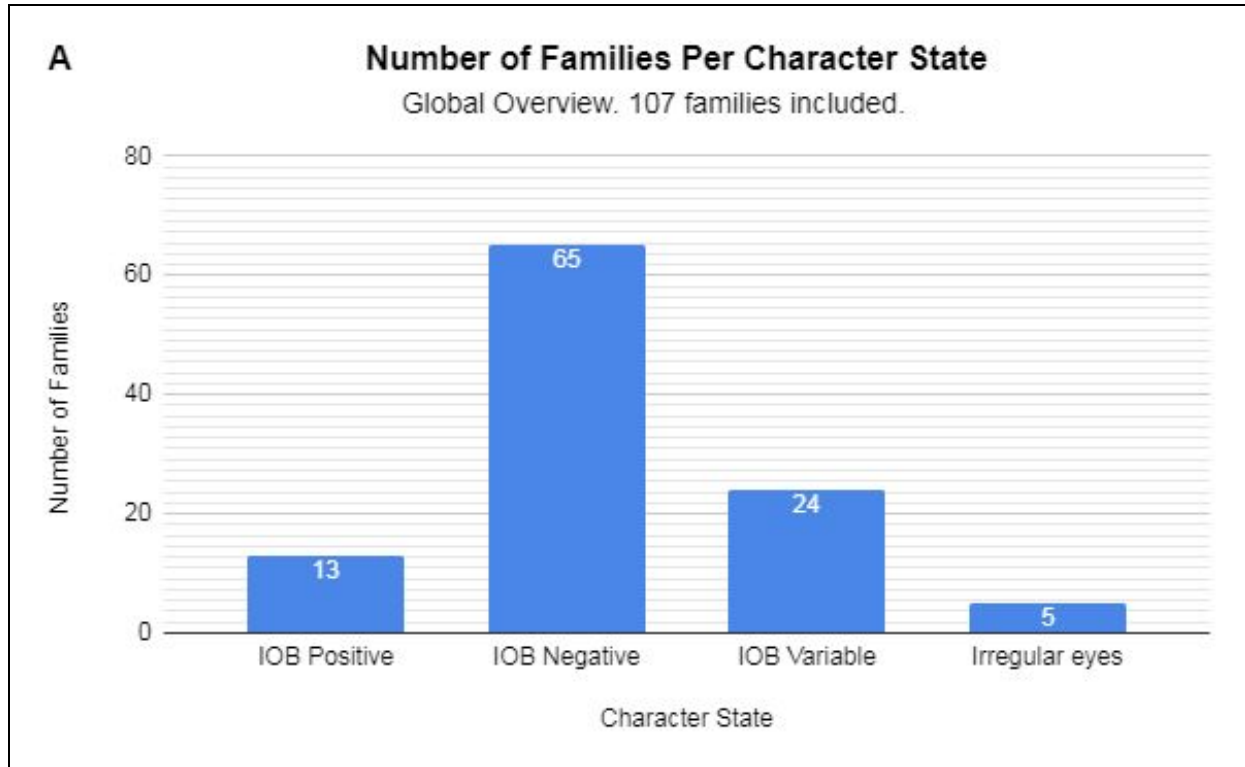


Figure 2: Quantification of IOB Character States. These graphs quantify the number of *Diptera* families representing each character state. IOB positive families have IOBs in all genera studied, while IOB negatives are consistently bare eyed. IOB variable denotes any family with at least one genera that is IOB positive and one that is IOB negative. Irregular eyed families are those whose unique morphologies make IOB presence/absence irrelevant, including *Blephariceridae*, *Ptychopteridae*, *Braulidae*, *Nycteribiidae*, and *Streblidae*. **A)** Analysis of all families covered in *The Manual of Nearctic Diptera*. **B)** Analysis of 35 families covered in *The Manual of Nearctic Diptera* (McAlpine 1981) as well as *The phylogenetic relationships among infraorders and superfamilies of Diptera based on morphological evidence* (Lambkin et al. 2011)

Phylogenetic Analysis of IOB variation.

Parsimony and likelihood reconstructions of IOB character state history yielded different results.

Parsimony analysis showed high probabilities of IOB positive ancestors while likelihood analysis gave consistently lower probabilities. However, despite the differences in the two methods, both were able to determine conclusively that the IOB character state is outgroup sensitive. The presence of a positive outgroup increases the likelihood of early common ancestors being positive or variable for the IOB trait no matter what reconstruction method was used. (Fig. 3 and 4) Furthermore, because IOB presence is known to exist variably in *Lepidoptera*, one of the orders most closely related to *Diptera*, it can be assumed that the inclusion of a positive outgroup is the most accurate representation.

All reconstructions showed a strong likelihood that the last common ancestors of all *Diptera* possessed IOBs. This was especially true in the more accurate reconstructions that included an IOB positive outgroup. It was only with distance from the last common ancestor that higher likelihoods of IOB absence were observed, indicating that absence may be the derived trait. Notably, absence could still be seen across the entire phylogenetic tree as well as in most stages of evolution after the earliest common ancestors.

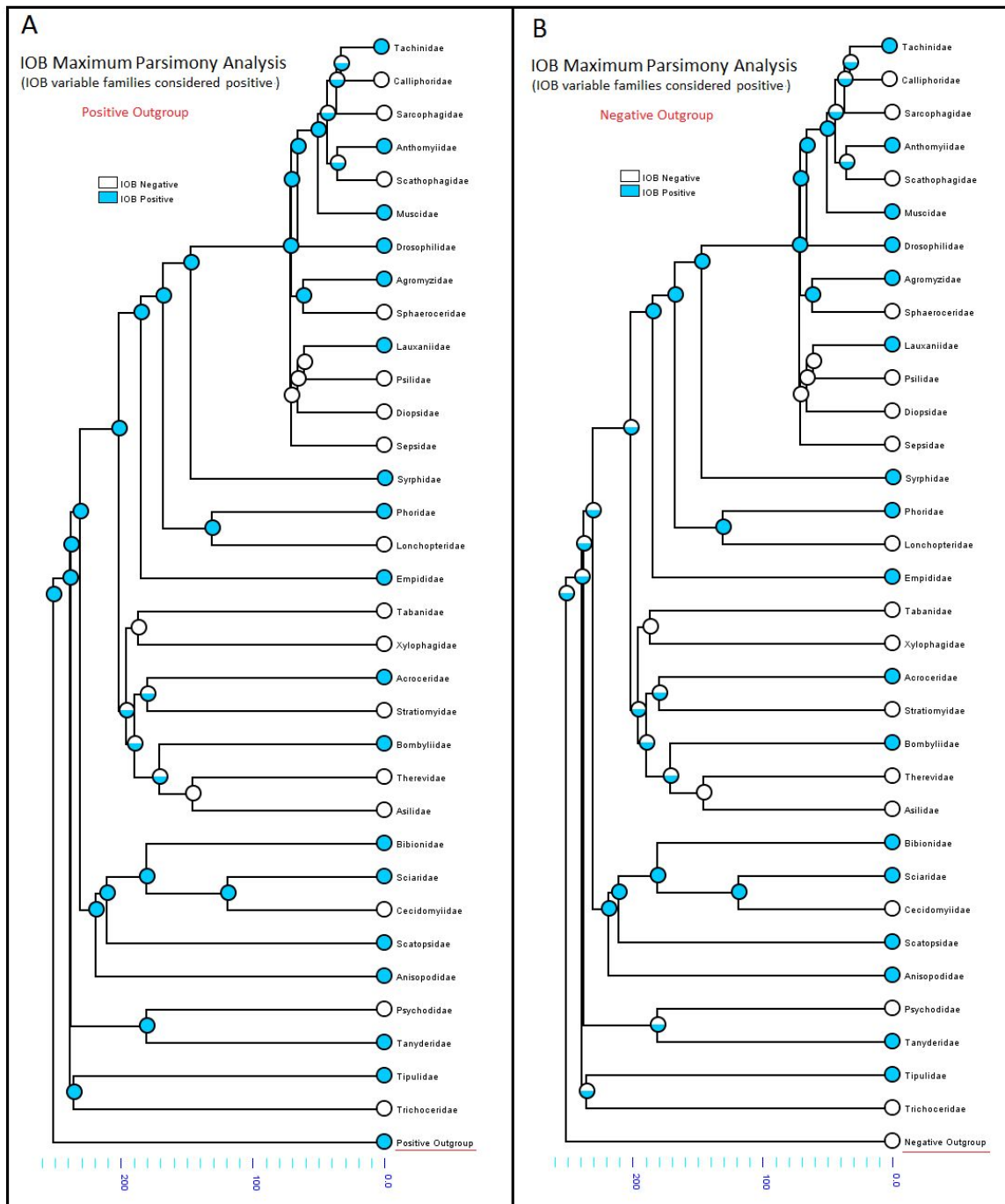


Figure 3: Parsimony Reconstruction of Ancestral IOB States. These phylogenetic trees were reduced to include only the 35 families for which there is morphological and phylogenetic data. Variable IOB families are considered IOB positive. Nodes are filled in according to the maximum parsimony likelihood of common ancestors being IOB positive. The scale on the bottom is measured in millions of years. **A)** Positive outgroup assumed. **B)** Negative outgroup assumed.

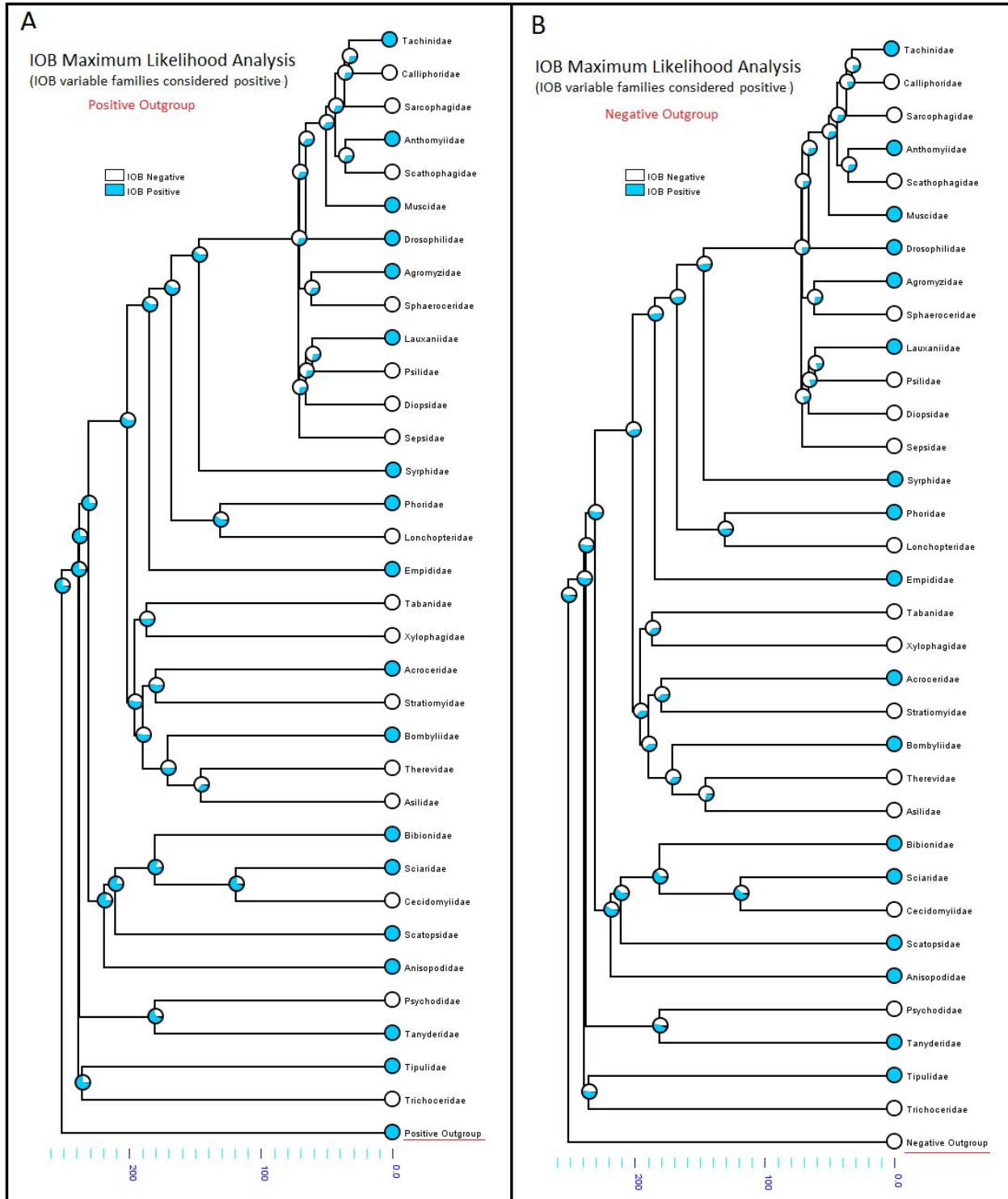


Figure 4: Likelihood Reconstruction of Ancestral IOB States. Nodes are filled in according to the maximum likelihood probability of common ancestors being IOB positive. The scale on the bottom is measured in millions of years. **A)** Positive outgroup assumed. **B)** Negative outgroup assumed.

Comparison of trait variability

Summary parsimony reconstructions of the 371 characters studied in Lambkin et al. (2011) indicated that the IOB trait variation was best explained by an exceptionally high number of losses. In analyses run under the assumption that IOB variable can still be considered IOB positive, there was an average estimate of 10 full trait losses for different reconstruction approaches. That was the highest number of average estimated losses for any of the traits studied. (Fig. 5A) The next 6 most frequently lost traits included two other types of bristle, the Scutellar bristles and the setae on the subapical aboral surface of mandible. Another notable trait was the development of the upper calypter, a small structure located at the base of fly wings (Crosskey 1993). With an average estimate of 6 losses, it was the second most commonly lost trait.

Results were less dramatic when parsimony analyses were run assuming that IOB variable families are negative for the trait. Considered in this light, it was less likely that dipteran ancestors possessed the trait and therefore less likely that it could be so frequently lost. IOB absence versus presence did not rank among the most commonly lost traits. It did however, continue to be closely associated with other bristle traits including the presence versus absence of Supra-alar bristles and Dorsocentral bristles.

The same analyses with likelihood reconstructions of ancestral states made it difficult to quantify the number of times the IOB trait was gained and lost over the course of evolution. When variable families were assumed to be positive for the trait, Likelihood reported 0 cases of

either trait gain or loss. This could be due to the fact that likelihood analysis could not confirm that any dipteran ancestors were fully positive or negative for the trait. With no certainty of ancestral states it could not accurately assume that any trait gains or losses occurred. Strangely, when IOB variable families were considered negative, Likelihood did report a relatively high number of trait gains. (Fig. 5B)

Character	Trait change	MP gain (V+ OG+)	MP loss (V+ OG+)	MP gain (V- OG+)	MP loss (V- OG+)
IOB	Presence / Absence	2	10	5	2
Upper calypter	Developed / Not Developed	0	6	0	6
Accessory gland	Continuation with Vasa Deferentia	2	4	2	4
Scutellar bristles	Presence / Absence	1	4	1	4
Subapical aboral surface of mandible	Setae Presence/ Absence	0	4	0	4
Mandibular chela:	Presence / Absence	0	4	0	4
Female Cerci	1 Segmented / 2 Segmented	0	4	0	4

Character	Trait change	ML gain (V+ OG+)	ML loss (V+ OG+)	ML gain (V- OG+)	ML loss (V- OG+)
IOB	Presence / Absence	0	0	5	0
Head Retraction	Retracted into Thorax / Not Retracted into Thorax	4	0	4	0
Body Segments	With / Without Complete Secondary Divisions	4	0	4	0
Frons differentiated into frontal vitta and lateral fronto-orbital plates	Presence / Absence	4	0	4	0
Posterior anepisternal bristles	Presence / Absence	4	0	4	0
Suprasquamal ridge	Setose / Bare	4	0	4	0
Postmetacoxal bridge	Presence / Absence	4	0	4	0

Figure 5: Comparison of Estimated Trait Gains and Losses Across Dipteran Ancestry. The table shows the average number of estimated trait gains and losses over the course of dipteran evolution. Values have been rounded to the nearest whole number. A positive outgroup has been assumed for all characters. **A)** Values according to maximum parsimony. Yellow indicates the amount of estimated trait losses when variable families are considered positive. **B)** Values according to maximum likelihood. Yellow indicates the estimated trait gains when variable families are considered negative.

DISCUSSION

Taken together, my findings support the conclusion that the presence of IOBs is a highly variable but ancestrally well founded trait and that absence of IOBs in modern dipteran species is therefore a result of evolutionary loss. Character state quantification revealed that there are more families within *Diptera* that can be considered IOB negative than IOB positive. However, there is also a solid representation of families that vary by genera and do not fit firmly into either category. If such variable families are considered to have retained the trait, the difference

between IOB negative and IOB positive families becomes far less significant. It was also notable that none of the character states observed were restricted to certain suborders of *Diptera*. Instead, all states seem to be spread over the phylogenetic tree fairly evenly. This implies that evolutionary loss or gain of IOBs can happen fairly quickly and circumstantially. If there is an ecological factor influencing how each family exhibits the trait, it is not yet known.

Furthermore, the ancient origins of IOB presence were confirmed by both parsimony and likelihood reconstructions. This was less apparent when negative outgroups were introduced. However, since true outgroups of *Diptera* are confirmed to display IOB presence, it is safe to assume that the reconstructions showing a high likelihood of IOB positive ancestors were most accurate. In some cases gains are seen in the reconstructions. For example, *Lauxaniidae*, *Acroceridae*, and *Bombyliidae* are all IOB positive families within clades that are primarily IOB negative. The immediate ancestors of these families have relatively high likelihoods of being IOB negative. It can be speculated that such families may have regained IOBs even after earlier ancestors stopped expressing the genes that produce them. However, reconstruction reports of trait gains were not nearly as frequent as loss so it is far more likely that most IOB positive and variable families simply retained the trait.

Comparison of IOB trait gain and loss to other morphological characteristics again proved this point by showing that the number of trait losses throughout evolution was exceptionally high compared to other traits that are likely more difficult to genetically alter. Some of the other most frequently lost traits included other types of bristles suggesting that a common genetic cause may be at play here.

Evaluating the above conclusions for variability across traits, the bias produced by reducing the sample size must be acknowledged. Outgroup data proves that the earliest ancestors of *Diptera* were still highly likely to be IOB positive. However, the reconstructions may have looked very different if a greater pool of morphological data had been available to allow the inclusion of a more representative amount of IOB negative families. If IOB negative families were closer to 60% of the total families studied, there would have been a higher likelihood of bristleless ancestors. Therefore, it would have been easier to see instances of the trait being lost and regained. Future morphological studies of other fly families will be of great aid to corroborate our understanding of IOB variation as well as its ancestral states with regards to other traits.

The limitations of parsimony and likelihood reconstructions are also a factor when evaluating the results of this experiment. Likelihood analyses are not commonly used for morphological studies because they work best with added genetic information. Parsimony, on the other hand, has been used to reconstruct the ancestry of everything from the coloration of blackbirds (Johnson and Scott 2000) to the shape of musical instruments (Tëmkin and Niles 2007). Parsimony analysis does a much better job with purely morphological data and, therefore, produced the most accurate results.

Future Objectives

In order to determine why certain families would lose the IOB trait, the next logical step is to determine how IOBs are used. There is as yet no firm evidence to suggest that IOBs serve a sensory function. However, it has been observed that IOBs are innervated and have peripheral

projections (Chayka and Mazokhin-Porshnyakov 1986). If IOB neurons were traced it is possible that a linkage to the brain could be discovered. The presence or absence of such a linkage would tell us for certain whether or not IOBs produce a real sense in flies. Certain aspects of fly eye morphology such as photoreceptors have already been proven to be sensitive to ecological factors (Gonzalez-Bellido et al. 2011). Determination of IOB sensory function is the first step toward understanding what sort of ecological factors might make the trait evolutionarily disadvantageous and why the it was lost so many times throughout evolution.

Another possible continuation would be to analyse the genetic regulation of the top ten most frequently lost traits in order to determine if they are related by a single pathway. Because the Notch signalling pathway is so heavily involved in eye development, it is my hypothesis that IOB loss is somehow related to Notch expression.

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