Strepsipteran Vision

By

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Global Abstract

Insect vision is renowned for being remarkably poor in terms of resolution, a situation worsened by low levels of light. In dim light, the heightened temporal resolution of insect eyes is also reduced, particularly in small species. Adult male Strepsiptera have among the fewest ommatidia (i.e., ocular facets) of any flying insect (10–150). The number is most diminished in species active in low light, which have fewer but larger ommatidia, and in smaller species. All well-characterized insect compound eyes perceive a single point per ommatidium, but given the extent of the reduction and other physiological traits, it is not clear whether each strepsipteran ommatidium only perceives a single point, or is ever able to re-solve additional detail in accord with the large number of photoreceptors in each respective "retinula" (little retina)—rather than 8, as is typical of other insect ommatidia, there are 50 to perhaps as many as 100 in diurnal species, such as *Stylops pacifica*, *Xenos vesparum*, and *X. peckii*. This is especially odd since light is plentiful during the day. (The number of photoreceptors per ommatidium is unknown for most Strepsiptera, including any crepuscular or nocturnal species.) The body of this dissertation consists of three chapters that help clarify and extend what is known about vision in Strepsiptera. Chapters 1 and 2 have already been published; chapter 3 is expected to be.

Chapter 1 highlights my collaboration with the Buschbeck lab (University of Cincinnati) to determine if Strepsiptera have the capacity to see in color. *Xenos peckii*, a diurnal paper wasp-infecting species, was only found to express two opsins, rather than the three typically found in insect compound eyes. We determined that the tow opsins maximally absorb in the long wavelength band (539 nm; green), and the UV band (346 nm). Strepsiptera lack ocelli, which when present, often express a separate UV and green opsin. Thus, I posit that between the compound eyes and ocelli, all flying insects have a short and a long wavelength band opsin, on account of their usefulness in quickly distinguishing ground from sky. Due in large part to their enormous eyes, we assumed adult male Strepsiptera must use vision to finally identify calling females (i.e., emitting sex pheromone) after having tracked them countless meters via olfaction.

Chapter 2 describes using a light trap for field collection of live *Elenchus koebelei*, a Strepsiptera active at dawn. Live collection was conducted to provide pristine specimens for future ocular structural comparison and analysis via electron microscopy. Prolonged collection demonstrated that reliable populations of Strepsiptera are available from hosts that inhabit fixed locations that only require single determination, unlike the more frequently casually encountered paper wasp-infecting species, whose individual nest sites are relocated each mating season. It furthermore provided the potential for direct interaction with flying specimens. In one such instance, I unsuccessfully attempted to aspirate an airborne *E. koebelei* (≈ 0.8 mm), which made it clear that whatever visual and other sensory strategies Strepsiptera use enable them to deftly avoid direct encounters with potential threats.

Chapter 3 centers on my analysis of what I believe to be the first-ever photographs of flying Strepsiptera, the nocturnal species, *Triozocera texana*, which I took in the field. From these and historical descriptions of various flying Strepsiptera, I learned that the often incongruous accounts of strepsipteran flight (e.g., that they fly quickly, that they fly slowly, that they are sporadic, that they are graceful, that they are excessively active, that they hover in place, etc.) are nearly all shared with sex pheromone plume-following moths. The only exceptions were the erratic flight of Strepsiptera confined to tight spaces, and the patrolling undertaken by adult males of species infecting diurnal solitary bees, the hosts of which overwinter in stable nesting areas. In these species, the typical application of olfaction at distance and sight in the vicinity of sex pheromone-emitting females is apparently inverted, although final determinations are still made chemotactically. Such males are larger and diurnal, and thus have bigger eyes with more, but smaller, ommatidia than other Strepsiptera (up from the 20–65 found in many others to ≈150 in the families Xenidae and Stylopidae). From a description of an incompletely eclosed flying *Hylecthrus rubi* (family Halictophagidae) dragging its dead host, I calculated its flight muscle to total mass ratio (FMR) to be *at least* 44%, which should be typical for Strepsiptera. That value just surpasses the most flight muscle-endowed sphinx moths (0.436) and is only known to be exceeded by the upper echelon of odonate FMRs (damsel- and dragonflies at 0.473 and 0.560–0.63, respectively). However, unlike those species, adult male Strepsiptera do not feed and die on the same day they eclose. Although their flightworthy wings appear to be teneral, Strepsiptera are clearly not weak fliers. Moreover, strepsipteran flight patterns and mate pursuit show no sign of being disadvantaged by their potentially poor eyesight, which does not ever appear to require super-ommatidial resolution, except perhaps when approaching an agile and agitated host bearing a calling female. Even then, however, it is not the female that is ever identified visually, but instead her host, with a taxon-dependent level of specificity.

The three mandatory chapters are followed by a coda that examines the possibility of Strepsiptera having a super-ommatidial resolution visual mode, but without the benefit of a comparative analysis of the ultrastructure of the eyes of a diurnal, a crepuscular, and a nocturnal strepsipteran species, as originally intended. It also discusses what additional information is required to fully characterize strepsipteran vision and how that could be achieved.

Global Introduction

Strepsipteran vision is poorly understood: Neither the spatial nor temporal visual capacity of any strepsipteran species is fully known. Neither the structure nor even the presence of multiple rhabdoms comprising the extended retinas of their enormous ommatidia has been documented.

Strepsiptera are endoparasitic on other insects for the vast majority of their lives. In all but the most basal extant family, adult females never leave their hosts (Pohl & Beutel 2008), and are either actually (Peinert et al. 2016) or essentially motionless (Hrabar et al. 2014), and therefore completely unable to discern form. Neither sex feeds as adults, but eclosed males are active, miniscule, delicate, and extremely short-lived. Thus due to lack of information, strepsipteran vision is usually ignored. However, when it has been considered, contrasting conclusions have been reached, ranging from spatial acuity in *Xenos vesparum* and *X. peckii*—diurnal paper wasp-infecting Strepsiptera—of only about 18° (Pix et al. 2000) to 9° (Maksimovic et al. 2007) (anatomically and behaviorally assessed; lower acuity assessment of Buschbeck et al. (2003) (histological analysis of *X. peckii*)), to about twice that of similarly-sized *Drosophila*[1] at around 2.4° (upper acuity estimate of Buschbeck et al. (2003) (histological analysis of *X. peckii*)), to the considerably higher acuity ostensibly necessary to spring onto the abdomens of planthoppers (Muir 1906), as occurs in *Elenchidae*—despite their having only about ⅓ as many ommatidia as *X. peckii*. Given that strepsipteran eyes are all morphologically similar, it follows that they should be functionally similar, as well. Although different families rely more fully on different optical information, the characteristics of the peculiar strepsipteran eye must suffice for the entire order.

Strepsiptera apparently evolved as nocturnal insects (Pohl & Beutel 2008), providing the impetus for the enlarged eyes and ommatidia of adult males. Nocturnal insects often employ superposition optics to obtain high-sensitivity while reducing, or even avoiding, sacrifices in acuity. How well this is achieved varies widely and in accordance with flight ability, from nocturnal beetles (poor) to hawk moths (great).

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[1] Which normally have a visual acuity of about 4.6°.

All compound eyes accept the on-axis light incident upon a given ommatidium, but unlike diurnal apposition optics, which completely blocks off-axis light, or nocturnal apposition optics, which accepts off-axis light (to increase sensitivity at the cost of acuity), superposition eyes redirect varying amounts of off-axis light to nearby ommatidia for which it is on-axis. However, the further off-axis the light, the less precise the redirection, so greater sensitivity eventually erodes acuity. Unfortunately, strepsipteran eyes are far too tiny to appropriately redirect off-axis light at all (Meyer-Rochow & Gál 2004), so they are unable to harness superposition optics.

Another way to improve light sensitivity that mitigates costs to acuity is neural superposition. As implemented in "higher flies" (Brachycera), neural superposition literally redirects the six peripheral rhabdomeres of an eight-rhabdomere open rhabdom (the two central rhabdomeres are "tiered," or stacked one on the other), so that they send the signals obtained from having absorbed off-axis light to different adjacent ommatidia for which it is on-axis (Braitenberg 1967). Although an incomplete form of neural superposition is suspected in most nematoceran flies (midges, gnats, etc.), heteropteran Hemiptera (true bugs with differently textured fore- and hindwings), cucujiform beetles (an infra-order of plant-eating beetles), and Dermaptera (earwigs)—all of which also have open rhabdoms—complete neural superposition is only otherwise known from Bibionidae, which also have open rhabdoms. Bibionidae is a family of nematoceran Diptera commonly known as March flies, that have evolved their own form of neural superposition[2] (Zeil 1979; Nilsson & Ro 1994). "Incomplete" neural superposition combines signal redirection (actual neural superposition) with neural pooling, and is used to allow a compound eye to adjust to profoundly different light conditions. (Complete) Neural superposition is basically a diurnal-only specialization of incomplete neural superposition (Nilsson & Ro 1994). However, Strepsiptera were poor candidates for either form because their progenitors inherited fused, rather than

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[2] In it, neural signals are distributed to 6 of the 12 second nearest neighbors, rather than to the inner hexagonal ring—as mostly occurs in brachyceran neural superposition (except that what would have been the bottommost recipient (and would have completed a hexagonal surround) is effectively displaced to the upper right corner, for whatever reason).

open, rhabdoms (Zelhof et al. 2006; Misof et al. 2014). Additionally, due to the short lifespans of eclosed adult males (some 4–5 h, of which they are able to fly about half that time), strepsipteran eyes are usually subjected to a single light regime. Therefore, having been ancestrally nocturnal (Pohl & Beutel 2008), an eye catering to high sensitivity would initially have been favored. Thus, Strepsiptera were compelled to evolve a different mechanism than either optical or neural superposition.

For acute vision, the least favorable approach is to have fewer larger ommatidia that contain fused rhabdoms, while keeping the eye the same size and increasing the curvature of the lenses to accept more off-axis light. This does increase sensitivity, but reduces acuity and also results in lower resolution because of the smaller number of ommatidia. Another approach is to facultatively pool the neural signals of several smaller facets. This has the advantage of preserving resolution in bright light, but because smaller ommatidia have relatively larger peripheries, it wastes more off-axis light than a single larger ommatidium would, and is therefore of limited use to truly nocturnal animals. Instead, strepsipteran ommatidia may have the capacity to function as simple eyes (such as our own), because each ommatidium contains its own potentially image-forming mini-retina. While a typical fused rhabdom has 8 entwined photoreceptors that all observe the same region in space, an *X. peckii* “eyelet” contains from 50–100 photoreceptors (Strohm 1910; Buschbeck et al. 2003). Strepsipteran photoreceptors are reticular, so they are physically connected end-to-end—and thus apparently subject to optical crosstalk—but they are not fused. The extent to which Strepsiptera have enhanced acuity and/or resolution rests largely on the nature of this photoreceptive network. Thus, a strepsipteran ommatidium may conceivably capture a tiny sub-image, rather than a single point, but whether or not that actually occurs is unknown.

Concerning sub-image formation, strepsipteran photoreceptor axons twist through 180° before reaching the lamina (Buschbeck et al. 1999, 2003), where visual processing begins. This counters the optical inversion that occurs from passing light through a normal lens. It also means strepsipteran ommatidia could potentially not only capture independent sub-images, but furthermore, that the sub-images could subsequently be united to form a panorama. Because it does not matter whether a single point is upside down or not, image reinversion is unimportant for visual coherency in ordinary apposition eyes. Even so, although it is not clear why,[3] rhabdomal axon twisting is known to occur in several species that have apposition optics and fused rhabdoms, including the cabbage white butterfly (*Pieris rapae*), drone European honey bees (*Apis mellifera*), and even the ruby meadowhawk (*Sympetrum rubicundulum*). This last example is especially noteworthy because dragonflies and damselflies (order Odonata) branched from other insects early in the evolution of flight. Additionally, the rotation exhibited by *S. rubicundulum* rhabdomal axons is uniformly 180°, while those of the other two exemplars are of varied extent and even direction. Therefore, the 180° inversion undertaken by *X. peckii* axons[4] could itself be the result of a carryover effect and may not signal meaningful retinotopic reconstruction at all. Lastly, strepsipteran retinas are curved, which helps to better capture what would otherwise be poorly focused light on the outskirts of each ommatidial field of view. However, that could occur for the purpose of increasing the sensitivity of single point-producing ommatidia, or for improving the acuity of the outer elements of enhanced resolution sub-retinas—or, potentially, both.

Finally, due to the dearth of photons, many nocturnal vertebrates do not have color vision. However, rhabdomal photoreceptors (primarily found in invertebrates) are more sensitive than ciliary ones (used in our eyes), but are also more energy intensive (Fain et al. 2010). Rhabdomal photoreceptors enjoy a larger dynamic range as well (Fain et al. 2010), so that the same type of photoreceptor supplies photic (color) and scotopic (dim light) vision, with only the wavelength range changing between photoreceptor “classes” (peaking in the long, medium, and short wavelength range). Thus in insect eyes, there

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[3] Perhaps it is useful when neural summation is applied to partially overlapping visual areas (neural pooling, **not** neural superposition), as could occur in incomplete neural superposition, among other cases.

[4] Post-basement membrane inversion differs from the twisting that normally occurs within non-DRA ommatidia, in that it occurs in the context of groups of photoreceptors served by the same lens. Photoreceptor twisting, which may or may not amount to 180°, reduces or removes the inherent polarization sensitivity of rhabdomere-based photoreceptors, enabling them to be more sensitive, as well as to avoid perceiving “false colors,” which could otherwise arise from confusing polarization sensitivity for wavelength conformance. Post-membrane inversion and individual (open rhabdom) or rhabdom (fused rhabdom) photoreceptor twisting are not mutually exclusive.

are no “cones” or “rods,” nor is there a Purkinje shift. These differences, coupled with temporal and/or spatial pooling, enable insects to see color in very dim environments (Kelber et al. 2002). However, to produce the very most sensitive and comparatively fast compound eyes, color vision may be lost (though ostensibly not also in the ocelli, if present) or reduced. Color vision could be used to help distinguish between a host and its calling female parasite, but if nocturnal species and those of Corioxenidae, whose females are always hidden beneath the hemelytra of their (true) bug hosts, are able to thrive without it, then color vision—and the opportunity for other more visually competent insects to intercept the signal—may be absent in diurnal Strepsiptera too.

The great question of strepsipteran vision is if any ommatidium ever acts as an actual simple eye and produces its own sub-image—rather than behaving as an enormous well-focused, super sensitive light collector—and if so, under what circumstances it is activated, how it is activated, what resolution it provides, and at what speed it operates. That last point touches upon the correspondence problem (deciding which of the overlapping points between “eyelets” coincide), which can be very resource and time intensive, and thus limits the likelihood of any verified super-ommatidial resolution from operating across multiple eyelets. The existence of super-ommatidial resolution is further complicated by the fact that the strepsipteran eye ultrastructure so far encountered has not been at all well-organized, which is not indicative of enhanced eyesight. However, those eyes have belonged to larger diurnal species (Stylopidae ((solitary) bee-infecting) and Xenidae (wasp-infecting)), and not to smaller or nocturnal ones, which would benefit most from improved acuity: smaller species because they have smaller eyes that have fewer facets as a consequence of diffraction, and nocturnal species because to enhance light capture, they have fewer larger ommatidia. Both conditions reduce resolution at the ommatidial level. Moreover, strepsipteran eyes are not known to encompass any internal specialized variations (e.g., no DRA, no acute zones, no peripheral drop off in acuity), which is also not indicative of high-quality vision (Gonzalez-Bellido et al. 2011). However, the ultrastructure of an entire strepsipteran eye has still never been probed. In many, typically nocturnal, strepsipteran species, the sizes of ommatidia *do* change in an ordered way across the eye, with ventral facets being larger than dorsal ones (Kinzelbach 1971)(personal obs.). However, that may simply serve to enhance light signals partially absorbed by the ground.

Because I had no direct experience of Strepsiptera, I sought mentorship from Dr. Elke Buschbeck, who originally proposed that Strepsiptera have super-ommatidial resolution (Buschbeck et al. 1999, 2003). Thus in Ch. 1, we investigated the capacity of the diurnal species *Xenos peckii* to perceive color. Thereafter, in the hope of elucidating the nature of strepsipteran photoreceptor tiering and the structure of strepsipteran rhabdoms, I obtained fixed and embedded heads and bodies of specimens of the crepuscular species *Elenchus koebelei*, the nocturnal species *Triozocera texana*, and the diurnal species *Xenos vesparum* for examination via electron microscopy. I acquired the former two species from live collection in the field (Ch. 2), and subsequence preparation in the lab, and the latter from the Zeil lab. I sought to contrast the ultrastructure of strepsipteran eyes from the three major light regimes to see if any specialized adaptations are present. Unfortunately, due to lack of funding, proper transmission electron microscopical analyses were never undertaken, and only several relatively low-res “landmark” thick cuts were made through *E. koebelei* eyes. However, while collecting specimens of the nocturnal species *T. texana* (also for expected TEM study), I became annoyed by entomologists who had never seen Strepsiptera fly—and often had never seen them alive at all—dispute claims I made about strepsipteran flight ability. Thus, in addition to bolstering my knowledge of optics, to produce supporting evidence, I photographed flying Strepsiptera (Ch. 3) because it had never been done before. Furthermore, after Hrabar et al. (2014) presented evidence that even males of the diurnal species *X. peckii* do not visually identify calling females, it became clear to me that vision in Strepsiptera primarily services flight. Therefore, much could be learned about strepsipteran vision from analyzing strepsipteran flight (Ch. 3).

Even if high acuity or enhanced resolution plays no part in strepsipteran vision, in interacting with live *E. koebelei* and *T. texana*, I saw that their sensory interplay and bodily dynamics provide the underpinnings for impressive aerobatics.

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RESEARCH ARTICLE

The unusual eyes of *Xenos peckii* (Strepsiptera: Xenidae) have green- and UV-sensitive photoreceptors

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Abstract

The highly specialized evolution of Strepsiptera has produced one of the most unusual eyes among mature insects, perhaps in line with their extremely complex and challenging life cycle. This relatively rare insect order is one of the few for which it has been unclear what spectral classes of photoreceptors any of its members may possess, an even more apt question given the nocturnal evolution of the group. To address this question, we performed electroretinograms on adult male *Xenos peckii*: we measured spectral responses to equi-quantal monochromatic light flashes of different wavelengths, and established *V*log*I* relationships to calculate spectral sensitivities. Based on opsin template fits, we found maximal spectral sensitivity (λ_{max}) in the green domain at 539 nm. Application of a green light to 'bleach' green receptors revealed that a UV peak was contributed to by an independent UV opsin with a λ_{max} of 346 nm. Transcriptomics and a phylogenetic analysis including 50 other opsin sequences further confirmed the presence of these two opsin classes. While these findings do not necessarily indicate that these unorthodox insects have color vision, they raise the possibility that UV vision plays an important role in the ability of *X. peckii* males to find the very cryptic strepsipteran females that are situated within their wasp hosts.

Key Words: Invertebrate vision, Color vision, Spectral sensitivity

Introduction

Strepsiptera are a small, curious order of obligate endoparasitic insects whose complex life histories have raised many unanswered questions. These include several aspects of their visual physiology, which is the subject of this investigation.

Strepsiptera have diverged so strongly from other insect orders that they are best known for the extreme difficulty of placing them phylogenetically (Kristensen, 1981; Wiegmann et al., 2009). Recent research has resolved the issue quite satisfactorily, however: there is now very strong morphological and genomic evidence supporting Strepsiptera as the sister group to Coleoptera (Cook, 2014; Niehuis et al., 2012). *Xenos peckii* Kirby 1813 is a diurnal species of Strepsiptera that uses paper wasps as its host. As in most strepsipteran species, adult female *X. peckii* never leave their host. Instead, adult females are larviform, lacking wings, eyes, and legs. Unlike any other known holometabolous insect, adult female Strepsiptera mature without pupating (Kathirithamby, 1989). Their neotenic bodies remain within their hosts, within which they give birth to live young. These triungulins are mobile and find new hosts by entering into wasp

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larvae of the same nest, or by riding uninfected wasps to other nests to enter larvae there (Hughes et al., 2003). Toward the end of summer, developing *X. peckii* breach the cuticle of the abdomen of their, by then, adult wasp hosts. Males pupate without exiting their hosts, and only later eclose, becoming airborne immediately. Mature, unmated females emit a sex pheromone (Cvačka al., 2012; Tolasch et al., 2012), which attracts adult males (Fig. i.1A) through olfaction. In so doing, females also protrude their cephalothoraxes out of the wasp abdomen, potentially providing an additional visual signal. Adult male *X. peckii* are about 4 mm long. They, like other male Strepsiptera, have a very well-developed flight apparatus (Pohl and Beutel, 2008), including halteres that are homologous with the forewings of other insects and are important for flight control (Pix et al., 1993). By means of their semicircular hindwings, they are able to fly immediately upon eclosing from the pupal case (Smith and Kathirithamby, 1984). Once airborne, with the assistance of their elaborate antennae and prominent eyes (Buschbeck et al., 1999, 2003), they search incessantly for a virgin female with which to mate. Males die within a few hours of eclosing, but females persist long enough for their offspring to mature. In the case of *X. peckii*, this includes overwintering, which they are able to induce even in unmated wasps by hormonal manipulation (Strambi and Girardie, 1973).

Strepsipteran eyes are remarkable. Unlike typical compound eyes, which consist of ommatidia that each collect information from a single point in space, the strepsipteran eye is constructed of a number of single-chamber eyes that are aggregated into a larger eye (Fig. i.1B). A single-chamber eye differs from an ommatidium in that it has a retina large enough to contain spatial information. In *X. peckii*, each eyelet has a retina that consists of about 100 receptors (Buschbeck et al., 1999), onto which a small image is projected. Because of the characteristics of lenses, the image is inverted within each eyelet. However, in *X. peckii*, the original orientation of each image is restored via downstream wiring (Buschbeck et al., 2003), allowing the eye as a whole to (potentially) produce a combined image of higher acuity (Maksimovic et al., 2007) than the 50 or so pixels that *X. peckii* would be able to represent if each of the eyelets only resolved a single point in space (as is typical for compound eye ommatidia).

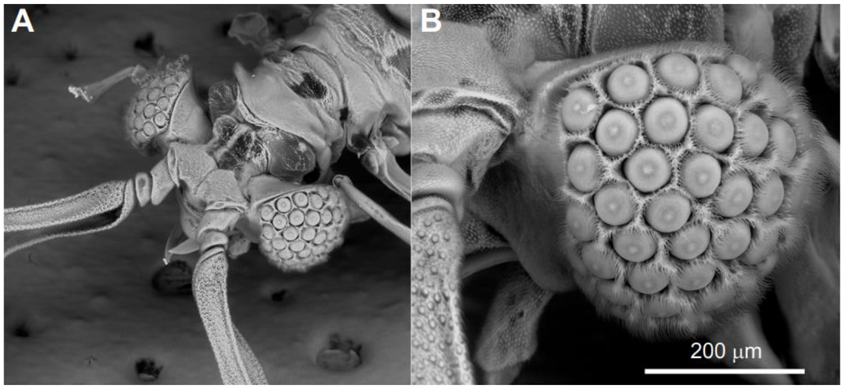

Fig. i.1. Twisted-wing parasites such as *Xenos peckii*, are characterized by 'eyelets' of unusually large diameter, each of which contains its own extended retina. (A) Overview of an adult male *X. peckii* head, illustrating the presence of two large eyes. (B) A magnification of the left eye illustrates the shape and position of individual eyelet lenses, each of which is surrounded by dense setae ('hairs').

While this extraordinary eye organization continues to inspire novel camera designs (Brückner et al., 2011; Druart et al., 2009; Keum et al., 2016), its evolution remains unclear. However, some insight can be gained from the fact that a large number of strepsipteran species appear to be nocturnal (Pohl and Beutel, 2008). Although rarely experimentally confirmed, the inability of adult male Strepsiptera to feed or drink (Pohl and Beutel, 2008), coupled with the relative frequency with which males (particularly those of basal clades) are caught in light traps (Kathirithamby, 1989; Khalaf, 1968; Shepard, 1979), the activity patterns of their hosts and the absence of sightings of free-flying males, all support strepsipteran nocturnal ancestry. Furthermore, several attributes of the strepsipteran eye – even those of diurnal species – are reminiscent of nocturnal insects, raising the possibility that even though *X. peckii* is a diurnal species, their extraordinary eyes owe their existence to a nocturnal evolutionary history (Buschbeck et al., 2003). As photons are limited at night, one might expect that a nocturnal lifestyle could lead to a reduction of photoreceptor classes, and there is evidence for such reduction, at least in mammals. Most mammals have dichromatic vision (Osorio and Vorobyev, 2008), but some nocturnal groups have become monochromatic (Kelber et al., 2003). When light is dim, available photons may be used to boost sensitivity rather than the ability to discriminate color. It is therefore plausible that the nocturnal ancestry of Strepsiptera led to the reduction or absence of color vision in this group. It is also notable that in insects known to have color vision, the color-mediating photoreceptors typically pass straight through the lamina, the first neuropil of the visual system, and terminate in the second layer, the medulla (Morante and Desplan, 2008). In contrast, photoreceptors that are associated with motion vision tend to terminate in the lamina (Heisenberg and Buchner, 1977). In *X. peckii*, all identified projections terminate in the lamina (Buschbeck et al., 2003), possibly indicating that color vision in this insect group is absent. However, more recent data have emerged indicating that apparently parallel visual pathways are not as clearly separated as has long been believed (Kelber and Henze, 2013). For example, in *Drosophila*, it has been demonstrated that the outer photoreceptors R1–R6 (which terminate in the lamina) can also mediate color vision (Schnaitmann et al., 2013). Despite severely reduced light levels at night, it has been noted that color vision in nocturnal insects is more common than historically believed (Kelber and Roth, 2006). For example, the hawk moth, *Deilephila elpenor*, can distinguish colors by dim starlight (Kelber et al., 2002). The majority of insects studied so far have three color channels, with photoreceptors specialized for absorbing light in the green, blue, and UV ranges (Briscoe and Chittka, 2001; Kelber, 2006; Osorio and Vorobyev, 2008). Taken together, the question of whether or not Strepsiptera have the visual machinery necessary to detect color arises. To address this question, we used extracellular recordings (electroretinograms, ERGs) of photoreceptor responses to equal-intensity but differently colored light flashes to investigate the spectral response properties of *X. peckii*, a diurnal strepsipteran, and among the best-known species in this order.

Materials and Methods

The strepsipteran *X. peckii* is relatively difficult to find, but in mid-summer 2012 we came across a fertilized female Strepsiptera within its queen *Polistes fuscatus* host. This female subsequently produced triungulins that allowed us to raise a generation of Strepsiptera. To do so, the host wasp was kept separately in a cool and dark environment, and over a period of 10 days she was periodically handled to elicit the emergence of triungulins. These were then picked up with a soft brush and placed directly onto *Polistes fuscatus* larvae of colonies that were reared separately in small wooden nest boxes. To access wasp larvae, nest boxes were cooled to 4°C. This allowed triungulins to be placed onto the nests without interference from the adult wasps that tend their larvae. Adult wasps were fed honey–water and freshly killed crickets. Once stylopized wasps emerged, they were monitored closely, and separated from the nest as soon as *X. peckii* puparia became visible.

In our laboratory, eclosed adult male Strepsiptera were only fully healthy for 2–3 h at room temperature. Therefore, one of the biggest challenges was to secure them immediately after emergence. To do so, we moved stylopized wasps into a dark chamber and then every morning, or every other morning, we placed them in separate containers under bright light that triggered the emergence of mature males. When multiple adult male Strepsiptera eclosed in rapid succession, some of them were placed in a refrigerator at 4°C for up to 3 h to keep them viable until we could record from them.

ERGs

To record ERGs from the *X. peckii* eye, each insect was immobilized by mounting it on a cover-slip using dental wax. A cotton wick inserted into a glass capillary tube filled with a solution of NaCl (0.9% w/v NaCl) served as the measuring electrode and was placed on the surface of the eye. The reference electrode was another glass electrode, also filled with NaCl solution and placed into the abdomen of the

Strepsiptera. All recordings were performed in a Faraday cage, on a TMC 66-501 vibration isolation table (Technical Manufacturing Corporation, Peabody, MA, USA) using standard electrophysiological equipment, including an A-M Systems Neuroprobe amplifier 1600 (A-M Systems, Inc., Sequim, WA, USA), Tektronix Oscilloscope 5111A (Tektronix, Inc., Beaverton, OR, USA) and an iWorx Data Aquisition System (HAI 118, iWorx Systems, Inc., Dover, NH, USA). Data were acquired at a sampling rate of 10,000 Hz and stored on a PC computer using iWorx LabScribe software (iWorx Systems, Inc.), and analyzed as outlined below using customized programs (available upon request) in MATLAB (The Mathworks, Inc., Natick, MA, USA). The eye was then stimulated with equi-quantal monochromatic light pulses and the voltage responses of photoreceptors were recorded. These light flashes were obtained from a 150 W xenon arc lamp coupled to an Oriel Cornerstone 130 ⅛ m 74000 monochromator (Oriel Instruments, Stratford, CT, USA). The intensity of the stimulus was controlled with a Newport circular variable neutral density filter 50Q04AV.2 (Newport Corporation, Irvine, CA, USA) operated with a Newport Newstep Controller NSC200 (Newport Corporation). The filter was mounted onto a Newport NSR-12 motorized rotator stage (Newport Corporation) and placed in line with the output slit of the monochromator. A converging lens (f=10 cm) was used to focus the light from the monochromator onto the tip of an optic fiber, the other end of which was positioned a few millimeters from the strepsipteran eye. Prior to the experiment, the intensity of the light at the tip of the fiber was calibrated using an Ocean Optics USB2000+ spectrometer (Ocean Optics, Inc., Dunedin, FL, USA). Specific neutral density filter positions allowed for equi-quantal light stimulation at different wavelengths.

To assess the spectral response, the intensity of monochromatic light flashes was pre-calibrated to 6.5×10^{13} photons cm^{-2} s^{-1} for all wavelengths (stimulus intensities ranged from 6.0×10^{13} to 6.8×10^{13} photons cm^{-2} s^{-1}). A typical spectral response recording consisted of equi-quantal monochromatic light stimuli ranging from 300 to 640 nm in 20 nm steps. To verify the stability of the recording, this was followed by a set of simulations in the opposite direction (640–300 nm). For most animals, additional recordings were taken later in the experiments, and data were averaged over up to four measurements for each animal. For each of these measurements, at each wavelength, three consecutive flashes (each 300 ms long with a 1.7 s interval) were presented. A 10 s time interval between consecutive wavelengths allowed the eye to recover between light stimulations of different wavelengths.

Immediately afterwards, responses to monochromatic light stimuli at 500 nm (near the putative peak) ranging from 4.8×10^{11} to 8.5×10^{14} photons cm^{-2} s^{-1} in 0.25 log steps were recorded to later generate the response–stimulus intensity (Vlog$I$) function.

As initial results showed a secondary peak around 350 nm, we performed additional measurements to determine whether UV sensitivity is independent of green sensitivity, or whether it merely reflects a typical beta peak of a green opsin (Stavenga et al., 1993). To do so, we re-measured the response to light pulses across the spectrum while using a green LED (525 nm; superbrightleds.com) to 'bleach' green receptors ('green-bleach'). As these measurements revealed a prominent peak in the UV range, the Vlog$I$ relationship was also established for the green-bleach paradigm for intensities of 380 nm light ranging from 4.78×10^{11} to 2.68×10^{14} photons cm^{-2} s^{-1}. Finally, a 380 nm UV LED (RL5-UV0315-380 from superbrightleds.com) was used to bleach out the majority of the response across the spectrum.

Analysis

Both the spectral response and Vlog$I$ results were analyzed using in-house MATLAB code. Briefly, data were first smoothed with the following function: (filter(ones(1,windowsize)/windowsize,1,data)), with windowsize=50. For each pulse, a baseline value was determined as the average of 100 points surrounding stimulus onset (see red points in Fig. i.2B). The response was defined as the average of 100 points (equaling 10 ms) surrounding the minimum response that occurred during each stimulation (see magenta points in Fig. i.2B).

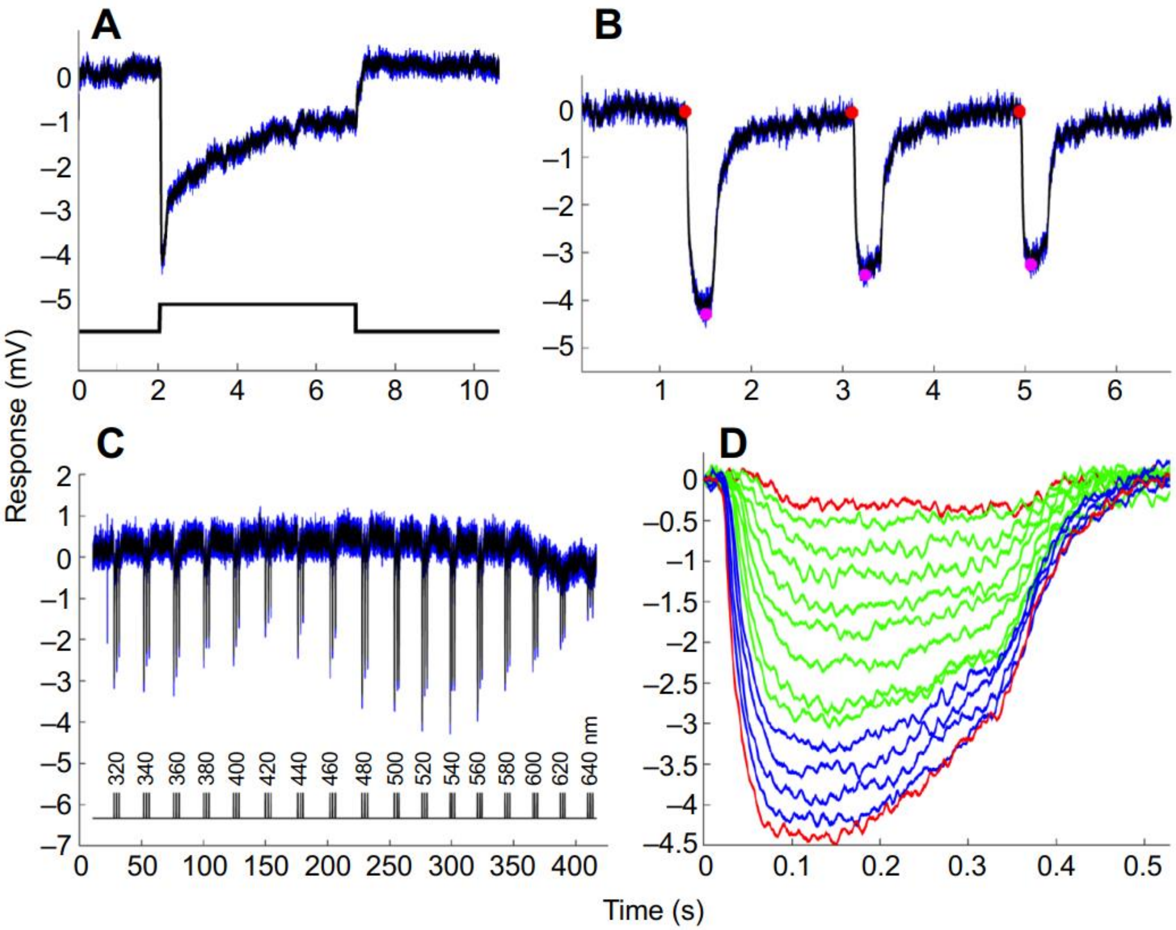

Fig. i.2. Examples of electroretinogram (ERG) responses. (A) Response to a bright green light pulse near saturation. As in other insects, the response is characterized by a fast transient component, as well as an extended receptor potential. (B) Example of the three consecutive responses that were the basis of our spectral response measurements. In this example, green (520 nm) light pulses were administered at 6.5×10^{13} photons cm^{-2} s^{-1}. Red dots indicate the base values and magenta dots indicate response values that were identified by our in-house analysis program. (C) Example recording of the entire spectrum, from 320 to 640 nm wavelength. At each wavelength, three pulses were administered as indicated in B. The bottom trace illustrates stimuli (and wavelength values) and the top trace illustrates the recorded response (in blue, with smoothed data in black). (D) Superimposed responses to green light of all intensities that were presented in our *V*log*I* measurements. The weakest and strongest responses are illustrated in red, and intermediate responses are in green and blue. The light intensity that was used for further analysis lies between the intensity that elicited the largest response that is plotted in green (4.8×10^{13} photons cm^{-2} s^{-1}), and the intensity that elicited the smallest response that is plotted in blue (8.5×10^{13} photons cm^{-2} s^{-1}), demonstrating that photoreceptors were not saturated in these measurements.

To validate the stability of our recordings, multiple measurements were plotted on top of each other. As the analysis revealed that for each wavelength the first pulse was systematically larger than the other pulses, the analysis was performed for the first pulse of the three stimuli only, as well as averaged across the three pulses. Because comparison of these two analyses revealed no systematic difference in regards to the spectral findings (Fig. i.3A,B), further analysis was completed by averaging the results of the three pulses.

To convert our spectral response measurements to spectral sensitivity curves, we used the hyperbolic Naka–Rushton (NR) function (Eqn 1), where V_{max} is the maximum response amplitude, I is the stimulus intensity, k is the stimulus intensity at $V_{max}/2$ and n is the slope of the function (Menzel et al., 1986; Naka and Rushton, 1966; Skorupski and Chittka, 2010):

$$\frac{V}{V_{\max}} = \frac{I^n}{I^n + k^n}. \tag{1}$$

Our *V*log*I* data were fitted to this function using the MATLAB curve-fitting tool *cftool* to obtain values for *k*, *n*, and $V_{\max}$. To establish the peak green sensitivity, the *V*log*I* data for 500 nm were used. To establish the UV peak, the *V*log*I* data were taken at 380 nm under green-bleach conditions. Each fit was then used to extrapolate the *V*log*I* curves for all other wavelengths. The spectral sensitivity curve was then determined as the reciprocal of the photon count required to elicit equal response amplitudes at wavelengths ranging from 320 to 640 nm. Finally, these spectral sensitivity data were fitted (with *cftool*) to the Govardovskii (Govardovskii et al., 2000) and Stavenga (Stavenga et al., 1993) rhodopsin absorption templates to find the maximal sensitivity of the opsin in question.

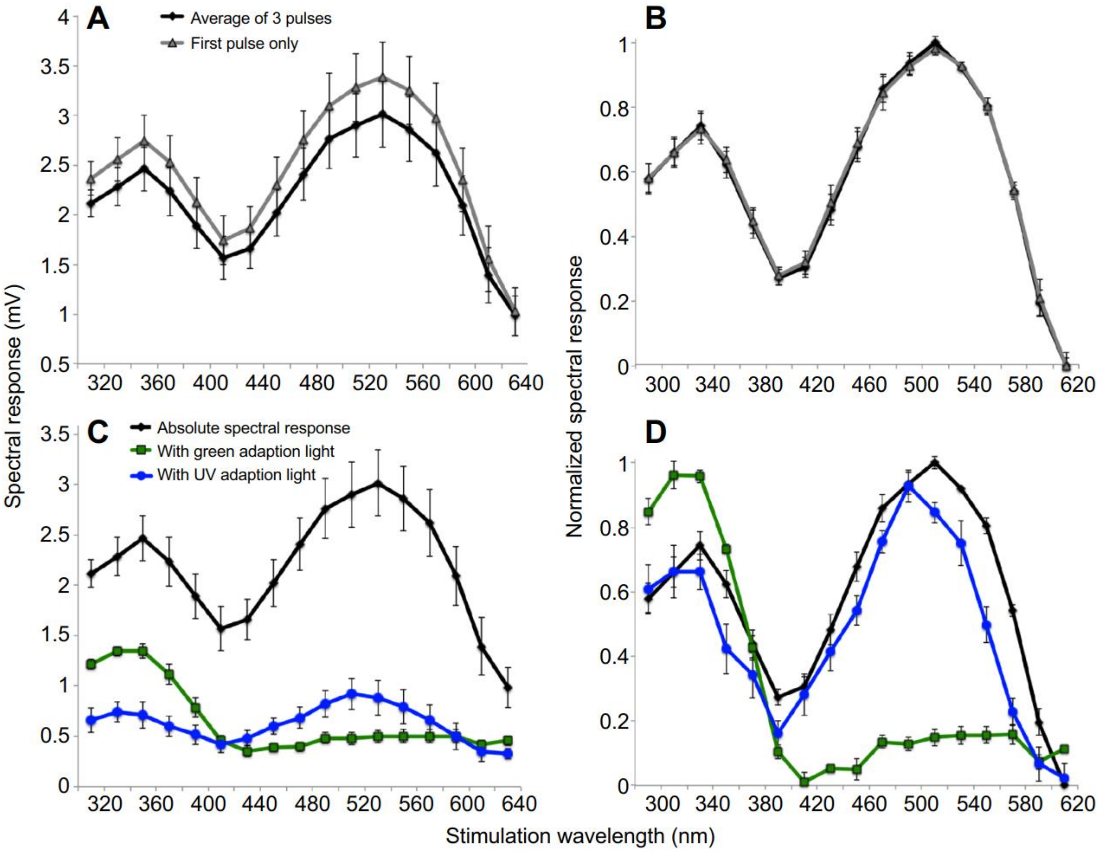

Fig. i.3. Spectral response recordings. (A) Average response curves of our analysis based on all three pulses, and of the first pulse only. (B) These two types of analysis yield essentially identical results when normalized. (C) Under strong selective stimulation of green-sensitive receptors (‘green-bleach’), a UV response remains. In contrast, a UV-bleach light greatly attenuates the entire response, indicating that the long wavelength (LW) opsin contains a beta peak. (D) Normalizing the data illustrates that the spectral characteristics of the response under the UV-bleach light are qualitatively similar to those of the non-attenuated response. All curves represent means±s.e.

Transcriptomics and phylogenetic analysis

The RNeasy Lipid Tissue Kit (Qiagen, Valencia, CA, USA) was used for RNA isolation of two intact animals. To assess the quality of RNA, extractions were subjected to spectrophotometric analysis via a NanoDrop 1000 Spectrometer (Thermo Fisher Scientific, MA, USA) where the A260/280 absorbance ratio yielded measurements of ~2.0 for RNA extracts, indicating that all RNA measurements were relatively pure.

RNA-seq utilized the Illumina HiSeq 2500 (75 bp) with a Ribo-zero preparation at Cincinnati Children's Hospital Core Sequencing Facility (Cincinnati, OH, USA). The raw read FASTQ files were assembled using SeqMan NGen default assembly parameters (DNASTAR. v. 12.0, Madison, WI, USA). Contig annotation was carried out using Blast2GO (BioBam, Valencia, Spain) with default parameters using the blastx database (Altschul et al., 1997). To contrast our mRNA sequences against other opsins, we used the blastx algorithm to predict the amino acid sequences of the opsins. Amino acid sequences of 50 known additional opsins from GenBank (Table S1) were aligned using the ClustalW algorithm (Saitou and Nei, 1987). This alignment was subjected to a neighbor-joining algorithm to perform a phylogenetic analysis as implemented in MEGA v. 6.06 (Tamura et al., 2007). Bootstrap values were derived from 1000 bootstrap replicates.

Results

ERGs and spectral response measurements

Our initial recordings of longer light stimuli revealed that the wave shape of the strepsipteran ERG looks like that of typical insect photoreceptors (Fig. i.2A). Near-saturation responses are characterized by a transient strong response, followed by an extended persistent activation. To measure the spectral response, three consecutive light pulses of equal wavelength and intensity were used, resulting in responses as illustrated in Fig. i.2B. Fig. i.2C illustrates the raw data for one recording from 320 to 640 nm, in which particularly strong responses are notable around 350 nm as well as around 540 nm. For further analysis (see below), and to ensure that our measurements were performed within the linear range of the receptor response, we also established the relationship between stimulus intensity and response at 500 nm. A set of response curves to each light intensity illustrates minor light intensity-related changes in the overall shape of the responses (Fig. i.2D). Spectral response measurements were performed at a light intensity of $\sim 6.5 \times 10^{13}$ photons cm^{-2} s^{-1}. This intensity elicited responses that were in the upper mid-portion of the receptor's range.

Our initial analysis revealed that the second and third pulse of each stimulus consistently showed a slightly smaller response, presumably because the receptors did not fully dark adapt between pulses. Independent analysis of only the first pulse, and of all three pulses, showed comparable results in regard to the spectral qualities of the data, with the main difference being that the three-pulse analysis led to slightly smaller response magnitudes than the first-pulse-only analysis (Fig. i.3A). However, normalization of the data led to essentially identical traces (Fig. i.3B), demonstrating that the two analyses are comparable with respect to spectral response properties of the strepsipteran eye.

Because our initial analysis revealed the presence of a peak in the UV region, we performed further tests to establish whether this UV response simply represents the beta peak of a long wavelength opsin or whether it could be the manifestation of an independent UV opsin. Specifically, we used a green-bleach light (at 525 nm) to saturate the green receptor. The rationale of this experiment is that constant activation of the green opsin leads to a constant response (both its UV and green components) independent of additional stimulation of opsins that are outside the range of the 'bleach light'. Fig. i.3C illustrates that under these conditions, a UV response remained (though reduced in size), whereas the green response was essentially absent, indicating that at least a portion of the initial UV response was independent of the green opsin. In contrast, the application of a UV-bleach light resulted in a strongly reduced response across the spectrum, indicating that all opsins that contributed to the initial response had a UV component; when normalized, this response curve regained a shape comparable to that produced by the original measurements (Fig. i.3D), suggesting that the UV-bleach attenuated the response approximately equally throughout the spectrum.

Establishment of spectral sensitivity maxima

To convert our spectral response measurements of the green peak to spectral sensitivity data to which opsin templates could be applied, we first established the photoreceptor response characteristic of a series of 500 nm light pulses of different intensities (4.8×10^{11} to 8.5×10^{14} photons cm^{-2} s^{-1}). Fig. i.4A illustrates these measurements for each of the seven male Strepsiptera that were measured, as well as the NR function (Naka and Rushton, 1966) fit that was used to calculate the *V*log*I* response, and to convert the data to spectral sensitivity curves. Govardovskii (Govardovskii et al., 2000) and Stavenga (Stavenga et al., 1993) opsin templates were then applied to the green peak (situated between 440 and 620 nm) of each spectral response curve. The Govardovskii template resulted in maximal sensitivities (λ_{max}) between 533.7 nm and 545.9 nm with a mean (±s.e.) peak sensitivity of 538.7±1.7 nm. The Stavenga template resulted in nearly identical results, with a λ_{max} between 533.6 nm and 546 nm and a mean (±s.e.) peak sensitivity of 538.7±1.7 nm. Template fits to these mean sensitivity values, as well as the mean (±s.e.) measurements are illustrated in Fig. i.4B. To establish the spectral sensitivity maxima of the UV opsin, we determined the photoreceptor response characteristic of a series of 380 nm light pulses of different intensities (4.78×10^{11} to 2.68×10^{14} photons cm^{-2} s^{-1}), while applying the green-bleach light. Fig. i.5A illustrates these measurements for each of the five male Strepsiptera that were successfully measured, as well as the NR function (Naka and Rushton, 1966) fit that was used to calculate the *V*log*I* response, and the conversion to spectral sensitivity curves. Two measurements were excluded based on electrical noise that confounded the analysis. The Govardovskii template resulted in λ_{max} values between 331.4 nm and 354 nm with a mean (±s.e.) sensitivity of 346.1±4.1 nm. Here too, the Stavenga template resulted in nearly identical results, with a λ_{max} between 331.3 nm and 353.8 nm and a mean (±s.e.) sensitivity of 345.9±4.1 nm. Template fits to these mean sensitivity values, as well as the mean (±s.e.) measurements are illustrated in Fig. i.5B.

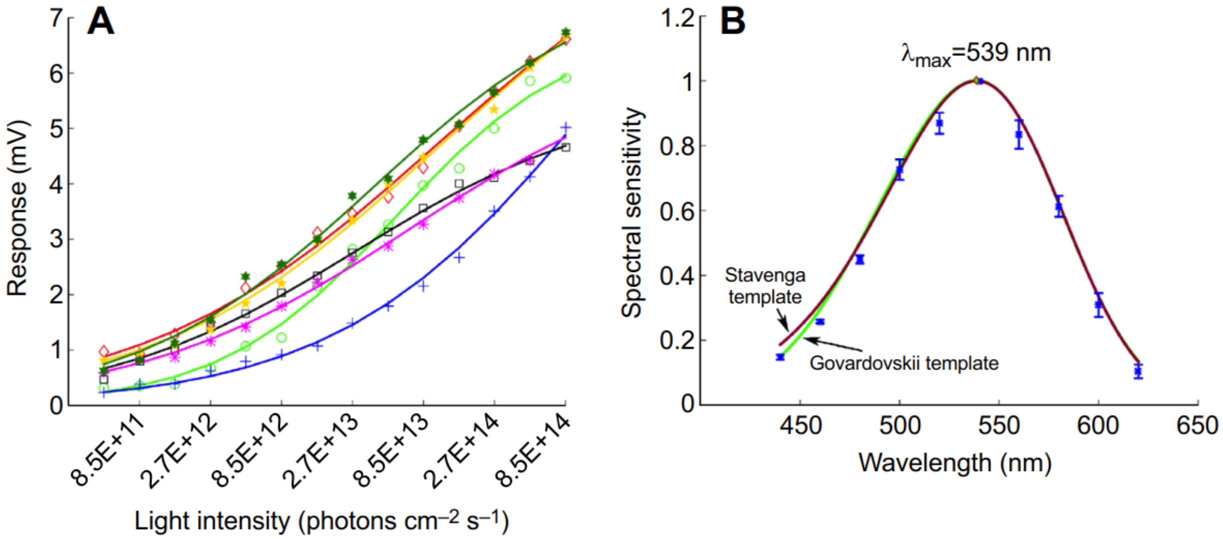

Fig. i.4. The spectral sensitivity of the green-sensitive opsin. (A) The *V*log*I* relationship for 500 nm light stimuli was established in seven Strepsiptera (measurements for each individual are illustrated with their own color and symbol) and fitted with the Naka–Rushton (NR) function (see Eqn 1; plotted in respective colors). (B) Values obtained from the NR function were used to calculate spectral sensitivity curves to which Stavenga (brown) and Govardovskii (green) templates were applied. Both fits resulted in a λ_{max} of 539 nm.

Transcriptomics and phylogenetic analysis of opsins

We used a molecular approach to independently investigate photoreceptor types that may be present in the strepsipteran eye.

Specifically, we identified possible opsins from a transcriptome of male *X. peckii*. The 23,308,238 reads, 75 bp in length, from this project have been deposited in the NCBI Sequence Read Archive. Their *de novo* assembly aligned a total of 9854 contigs. One tool utilized to assess the assembly quality was the contig N50, which resulted in an average length of 1253 bp, which on average was represented 12 times. From the de novo assembly, a total of 6879 contigs were assigned an annotation, including two opsin proteins: one long-wavelength sensitive and one UV sensitive (see below for GenBank accession numbers). To determine the relative expression of each opsin, we mapped the raw reads back to a templated assembly of the two opsin sequences. The long-wavelength opsin had 8100 sequences that mapped back, whereas the UV opsin only had 630. Our transcriptome did not resolve a blue-sensitive opsin, and there was no evidence for additional long-wavelength or UV opsin types, or any other opsin. We further investigated these categorizations, confirming the presence of a 7-transmembrane class 1 receptor, a sequence typical for opsins, and performed a phylogenetic analysis (Attwood and Findlay, 1994). As shown in Fig. i.6, opsins that share similar spectral characteristics cluster more closely to each other than opsins from different spectral classes. Our phylogenetic tree resulted in monophyletic clades for all LW opsins and for all UV opsins. In our analysis, the long-wavelength opsin (*Xenos peckii* LW) is nested well within the LW opsins clade, and the UV opsin (*Xenos peckii* UV) is nested in the UV clade.

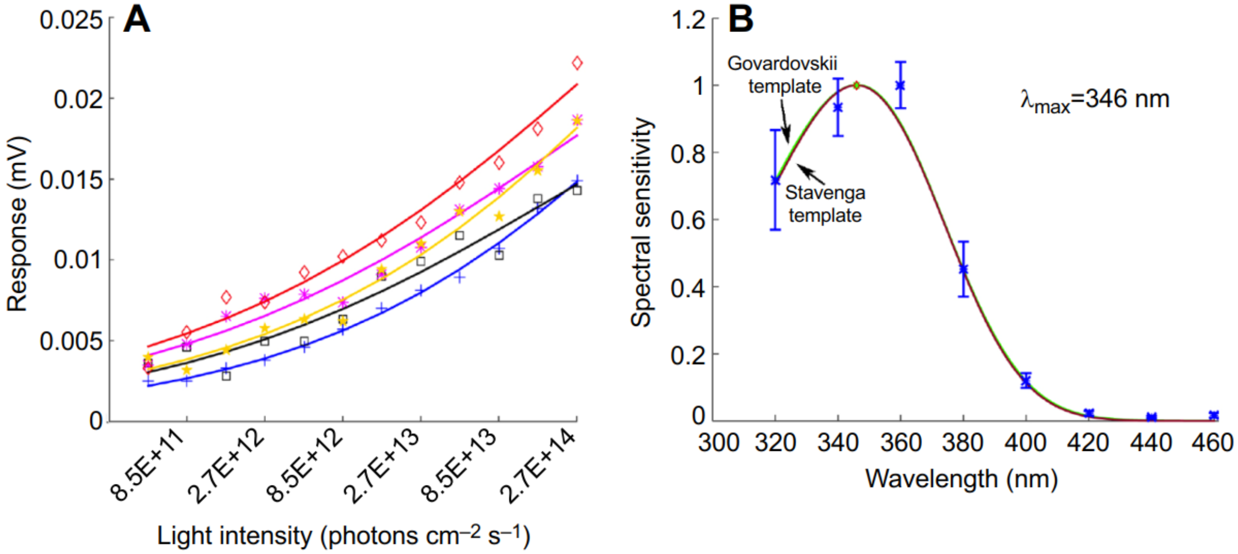

Fig. i.5. The spectral sensitivity of the UV-sensitive opsin. (A) The *V*/log*I* relationship for 380 nm light stimuli was established in five Strepsiptera (measurements for each individual are illustrated with their own color and symbol) during the application of a bleach light, and fitted with the NR function (see Eqn 1; fits are plotted in respective colors). (B) Values obtained from the NR function were used to calculate spectral sensitivity curves to which Stavenga (brown) and Govardovskii (green) templates were applied. Both fits resulted in a λ_{max} of 346 nm.

Discussion

The ability to see and discriminate objects on the basis of their color is an important attribute for the ecology of many organisms. Most insects are thought to have trichromatic vision, the presumably ancestral form, while some (including multiple groups of butterflies) have even evolved tetrachromatic vision (Briscoe and Chittka, 2001; Eguchi et al., 1982), with the addition of a red channel (Bernard, 1979). The ability to differentiate objects based on their color can be important for many aspects of their lives, including the ability to efficiently locate food sources such as flowers, select oviposition sites and find mates. Finding a mate is the most important challenge for an adult male Strepsiptera, whose few hours of eclosed life are only concerned with mating. In *X. peckii*, the larviform female is situated primarily within the abdomen of her wasp host. Although it recently has become clear that she actively participates in attracting a male (Hrabar et al., 2014), only a small and rather cryptic portion of her body is exposed. Still, the male finds her often enough to propagate the species, and his unique strepsipteran eye type (Buschbeck et al., 1999, 2003) may play an important role in that. Given the unorthodox eye organization and the lack of data in regard to what spectral classes of photoreceptors might be present in them, it has been difficult to hypothesize if color vision could be involved. In fact, Strepsiptera are among the few holometabolous insect orders for which spectral sensitivity data had been wholly absent, even though their unconventional eyes make them particularly interesting.

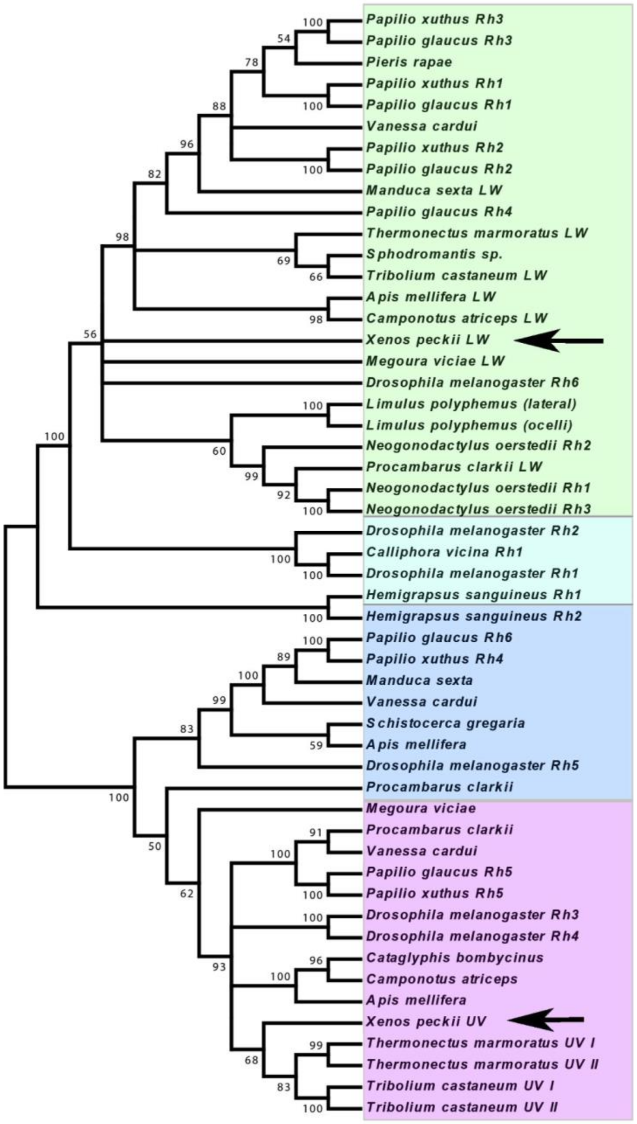

Fig. i.6. Phylogenetic analysis of opsins. A neighbor-joining phylogenetic tree (with bootstrap values) was produced from 50 amino acid sequences obtained through GenBank, as well as our own two *Xenos peckii* sequences, illustrating that the *X. peckii* LW opsin is nested well within the long wavelength clade, and the *X. peckii* UV opsin is well nested within the UV clade.

In part, Strepsiptera have been understudied because they are relatively difficult to find, and the short lifespan of adult males imposes additional challenges for research projects that rely on live specimens, because all data need to be collected within a few hours of their emergence. In this study, we succeeded in lab-rearing a population of Strepsiptera, and in measuring the spectral response characteristics of a representative set of adult males. Our initial measurements showed maximal responses to green light, with a secondary response in the UV domain. Based on our calculated spectral sensitivity and fits to both the Govardovskii (Govardovskii et al., 2000) and Stavenga (Stavenga et al., 1993) opsin templates, the maximal spectral sensitivity is 539 nm, well in line with long-wavelength receptors of other insects. In fact, with typical λ_{max} values of ~530 nm, insects so far have remarkably consistent peak green sensitivities, despite a large variety of ecological backgrounds (Briscoe and Chittka, 2001). Our assessment of the green sensitivity peak is particularly robust, as all specimens were measured at least twice, and often four times, with comparable results. With fewer measurements (for one specimen we only had one set of measurements and for the remaining specimens we had two), and smaller signal-to-noise ratios, our UV opsin analysis is slightly less robust. In addition to extinguishing the green peak, the green-bleach also reduced the size of the UV peak, indicating that the green opsin has some sensitivity in the UV (as is typical for long-wavelength opsins). Nevertheless, our analysis showed robust results, with both applied opsin templates suggesting a λ_{max} of 346 nm. Like the green sensitivity, the strepsipteran UV sensitivity lies well within the range of UV sensitivities of other insects, and is quite comparable to their typical value of around 350 nm (Briscoe and Chittka, 2001).

It is noteworthy that our initial measurement included stimulation at 300 nm that resulted in a surprisingly high sensitivity, as though Strepsiptera have sensitivity to UVB in addition to UVA. However, for technical reasons the 300 nm stimulus of our setup was least precisely calibrated, so we therefore did not sufficiently trust those data to include them in this publication. Furthermore, no UVB opsin was implicated in our transcriptomic analysis. Nevertheless, it would be worthwhile to further investigate the spectral response characteristics of *X. peckii* in the very short-wavelength domain, especially in the light of recent findings that UVB sensitivity is important in some other arthropods. For example, it plays a role in communication in jumping spiders (Painting et al., 2016), and a UVB receptor of slightly longer wavelength than would be predicted for *X. peckii* has been identified in certain stomatopods (Kleinlogel and Marshall, 2009). It also has been suggested that a powerful cut-off filter could convert a UVA receptor into a UVB receptor in thrips (Mazza et al., 2010).

Although extracellular methods can never be completely conclusive, our data are most consistent with the absence of a blue receptor. Most telling are our recordings with a 520 nm bleach light, resulting in a relatively narrow spectrum (its width at half height was less than 50 nm). As blue receptors have a typical λ_{max} of ~440 nm, it is unlikely that the green-bleach light would have bleached out a blue opsin if it were present. Furthermore, our measurements show that *X. peckii* response curves are at their minimum at 440 nm (Fig. i.3D), making it very unlikely that a blue-sensitive opsin contributed to the measured response curves in any way. Despite ancestral trichromacy, the absence of a blue-sensitive opsin has been noted in several insect orders, including representatives of Coleoptera, Hymenoptera, Neuroptera and Blattodea (Briscoe and Chittka, 2001). Particularly noteworthy is that in the strepsipteran sister group Coleoptera, the absence of a blue-sensitive opsin has been reported more often than its presence, including in the flour beetle *Tribolium castaneum* (Jackowska et al., 2007), and at least in the larval form of the diving beetle *Thermonectus marmoratus* (Maksimovic et al., 2009, 2011). In some fireflies, blue opsins also may be absent, or at least restricted to certain areas of their visual fields (Lall et al., 1982). This group of beetles is also known for variable green sensitivity in diurnal and nocturnal species, both through tuning of screening pigments and shifts in opsin sensitivity (Cronin et al., 2000). Overall, our findings raise the possibility that blue opsins are frequently absent within the entire coleopteran–strepsipteran clade.

In *X. peckii*, the condition of two photopsins is further supported through our transcriptomics analysis. Our initial BLAST results identified a long-wavelength- and a UV-sensitive opsin. The placement of these two opsin genes in our phylogenetic analysis confirmed that prediction, as in both cases strepsipteran opsins are well-nested within opsins of the same spectral class. Though spectral characterization of Coleoptera has been limited to date, it is satisfying to note that the *X. peckii* UV opsin is positioned at the base of the beetle clade, which is in line with current phylogenetic theory that places Strepsiptera as sister group to Coleoptera (Niehuis et al., 2012). Finally, we would like to emphasize that the presence of two distinct opsins does not mean that *X. peckii* has actual color vision. Color vision requires direct comparison of identical visual fields, the possibility of which largely depends on whether or not UV and green receptors project into the same eyelets. Backfills from portions of the eye showed that photoreceptors of respective eyelets terminated in the lamina (Buschbeck et al., 2003), as though these eyelets were characterized by only one receptor type. But UV and green receptors could be intermingled, leading to similar histological projections, or, alternatively, there could be specializations within the eyelet array, such as a dorsal rim area, which in other insects is converted to a UV-rich polarization sensor (Dacke et al., 2002; Labhart, 1980). Based on the number of reads that mapped to the two opsins, the green opsin appears to be more widely expressed than the UV opsin, but further molecular studies, such as expression analysis, are necessary to resolve this question. Based on the opsin sequence, such studies can now be executed when additional material becomes available. True color vision also depends on the presence of a neural substrate that can adequately process photoreceptor input. However, the presence of distinct UV and green opsins suggests that UV–green coloration could play a significant role in strepsipteran ecology, such as helping the male to find the female. Toward that end, it would be interesting to determine whether the *X. peckii* female, which is rather cryptic in the visual spectrum, selectively reflects UV. If so, this could help explain another aspect of the complex life cycle of these extraordinary insects.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.J. wrote portions of the manuscript and assisted in the data collection. S.P.N. collected the majority of the physiological data and A.S. worked on transcriptomics and bioinformatics. E.K.B. organized specimens, conceived and designed experiments, analyzed the physiology data and drafted and revised the manuscript.

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Data availability

Transcriptomic data were deposited in the NCBI Sequence Read Archive, and are available under SRP090411. Opsin sequences were submitted to NCBI GenBank and are available under the following accession numbers: BankIt1954825 long-wavelength, KX898496 and BankIt1954825 UV-wavelength, KX898497. ERG analysis software will be shared upon request.

Supplementary information

Supplementary information is available online at
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Light trap capture of live *Elenchus koebelei* (Strepsiptera: Elenchidae)

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Abstract

Strepsiptera are a small order of obligate endoparasitic insects. Adult females are neotenic and never leave their host, instead bearing motile young that seek out their own insect hosts to infect. Males eclose without killing their hosts. In their 4 h adult lifespan, they fly off to search for mating opportunities, assisted by unconventional eyes with few, but large, ommatidia. Such distinctive features make Strepsiptera interesting in their own right, but also offer an opportunity to better understand evolutionary innovation. Unfortunately, Strepsiptera also are minute, reclusive, and difficult to obtain, severely reducing the study thereof, especially species not infecting solitary bees or social wasps. Here we describe methods for the successful capture of a strepsipteran species. We placed an ultraviolet light trap among *Spartina alterniflora* Loisel (Poaceae) shoots to attract adult male *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae) in salt marshes in the southeastern United States. In 72 d of sampling, 488 adult males were captured between 30 min before and 15 min after sunrise. None arrived more than 63 min before or 36 min after sunrise. The majority of *E. koebelei* were caught at wind speeds ranging from 0 to 10 km/h; however, a light breeze of about 1.5 km/h appears to be preferred. The highest daily catches occurred when the temperature was between 23 and 26 °C. No Strepsiptera were caught at temperatures below 17 °C. With 521 adult male *E. koebelei* caught in a single light trap, our results show this little-known parasite may be reliably obtained, enhancing opportunities for further study.

Key Words: temperature; time; wind speed; *Spartina alterniflora*; twisted-wing parasite; crepuscular

Resumen

Strepsiptera es una pequeña orden de insectos endoparasitarios obligados. Las hembras adultas son neoténicas y nunca abandonan a su hospedero, sino que tienen jóvenes móviles que buscan a sus propios insectos hospederos para infectar. Los machos eclosionan sin matar a su hospedero. En su vida adulta de 4 horas, vuelan para buscar oportunidades de apareamiento, asistidos por ojos no convencionales con pocos, pero grandes, ommatidios. Tales características distintas hacen que Strepsiptera sean de interés por ellos mismos, pero también ofrecen una oportunidad para comprender mejor la innovación evolutiva. Desafortunadamente, los estrepsípteros también son pequeños, solitarios y difíciles de obtener, lo que reduce drásticamente el estudio de los mismos, especialmente las especies que no infectan a las abejas solitarias o las avispas sociales. Aquí describimos métodos para la captura exitosa de una especie de Strepsiptera. Colocamos una trampa de luz ultravioleta entre los brotes de *Spartina alterniflora* Loisel (Cyperales: Poaceae) para atraer al macho adulto de *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae) en las marismas del sureste de los Estados Unidos. En 72 días de muestreo, 488 machos adultos fueron capturados entre 30 minutos antes y 15 minutos después del amanecer. Ninguno llegó más de 63 minutos antes o 36 minutos después del amanecer. La mayoría de los *E. koebelei* fueron atrapados a velocidades de viento que van de 0 a 10 km/h; sin embargo, parece preferible una ligera brisa de aproximadamente 1.5 km/h. Las capturas diarias más altas ocurrieron cuando la temperatura estaba entre 23 y 26°C. No se capturaron los estrepsípteros a temperaturas inferiores a 17°C. Con 521 macho adultos de *E. koebelei* capturados en una sola trampa de luz, nuestros resultados muestran que este parásito poco conocido puede obtenerse de manera confiable, mejorando las oportunidades para un estudio posterior.

Palabras Clave: temperatura; hora; velocidad del viento; *Spartina alterniflora*; parásito de alas retorcidas; crepuscular

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The order Strepsiptera consists entirely of obligate parasites of other insects [Strepsiptera: New Latin, from Greek strepsi- = "twisted" and pteron = "wing"]. The family Elenchidae parasitizes planthoppers (Homoptera: Delphacidae). Adult females have larviform bodies lacking wings, legs, and eyes, and never leave their host insect. They are neotenic, giving birth to motile male and female offspring that crawl or spring onto plants and animals seeking suitable insect hosts to enter. Adult males have the eponymous twisted "wings" of the order (actually halteres). In search of mating opportunities, they emerge from their own hosts and fly with the aid of eyes resembling tiny blackberries (Fig. ii.1). The male inseminates a portal on the cephalothorax of the female (Kathirithamby 1989), which in the case of *Xenos peckii* Kirby (Strepsiptera: Xenidae) is known to be protruded further forth from the living wasp host and inflated to increase perceptibility at times when males are apt to fly (Hrabar et al. 2014). The vast majority of knowledge about Strepsiptera pertains to diurnal species in the genus *Xenos* that infect *Polistes* (Hymenoptera: Vespidae) wasps. This paper introduces a black light technique for capturing the crepuscular strepsipteran *Elenchus koebelei* Pierce (Strepsiptera: Elenchidae). Insights into *E. koebelei* natural history and physiology based on the technique also are presented.

Fig. ii.1. Above: Image of an adult male *Elenchus koebelei* standing on an anesthetic stage. Note its bifurcated antennae, black tapioca-like eyes, modified forewing that forms a haltere (only 1 of the pair is visible), silver sheen hindwings (iridescent in color images), and extensive thorax. Below left: An *E. koebelei* positioned above a penny for size comparison. [Unlike in this image, *E. koebelei* fly upright when closing in on a calling female, with the abdomen tip pointed forward (Muir 1906).] Adjacent are 3 pictures of visibly stylopized planthoppers. Pupating male *E. koebelei* bulge from the sides of their hosts. The arrows indicate puparia.

Typically, female Strepsiptera mate only once (Hughes-Schrader 1924; Kathirithamby 1989), with some evidence of the male dying very soon after mating (Kathirithamby 1989). Many specifics of the life history of *E. koebelei* remain unknown, but based on *E. tenuicornis* Kirby (Strepsiptera: Elenchidae), it is expected that after several weeks in summer (when development times are shortest), the female internally births about 1,500 (Hassan 1939; Kathirithamby 1989) first-instar larvae that later crawl out of her "brood canal" to find new hosts. The brood canal is an initially sealed passage between the female parasite and her cast-off but partially encompassing exuviae, leading to the outside world. The male ruptures this while mating. Eclosed male Strepsiptera only live for a few h (Hubbard 1892; Cook 2014). "Calling" adult virgin females release a mate-attracting pheromone (Cvačka et al. 2012; Tolasch et al. 2012). Insects parasitized by Strepsiptera are referred to as "stylopized."

Elenchus koebelei are minute Strepsiptera (< 1.5 mm) that infect *Prokelisia marginata* Van Duzee 1897 and *P. dolus* Wilson 1982 (Hemiptera: Delphacidae), planthoppers which in turn feed on *Spartina alterniflora* Loisel (Poaceae) in subtropical salt marshes of the southeastern United States (Khalaf 1968; Stiling et al. 1991a). In summer in northern Florida, the planthopper hosts take about 6 wk to mature (Stiling et al. 1991b) and there are at least 5 overlapping generations per yr (Stiling et al. 1991b; Denno 1994). Studying strepsipteran eyes requires live specimens, which necessitated the development of new capture techniques. However, because the majority of Strepsiptera are known from light trap by-catch (Green 1902; Meadows 1967; Shepard 1979), similar methods can be used to catch live males of virtually any known crepuscular or nocturnal Strepsiptera.

Materials and Methods

It was expected that *E. koebelei* would be attracted to ultraviolet electromagnetic radiation from earlier work on *X. peckii* Strepsiptera (λ_{max} = 346 nm) (James et al. 2016). Accordingly, we designed and constructed portable light sources from a pair of 15 watt T8 UV lamps (diam: 2.54 cm; 1 in). One was a black light (BL); the other black light blue (BLB), using General Electric 35884 and 35885 GE F15T8 fluorescent tubes, respectively (45.72 cm; 18 in) (General Electric Corp., Fairfield, Connecticut, USA). Later, when collecting at Wakulla Beach, Florida, we replaced the original black light blue with a longer GE 10531 F40BLB, a 40 watt T12 black light blue (dia.: 3.81 cm; 1.5 in; length: 121.92 cm; 4 ft). All GE black lights have peak emittance at 368 nm (General Electric 2017), but black light lamps provide more visible light. This makes collecting easier, but may not be as attractive to Strepsiptera. General Electric T12 rapid start ballasts were used with all of the lamps. This likely increased the UV output of the T8 bulbs, at the anticipated cost of reduced lifespan. To provide waterproofing, silicon sealed PVC caps were placed over the wiring at the ends of each of the lamps. The lights were hung vertically atop a white sheet draped over a frame of PVC tubing, as shown in Figure 2. Batteries and ballast were protected by a plastic bucket that could rest on the ground at low tide, or be suspended from the trap by wire at high tide. Electrical contacts were soaked in undiluted white vinegar to remove saltwater-induced corrosion. A toothbrush also was used to help clean heavily corroded surfaces. By attaching shorter legs, the base of the frame could sit beneath the canopy of *S. alterniflora*. In the less dense *Spartina* of Wakulla Beach, this coupled with wetting the trap base greatly enhanced *E. koebelei* catch.

A Taylor 9840N Instant Read Digital Thermometer (Taylor, Oak Brook, Illinois, USA) was used to take local temperature measurements. Beginning in late 2015, a Kestrel 5500 Weather Meter (KestrelMeters.com, Minneapolis, Minnesota, USA) was used to record local wind speed, temperature, and humidity.

The custom light trap was used to collect *E. koebelei* Strepsiptera, where previous sweep netting in stands of *S. alterniflora* had revealed substantial numbers of stylopized *Prokelisia marginata* or *P. dolus* planthoppers. Strepsiptera were collected in late Aug through Sep, and a few d in Oct and Nov in 2013; in 2014, only in Oct; and in 2015, in Jul through early Aug (Table ii.1). The main collection sites were the north branch of Guana Tolomato Matanzas National Estuarine Research Reserve, also known as GTM Research Reserve (GTM) (30.0702°N, 81.3447°W), in St. John's County, Florida, just off the Guana River near the Atlantic Coast, and Wakulla Beach (WB) (30.1050°N, 84.2616°W) in Wakulla County, Florida, on the Gulf of Mexico (Fig. ii.3).

Elenchus koebelei males that landed on the light trap were captured and placed into vials via an aspirator or a moistened fine-tipped paintbrush. After returning from a field site, author James identified species under a dissecting microscope by immobilizing captured insects with a FlyStuff Flypad (10.1 × 14 cm; 4.0 × 5.5 in) (Genesee Scientific Corp., San Diego, California, USA) connected to a CO_2 beer regulator paired with a paintball CO_2 tank. Use of a portable CO_2 cylinder (590 or 710 ml; 20 or 24 oz) allowed sample processing in unconventional laboratory settings. Tanks can be refilled inexpensively at scuba diving or paintball shops.

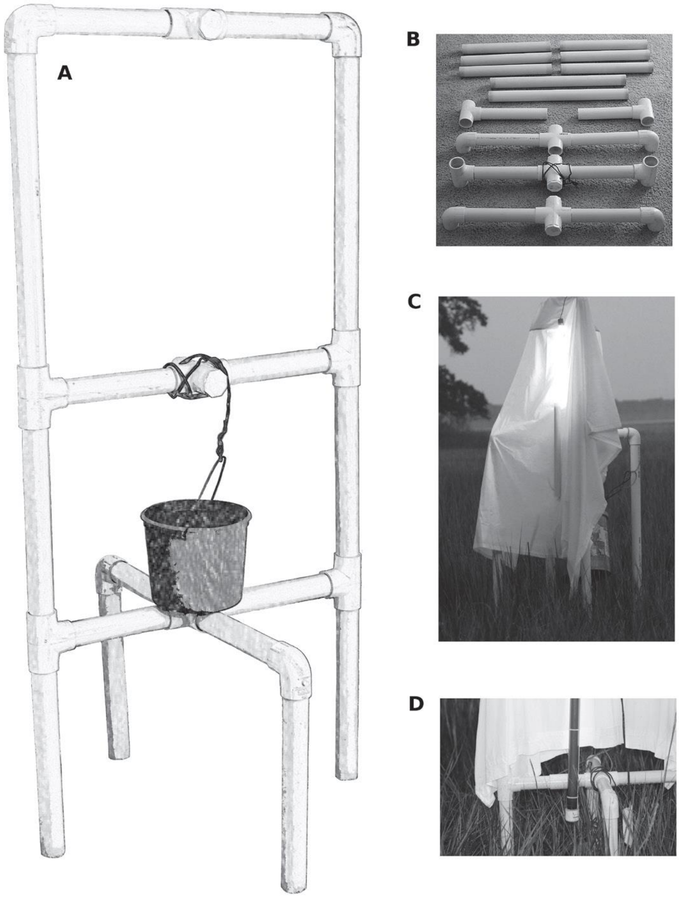

Fig. ii.2. The light trap. A. Schematic of the PVC skeleton. Electronics were placed in the bucket, which could be suspended from the trap at high tide. B. PVC parts. The parts were cemented together (as shown) for strength and ease of construction. C. Trap with sheet and lights in place. In taller grass, longer trap legs can be used to help provide a crease into which attracted Strepsiptera can fly, walk, or fall. This reduces specimen loss through desiccation or drop-off into grass. In short grass, it may be better to wet the trap base and use a long ultraviolet light. D. The base of the trap when used with the long light.

Sunrise is the most relevant timing event for animals that are active around dawn (matinal). Measuring time relative to sunrise allows capture times to be compared at different dates within a field season, between different field seasons, and between separate sites. Although the length of twilight can change dramatically depending on latitude and the time of yr, in northern Florida the duration of civil twilight—the time before sunrise when artificial illumination is not necessary to clearly distinguish terrestrial objects—remained between 23 and 27 min through summer and fall, with an average length of 24:44 (min:s). The duration of nautical twilight—the phase of twilight preceding civil twilight, when the horizon is distinct, but artificial light is necessary to see acceptably on moonless mornings—through the same filed season ranged between 27 and 33 min, with an average of 29:20 (min:s). Astronomical twilight (defined below in *Light sensitivity*) ranged from 27 to 35 min, and averaged 28:53 (min:s). The averages correspond to the twilight times labeled in Figures ii.4 and ii.5.

Variation in the start and duration of collection times is documented in Table ii.2. Effective start and duration times were determined in 2013. That information was exploited in sub-sequent seasons to reduce effort and increase catch. Each season an early start time and long duration was revisited, reaffirming the soundness of abbreviated schedules (Fig. ii.4). Due to processing limitations in 2015, the site was often abandoned early even though additional Strepsiptera could still have been collected.

Species identification

There are several ways to identify adult male *E. koebelei* in the field, even if one has never seen a living Strepsiptera before. First, adult Strepsiptera are not skillful walkers. Because of this and their general frenetic nature, energetic adult males tend to use their wings to assist them in walking. The most telltale sign of an *E. koebelei* is the arc their vibrating wings sweep out. It is difficult to describe, but utterly unlike any other flapping pattern. Once seen, it is immediately identifiable. Second, when less energetic, *E. koebelei* often walk with their wings held together above the thorax. However, the wings swing from side-to-side when they step because their legs are specialized for grasping onto a female, rather than for mobility. Third, although *E. koebelei* are tiny, often their bifurcated antennae can be recognized. When walking, they are extended forward with the branches separated, as with *E. tenuicornis* (Hassan 1939). Initially, one may find it helpful to use a magnifying glass to assist with in-field identification, but after a few sightings, it is normally much more efficient to proceed with the naked eye.

Results

We captured 521 adult male *Elenchus koebelei* using the mobile light trap: 284 from the Guana Tolomato Matanzas Research Reserve on the Atlantic, and 237 from Wakulla Beach on the Gulf of Mexico. These Strepsiptera were caught primarily in mid-Jul through Oct (Table ii.1). Male elenchids have a short eclosed adult lifespan of about 4 h (Cook 2014). Although some Strepsiptera were processed in the field, the remaining live specimens were vigorous at least 2 h after capture, allowing sufficient time for processing or experimentation in the lab.

In 2014, we were available to trap *E. koebelei* only from Oct to Nov, the latter portion of the field season in a yr in which cool temperatures arrived early. During the 3-yr study, no Strepsiptera were captured on 14 mornings (Fig. ii.6; Table ii.1), 11 of which occurred in 2014. Nonetheless, that yr was indispensable for determining the lowest temperature at which *E. koebelei* eclose (Fig. ii.7).

Table ii.1. Collection of adult male *Elenchus koebelei* occurred in late Aug through Sep and on a few days in Oct and Nov in 2013, only in Oct in 2014, and from mid-Jul to mid-Aug in 2015. Wakulla Beach (WB) is on the Gulf Coast of Florida, nearly due south of Tallahassee. Guana Tolomato Matanzas National Estuarine Research Reserve (GTM) is on the Atlantic Coast of Florida, due east of Wakulla Beach.

Site	Year	Sampling days	*Elenchus koebelei* caught	Average caught per sampling day	Average caught live per sampling day
GTM	2013	20	120	6.00	5.25
	2014	11	11	1.00	1.00
	2015	20	153	7.65	4.55
WB	2013	17	236	13.94	13.24
	2014	4	0	0.00	0.00

In 21 field days at Wakulla Beach, the number of live captures per day had a minimum of 0, a lower quartile of 0, a median of 1, a third quartile of 15, a maximum of 60, and a total live catch of 225, with an approximately 62% chance of catching at least 1 live Strepsiptera each morning. However, without including results from 2014, the lower quartile, median, and third quartile improve to 1, 3, and 18, respectively, and the chance of catching a live male rises to 13 out of 17, about 76%. In 51 field days at Guana Tolomato Matanzas Research Reserve, the minimum number of *E. koebelei* captured live was 0, the lower quartile was 1, the median 3, the third quartile 6, the maximum was 23, and the total live catch was 207, with a 45 out of 51 (about 88%) chance of capturing at least 1 live eclosed male each morning. Without 2014, the lower quartile, median, and third quartile improve to 2, 3.5, and 6, respectively, with a 39 out of 40 (97.5%) chance of catching a live male on any sampling day. These values are based on the 432 *E. koebelei* that were collected alive (Strepsiptera can quickly dry out on hot ultraviolet lights and die just before collection), including those caught at unrecorded times.

Most (488 out of 521, about 94%) *E. koebelei* arrived at the apparatus between 30 min before and 15 min after sunrise, as shown in Figure 5. None were captured more than 63 min before sunrise, or 36 min thereafter (Supplemental data). On average, the light trap was operational beginning 49 min before sunrise until 21 min after sunrise.

Apart from finding an area with visibly stylopized planthoppers, the most important determinants of sampling success are temperature (500 out of 521, about 96%, were caught at temperatures ranging from 21.7–26.1 °C; 71–79 °F), wind speed (485 out of 521, about 93%, were caught at wind speeds from 0–11.3 km/h; 0–7 mph), and timing (488 out of 521, about 94%, were captured from 30 min before to 15 min after sunrise).

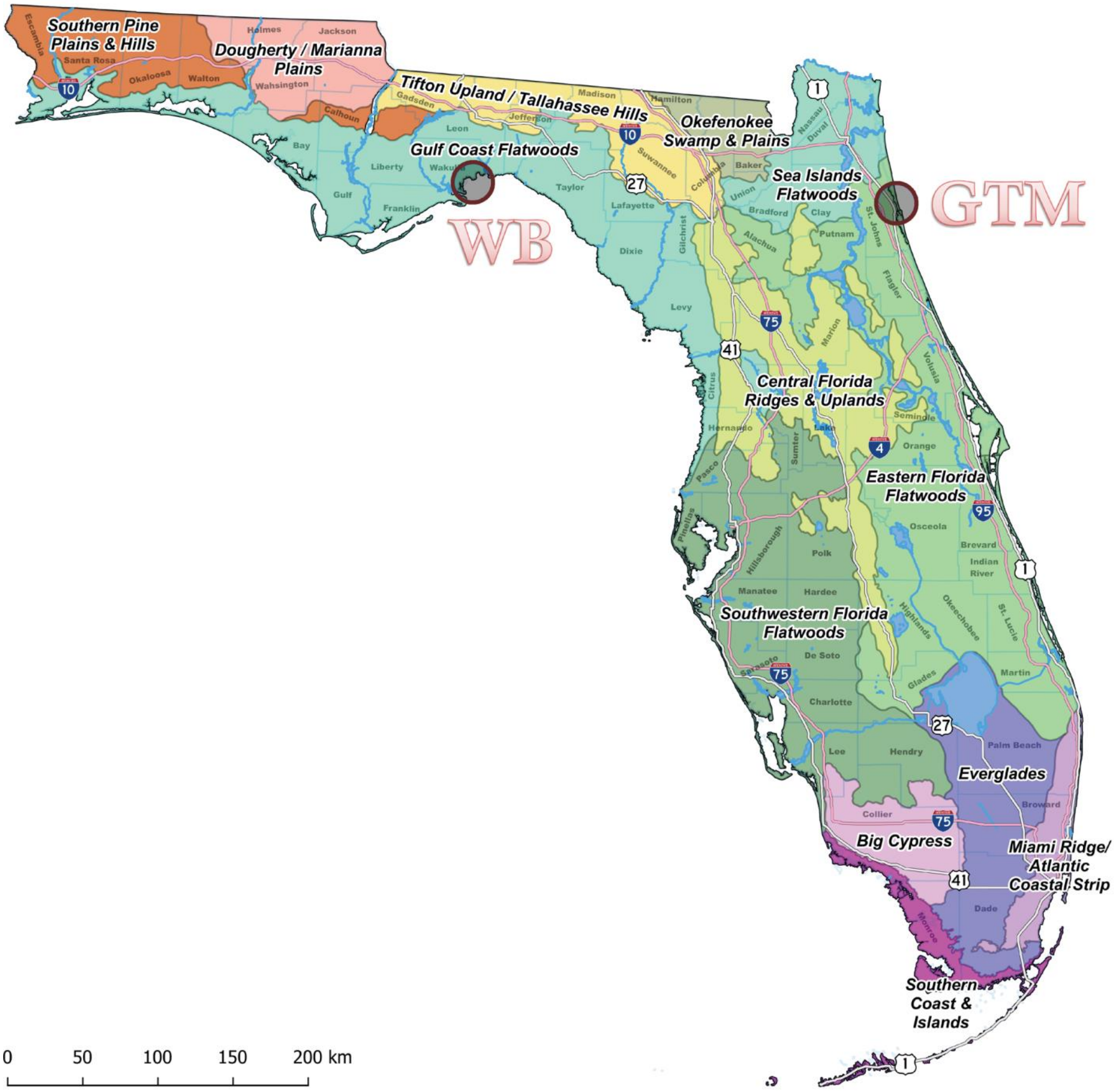

Fig. ii.3. Collection sites: The north branch of the Guana Tolomato Matanzas National Estuarine Research Reserve in Saint John's County, near Florida's Atlantic Coast, and Wakulla Beach, on the Gulf Coast in Wakulla County. [Produced with assistance from Eco-Regions of Florida. Level IV Ecoregions graphic developed by the Watershed Monitoring Section, Division of Environmental Assessment and Restoration, Florida Department of Environmental Protection, Tallahassee, Florida. Sourced from Griffith et al. (2001). Adapted with permission.]

Discussion

Having live Strepsiptera allows for experimentation and detailed investigation. We used a portable light trap to provide reliable access to live adult male *E. koebelei* during the species' mating season. Because Strepsiptera are known to be caught in light traps, the same general approach should be applicable to any Strepsiptera that flies in low light. However, one must first determine where they are and when they are active.

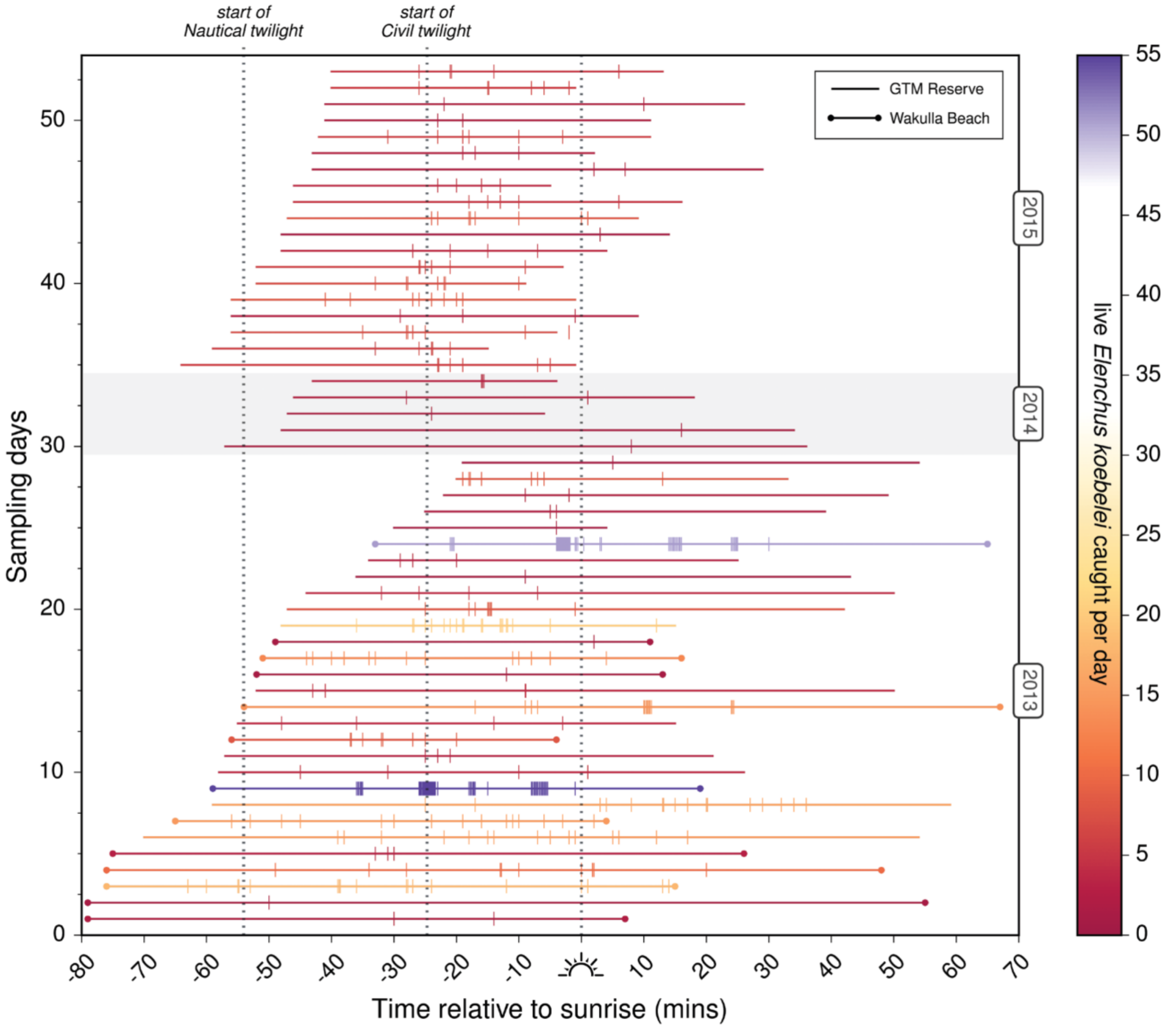

Fig. ii.4. Successful sampling days, grouped by year and ordered by start time. The figure displays the delay between the beginning of sampling and the first live capture, the time between live captures, and the time after the last live capture until disbanding of daily sampling. Seven captures were made during astronomical twilight, all of which occurred at Wakulla Beach: 5 on one morning, and 2 on another. There were no sampling days with live catches between 24 and 50 at either site.

Suitable habitat

Prerequisites for catching sizable numbers of *E. koebelei* are persistent stands of *S. alterniflora*, the larger, the better, and an abundance of *Prokelisia marginata* or *P. dolus* planthoppers, or both at once. It is also very important that sweep netting yield positive results prior to attempting light trap collection. Before realizing the primacy of this qualification, we sampled without success at a few waterfront locations in Franklin and Wakulla counties in 2013. Sweep netting also helps one to know where to position a light trap at a given site. Given the presence of visibly stylopized planthoppers, there is every reason to expect live eclosed *E. koebelei* will be captured at the site. Finally, promising sites may be worth revisitation, because early in a season hosts may be infected without external indication. New locations may even be colonized during particularly productive years.

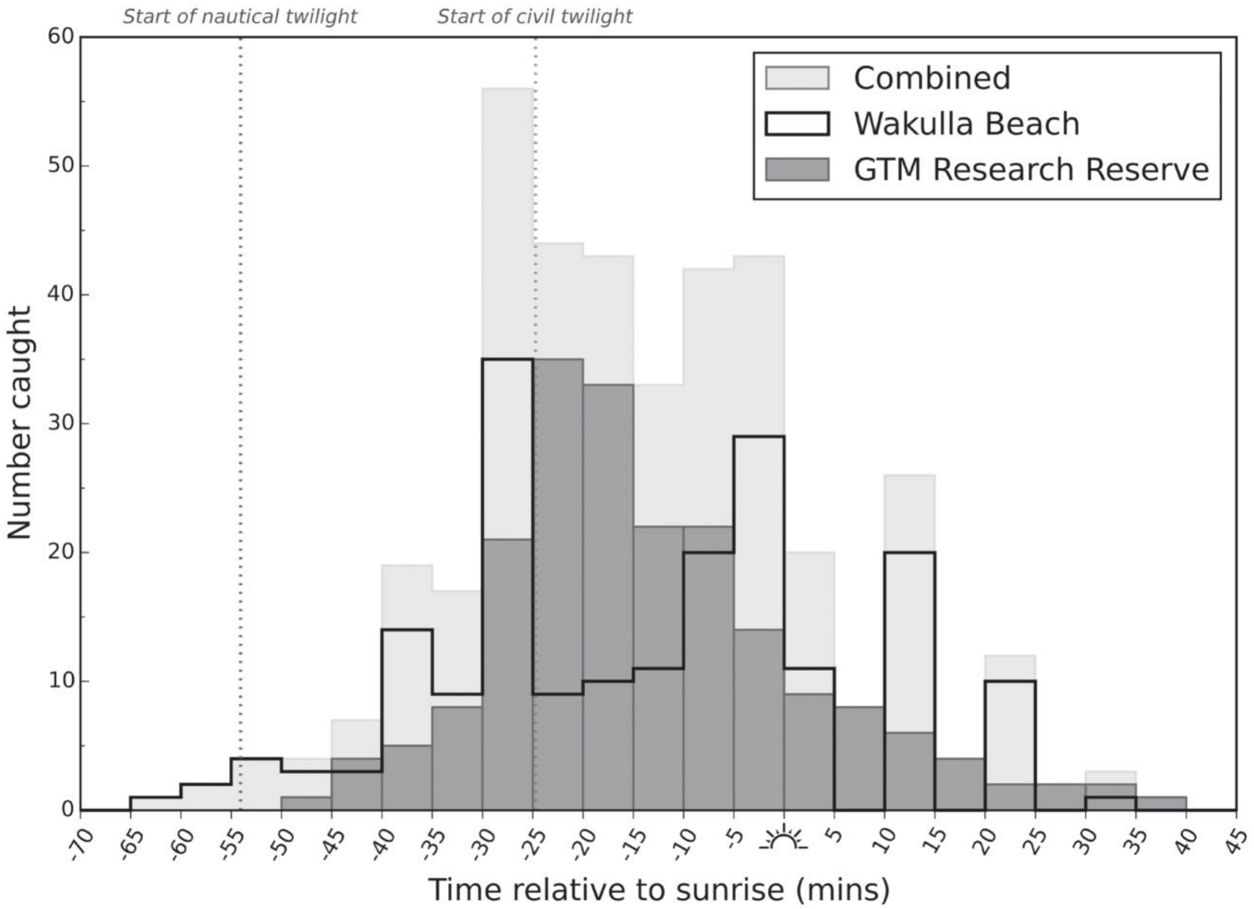

Fig. ii.5. Live *Elenchus koebelei* males caught over a 3-year period plotted against minutes relative to sunrise. Most eclosed males were caught between 30 min before sunrise and sunrise itself. None were caught more than 63 min before or 36 min after sunrise. Though wind-induced fluctuations occurred at Wakulla Beach, the range of capture times at both sites were similar, and peak catch times appear strongly influenced by morning civil twilight. Of the 521 adult male *E. koebelei* caught over the course of the study, only the 391 captured alive at known times are included in the graph.

Diel activity

At the onset of this study, the active period of *E. koebelei* was unknown. We hypothesized that the species is crepuscular, given that:

1. Host activity patterns are important indicators of strepsipteran activity, and delphacid planthoppers, especially the brown planthopper, *Nilaparvata lugens* (Stål 1854) (Hemiptera: Delphacidae), are known to migrate at dusk or dawn (Pender 1994; Qi et al. 2014)
2. Muir (1906) found that all specimens of the closely related species, *E. tenuicornis*, that he reared out in Hawaii eclosed between sunrise and 7 AM. [Time reckoning precedes the introduction of Daylight Saving Time.]
3. Eclosed *E. koebelei* are not encountered in diurnal sweep netting, even when net mesh sizes are suitably small and the percentage of stylopized hosts may approach 40% (Stiling et al. 1991b).

It is possible *E. koebelei* is diurnal. However, that is unlikely because on several mornings the final catch of the day occurred before sunrise (Fig. ii.4). In fact, more than 50 live *E. koebelei* were caught before daybreak on 2 separate mornings (Fig. ii.4; Supplemental data). Although the detectability of a light trap is reduced with increasing ambient light (particularly at distance), at Wakulla Beach we observed 2 additional peaks in captures that occurred from 10 to 15 min after sunrise, and from 20 to 25 min after sunrise, indicating that the trap was sufficiently conspicuous to attract *E. koebelei* well after sunrise. (This also suggests that the Strepsiptera did not have far to fly: at even moderate distances after sunrise, artificial light should fade into the background.) It is also possible *E. koebelei* is nocturnal. However, we conclude that that is highly unlikely, given the brief lifespan of eclosed adult male Strepsiptera and that some specimens were captured over 30 min after sunrise.

Table ii.2. Catch statistics and variation in *Elenchus koebelei* sampling duration. On average, all Strepsiptera were caught within a 20 minute period each day at Guana Tolomato Matanzas National Estuarine Research Reserve (GTM), and in just over a half hour at Wakulla Beach (WB). Due to logistics and inclement weather, the 2014 field season began late and was curtailed (no specimens were collected from WB, and only a few from GTM). Both locations are coastal sites in Florida—GTM on the Atlantic, and WB on the Gulf.

Year	Site	Avg start of sampling before sunrise (minutes ± SD)	Avg minutes sampling	Avg minutes until first catch	Avg max {abs max} minutes between catches	Avg minutes from first to last catch	Avg minutes sampling after last catch
2013	GTM	42 ± 17	81	21	14 {32}	19	33
2013	WB	59 ± 26	85	27	13 {19}	32	29
2014	GTM	48 ± 7	66	36	14 {20}	7	19
2014	WB	51 ± 14	49	—	—	—	—
2015	GTM	49 ± 8	55	24	12 {32}	19	11

Because *E. koebelei* are active at dawn, they are visually capable of flying at dusk as well. However, on the practically windless evening of 27 Sep 2013, no *E. koebelei* were captured or sighted in sampling at Wakulla Beach, where the insects were known to be present. Sampling began 13 min before sunset and ended 70 min thereafter. On that evening, dragonflies were flying in great numbers, however, some as long as 28 min after sunset. Although their hunting success would decline sharply with increasing darkness, dragonflies would likely pose a strong threat to *E. koebelei* at dusk. It is unknown how the eyeless calling females would be aware of dragonflies at whatever density, or even the sighted males—before having cast off their pupal caps and therefore committing to eclosure. Judging from their treatment of other variable parameters such as moon illumination and tide height (see below), the strepsipteran approach appears to be to ignore them in favor of consistent factors. It is therefore most likely that adult *E. koebelei* are active only around dawn (i.e., strictly matinal), when most bats are finished feeding, and larger predatory insects are not yet active.

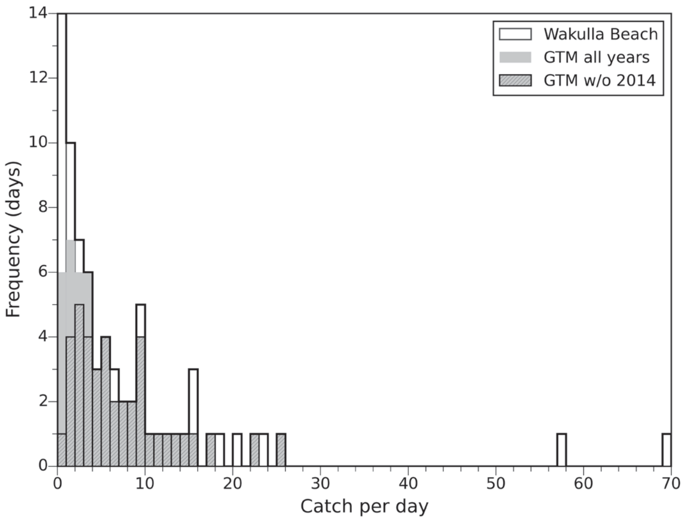

Fig. ii.6. Stacked frequency distributions of captured *Elenchus koebelei* males. The white area under the outline illustrates the number of days with a given catch at Wakulla Beach, the gray area depicts the same for all years at Guana Tolomato Matanzas National Estuarine Research Reserve (GTM), and the hashed gray area (outlined in black) represents the portion of the catch at GTM without 2014. Lastly, the broad outline represents the combined catch frequencies from both sites for all 3 years, 2013–2015. More than half the days with no catches occurred in 2014, when sampling began in mid-Oct.

Other conditions influencing catch

Factors such as temperature, timing, wind speed, and precipitation, significantly influence *E. koebelei* catch. In this study, most data were collected and disseminated by area weather stations. Such records are less accurate than local measurements, but are more readily available, are still very useful in determining sampling success, and provide data before arriving at a site, which can save a great deal of effort. Over the course of the study, a local thermometer was nearly always on hand. After 4 Aug 2015, a portable weather station also was available to record local wind speed, temperature, and humidity.

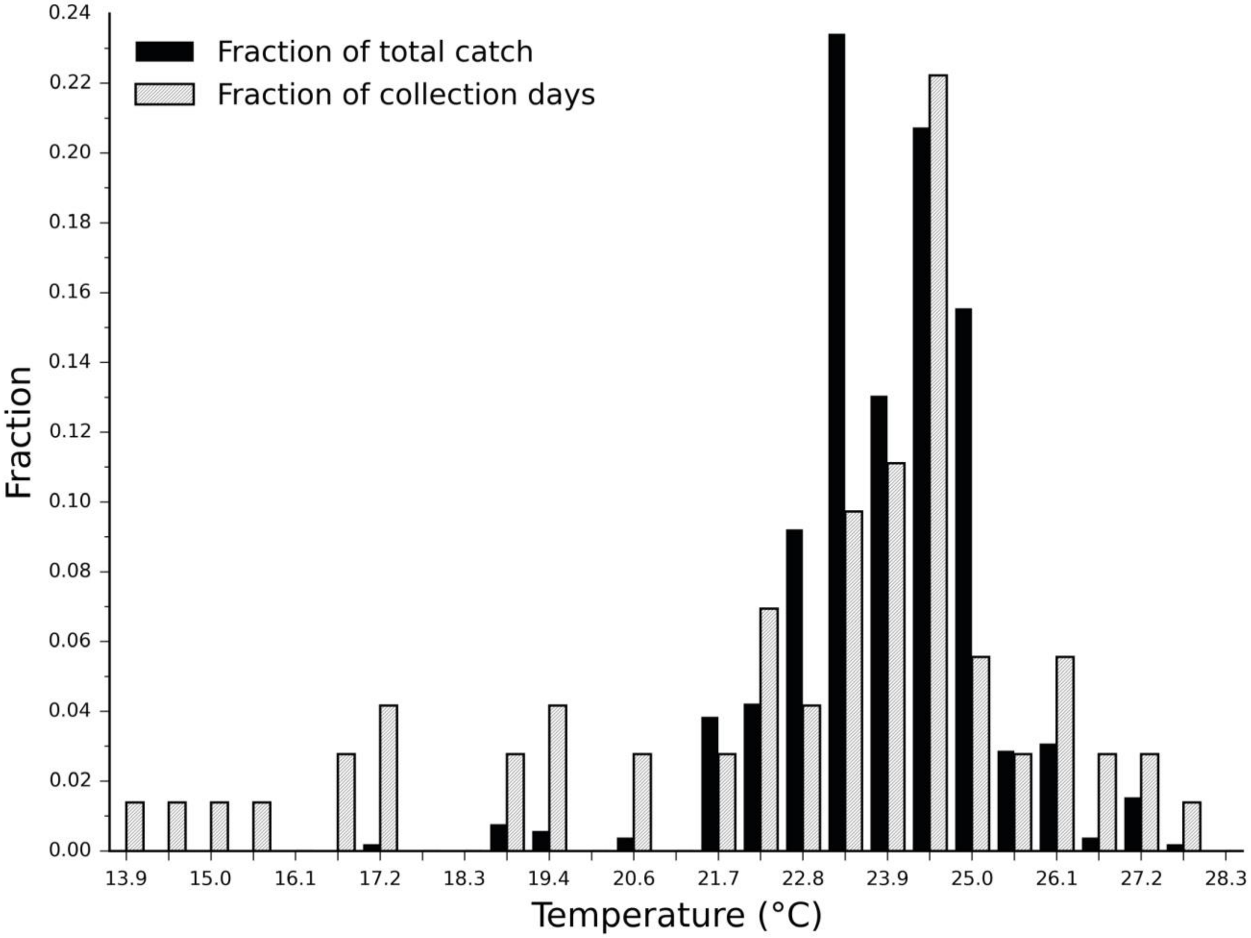

Fig. ii.7. Influence of initial temperature on total catch. Black boxes indicate the fraction of total Strepsiptera captured at the given temperatures; adjacent hashed boxes represent the fraction of mornings at each temperature. The most productive days were those with dawn temperatures ranging from 22.8 to 25 °C (73–77 °F). *Elenchus koebelei* was not found to fly on mornings when the temperature was below 17.2 °C (63 °F). The ratio of *E. koebelei* caught to collection days drops off dramatically for temperatures above 25.5 °C (78 °F).

Temperature

The most productive temperature range for catching *E. koebelei* was found to be from 21.7 to 25.6 °C (71–78 °F), within which 93% of all *E. koebelei* were caught. No *E. koebelei* were captured at temperatures below 17.2 °C (63 °F). *Elenchus koebelei* eclosed at the highest temperature encountered (27.8 °C (82 °F)); however, sampling efficiency declined sharply for temperatures above 25.6 °C (78 °F) (see Fig. ii.7.) Avoiding eclosing when it is warmer may help protect *E. koebelei* from attack by aerial predatory insects, which generally have higher take-off temperatures.

Wind

Elenchus koebelei are quite capable fliers, but they are minute and lightweight, so substantial local winds could blow them well off course, and also obscure the location of calling females. Furthermore, it is likely ‘strong’ wind is the predominant factor in patchy strepsipteran distribution. In the absence of a local wind meter, the Beaufort scale or the wind speed from an area weather report can be used. Wind speeds from about 1.6 to 9.7 km/h (1–6 mph) appear to be best (Fig. ii.8). Still air conditions were found to be good, and wind speeds from 9.7 to 12.9 km/h (6–8 mph) were feasible. *Elenchus koebelei* rarely flew at wind speeds above 16 km/h (10 mph). No Strepsiptera were ever captured when the trap’s collection sheet billowed enough to destabilize it. However, when stronger winds died down several min before dawn, males still eclosed and were caught. At Guana Tolomato Matanzas Research Reserve, wind speed was greatly reduced and regularized by nearby trees, and eclosed *E. koebelei* exhibited a smooth single-peaked distribution of catch times relative to sunrise. At Wakulla Beach, the frequency of windy conditions between sampling days greatly decreased sampling efficiency. Within sampling days, the prevalence of wind gusts likely prevented a unimodal distribution (Fig. ii.5).

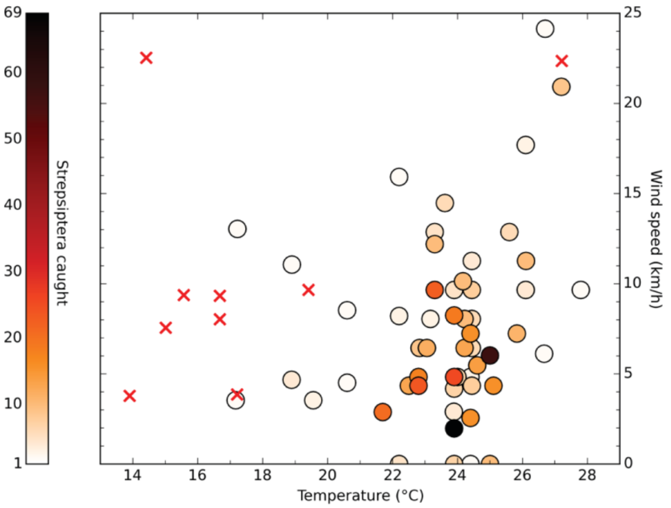

Fig. ii.8. Daily Strepsiptera catch versus temperature and wind speed. Ninety-three percent of the *Elenchus koebelei* were caught at temperatures between 21.7 to 25.6 °C (71–78 °F) inclusive. Strepsiptera catch suffered markedly when it was too cold. Similarly, most *E. koebelei* were captured when the wind was blowing slightly, likely due to the role sex pheromones play. The ×’s indicate conditions in which no Strepsiptera were caught but sampling was attempted. Graphed wind speeds were measured at area weather stations, rather than locally.

For consistency, all analyses were done on wind data from area weather stations. However, those may overstate the wind speed in protected areas, such that still wind conditions could in fact yield the best results. Unfortunately, on-site wind speed data were available only for the last 7 d of trapping in 2015. Over that time period, they averaged 5.6 km/h (3.5 mph) less than area wind measurements, with 5 d registering 0 km/h (Supplemental data). To obtain a more in-depth treatment, wind speed should be monitored continuously and matched against individualized catch times.

Timing

We found it best to have the trap completely operational from 35 min before sunrise to about 15 min after sunrise at Guana Tolomato Matanzas Research Reserve, where strong breezes are rare. However, at Wakulla Beach, a windier site, sampling from 45 min before until 25 min after sunrise produced better results. These findings should generalize to other locations.

Rain

Though very light drizzle was tolerated, *E. koebelei* were found to prefer mornings free of precipitation. Males appear to be highly sensitive to barometric pressure, such that they did not fly on mornings that seemed very promising, but suddenly degenerated into substantial rainfall. However, when rain was light enough to be of no concern for shorting out exposed electrical connections, conditions were also suitable for unhindered *E. koebelei* flight and collection.

Tide

Tide height was found to be of little significance, particularly at Guana Tolomato Matanzas Research Reserve. It may increase the difficulty of collection, but does not appear to actually influence strepsipteran eclosion. There is some indication that the host insect, *P. marginata*, may avoid immersion, ostensibly to escape consumption by predatory fish. At Guana Tolomato Matanzas Research Reserve, the Pearson correlation coefficient between tide height and the number of *E. koebelei* caught was 0.12, with an estimated 62% chance of having arisen randomly. At Wakulla Beach, the correlation coefficient was –0.29 with an estimated 21% chance of having arisen randomly. However, when corrected for the range of water heights that occurred during collection episodes, the correlation coefficient fell to –0.02 (95%) and –0.21 (37%), respectively. The persistent slight negative correlation found at Wakulla Beach was due to the catch characteristics of the 2 extremely high yield days, and not due to consistent differential tide-based eclosing of male *E. koebelei*.

Moonlight

Only moon rise, moon set, and lunar visibility as a function of phase were recorded consistently. No data on cloud cover were noted regularly, and a light meter was not available to record relative brightness. However, within these confines, it was repeatedly found that *E. koebelei* did not eclose differently despite the greatly increased availability of light during full, and nearly full, moons. This lack of response suggests strongly that in addition to being completely incapable of visually perceiving shape, adult female *E. koebelei* also do not adjust their calling times on the basis of lunar light intensity.

Moonlight intensity does appear to influence catch, however, albeit negatively. That is, as found for other nonaquatic insects, the number of Strepsiptera eclosing ostensibly remains constant with respect to moonlight, but on brightly moonlit nights, the light trap has to contend with increased ambient light, and is therefore less efficient (Williams et al. 1956). Because *E. koebelei* is crepuscular, the effect is less pronounced than what could be expected of nocturnal insects. However, it is worth noting that both cases of catches during astronomical twilight occurred on moonless nights.

Light sensitivity

Eclosed *E. koebelei* were most often captured during twilight, the period between night and day when no sunlight reaches an observer directly, but sunlight redirected by the atmosphere still does (unlike at night). Twilight is divided into 3 formalized stages, based on the sensitivity of the human eye (timeanddate.com n.d.; US Naval Observatory 2011). However, despite this origin, the subdivisions also have relevance to *E. koebelei*, as can be seen in Figures ii.4 and ii.5. In the morning, twilight begins at dawn and ends at sunrise. Astronomical twilight is the first stage of morning twilight; it occurs when the geometric center of the sun is between 18° (inclusive) and 12° (exclusive) below the horizon. At the beginning of astronomical twilight, the intensity of scattered sunlight is less than that from weak stars, and remains barely perceptible for a considerable interval thereafter (US Naval Observatory 2011). Throughout this stage of twilight the horizon is indistinct to a human observer. Nautical twilight occurs when the sun is between 12° and 6° below the horizon. It is named for nautical navigation by star and horizon sighting because in nautical twilight, the horizon remains discernible even on moonless nights, but most stars visible to the naked eye can also be seen. In the absence of moonlight, during nautical twilight artificial illumination is required for most outdoor activities. Civil twilight occurs from when the sun is 6° below the horizon until sunrise. Under good atmospheric conditions in civil twilight, artificial illumination is not necessary to clearly distinguish terrestrial objects, and only the brightest celestial bodies can be seen by the naked human eye. Sunrise begins the moment any portion of the solar disk breaches the horizon.

At Guana Tolomato Matanzas Research Reserve, the largest number of Strepsiptera were caught from 25 to 15 min before sunrise, the beginning of which roughly coincides with the onset of civil twilight. At Wakulla Beach, the majority of eclosed *E. koebelei* were caught in 2 waves, the first from 30 to 25 min before sunrise, just prior to the start of civil twilight, and the second from 5 minutes before sunrise until sunrise itself, which marks the end of civil twilight (Fig. ii.5). Although the boundaries of twilight correspond to human visual sensitivity, it appears that civil twilight had strong bearing on the mating dynamics of both strepsipteran populations nonetheless, though at Wakulla Beach the preferred flight time may have been split to better coincide with troughs in wind activity. Unlike at Guana Tolomato Matanzas Research Reserve, the site at Wakulla Beach was not partially enclosed by trees, thus allowing for stronger winds and also greater ambient light intensity, either or both of which may have encouraged *E. koebelei* to eclose at altered times. Accordingly, Strepsiptera at Wakulla Beach were more apt to fly during nautical twilight, and only *E. koebelei* at Wakulla Beach were ever found to fly during astronomical twilight (Table ii.3). Surprisingly, more *E. koebelei* were collected after sunrise at Wakulla Beach than at Guana Tolomato Matanzas Research Reserve. That may have been due to a post-dawn reduction in wind at the site.

Site	Phase of dawn	*E. koebelei* caught at known times	Proportion
GTM	During astronomical twilight	0	0.00
	During nautical twilight	42	0.21
	During civil twilight	123	0.62
	After sunrise	34	0.17
	Total	199	1.00
WB	During astronomical twilight	7	0.04
	During nautical twilight	66	0.34
	During civil twilight	77	0.40
	After sunrise	42	0.22
	Total	192	1.00

Table ii.3. Adult male *Elenchus koebelei* captured during different phases of dawn. *Elenchus koebelei* had a propensity to approach the light trap during civil twilight at both sites. However, because there were no nearby bordering trees, it was significantly windier and also brighter at Wakulla Beach (WB). Those conditions appear to have extended flight times even into astronomical twilight at the site.

The light sensitivity of *E. koebelei* has not been determined experimentally, but given these findings, we hypothesize that *E. koebelei* see at roughly 1 twilight stage brighter than humans do, such that our astronomical twilight corresponds to their nautical twilight, human nautical twilight is the civil twilight of *E. koebelei*, human civil twilight is their dawn, and full dawn and beyond may require significant internal shielding in *E. koebelei*, which could otherwise adversely affect their visual acuity.

Wing expansion

It typically took several minutes for any *E. koebelei* to visit the light trap regardless of when sampling began (Fig. ii.4). Furthermore, their wings are not curled as one would expect had they already been expanded within the pupal case, as is typical of male Strepsiptera. As a result, *E. koebelei* walk with wings held aloft. Because of these attributes, we hypothesize that *E. koebelei* do not fly immediately upon eclosing—unusual for Strepsiptera—but first expand their wings in the manner of other flying insects (although much more quickly), as Hassan (1939) reported of *E. tenuicornis*, or perhaps more likely, further expand them. After all, *E. koebelei* are not under any threat from their hosts. It may be that during this time, *E. koebelei* can become fixated on a present light trap. If so, this would help explain the effectiveness of deploying the trap several minutes before the first Strepsiptera were expected (Fig. ii.4), and perhaps the unit’s strong appeal so long after sunrise, as well. One also wonders if this approach improves immediate flight performance, which could be important for avoiding inadvertently flying into the water, and what effect such a ‘warm-up period’ might have on vision.

On several occasions we noted that moving the trap a few meters revived flagging catch totals. This indicated that many *E. koebelei* did not have far to fly to arrive at the trap, yet despite their considerable flight velocity, it still took several minutes for the first of them to do so. Although the unaccounted-for time could have been occupied by mating, many captured *E. koebelei* had very noticeably distended abdomens. Because Strepsiptera do not feed as adults and may spend substantial time in a pharate state awaiting appropriate conditions, the distension was probably due to sperm reserves, indicating that the males had not yet mated. Additionally, the act of strepsipteran mating is unlikely to last long (Muir 1906). Furthermore, in at least 2 strepsipteran species, it has been noted that males died just a few minutes after mating (Kathirithamby 1989; Beani et al. 2005) (no data on how old those male were, however). Our (potentially unmated) specimens normally survived for hours when allowed to do so. This occurred without regard to the distension of the abdomen.

Resistance to drowning

Unlike gnats, alate ants, and other flying insects attracted to the light trap, *E. koebelei* did not drown when water affixed their bodies to the trap's support beams. This was discovered accidentally after a rainstorm, when the erected trap was carried through wet grass, and exploited thereafter. In stunted, less dense Spartina such as that at Wakulla Beach, attaching shorter legs to the trap to adjust for the reduced canopy height and wetting the trap base, together act as another means of ensnarement. Capturing Strepsiptera in this manner tended to ruin their remarkable wings, but did assist in obtaining larger numbers of live Strepsiptera at Wakulla Beach, particularly on the 2 mornings with the greatest live catch counts: 63 on 17 Sep 2013, and 57 on 22 Sep 2013 (57 and 51 caught live at known times, respectively). Strepsiptera can survive at least 29 min in such conditions.

This ability to avoid immersion asphyxiation may relate to the poorly understood strepsipteran "balloon gut" (Pohl & Beutel 2005; Beutel & Pohl 2006), or to the thinness of the strepsipteran cuticle, such that some species may be able to breathe through it, as they ostensibly do as fully embedded larvae. Although their thoracic spiracles have remained intact, the reduction of all abdominal spiracles but the first one that has occurred in the adult males of Elenchidae and many other strepsipteran clades is worth noting (Pohl & Beutel 2005).

Vision

There is discrepancy over whether Strepsiptera see well with so few facets, which some expect to effectively act as pixels (Pix et al. 2000), while others hold that each 'ommatidia' should behave as a separate image-forming eyelet (Buschbeck et al. 1999; Maksimovic et al. 2007). The capture of large numbers of eclosed males below the grass canopy at Wakulla Beach suggests that *E. koebelei* can fly between grass blades before sunrise, and therefore must see well enough to do so. In addition to helping protect against larger insect predators, flying through Spartina should provide some buffer against wind, and also is where one might expect planthoppers—and calling females—to be located.

Caveat and commendation

The data leading to this report were amassed as by-products of collection forays for detailed eye studies. Because of this, there were lapses in its procurement, such as not having an anemometer collecting continuous local wind measurements, not beginning collection at the same time relative to sunrise each morning, and, due to processing limitations, not taking the maximal number of Strepsiptera available for collection each day in 2015. Despite these deficiencies, to the authors' knowledge, the Supplementary Data of this report comprise the most complete set of capture characteristics from any Strepsiptera collection effort so far conducted.

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The authors made use of the following free, open source, or freeware software in the creation of this paper: matplotlib 1.5.1 (Hunter 2007), JabRef 3.3, Docear4Word 1.30, LightZone 4.1.7, GIMP 2.8.14, and XnSketch 1.18.

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Additional information

A photostacked image of a critical point dried *Elenchus koebelei* was provided by author Marisano James and appeared as the cover image for the corresponding issue of *Florida Entomologist*. That cover has been reproduced on the following page.

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Insights into strepsipteran flight

Marisano James[1,*]

Abstract

Having virtually no recorded information depicting airborne Strepsiptera is a conspicuous gap in our knowledge of these insects, especially given the divergent descriptions of their flight, the peculiarity of the strepsipteran wing planform, and the ability of adult males to immediately fly upon exiting the puparium. Adult females are either non-motile and never leave their hosts (all but the least derived extant family), or are only capable of walking less than a meter over several consecutive evenings (least derived extant family). Adult males abandon their own hosts, dispersing by flight for the sole purpose of mating, their productive emerged lifespans totaling but a few hours of often feverish activity, as neither male nor female adult Strepsiptera feed. These conditions essentially ensure that outcrossing—sufficient genetic diversity being indispensable for parasites—is only achieved by means of the male flight apparatus. I used a self-modified commercially available insect photography system to capture images of airborne *Triozocera texana* (Strepsiptera: Corioxenidae), a nocturnal strepsipteran widespread in central Oklahoma, and analyzed the photographs it collected to assess strepsipteran flight, the bases of which are best characterized by low wing loading and low power loading. Although previous researchers described strepsipteran flight incongruously, apart from patrolling and the erratic flight exhibited when narrowly confined, the descriptions all fit neatly under the rubric of sex-pheromone plume following, which has been observed in detail in moths. In patrolling, undertaken by adult males of diurnal species whose itinerant hosts overwinter in stable nesting areas, the application of vision and olfaction are

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apparently inverted, such that vision predominates at (moderate) distance, while olfaction is used for identification, followed by touch for final verification, as usual. This inversion may have contributed to several diurnal species acquiring additional ommatidia. Finally, although their flight mode shifts with perceived distance from pheromone-emitting females, because of their enormous flight muscle assemblage, Strepsiptera should in no sense be considered weak fliers.

Keywords: wing loading, power loading, flight muscle ratio (FMR), halteres, sex pheromone, insect photography rig

Introduction

I discovered how to catch male *Elenchus koebelei* Strepsiptera one morning not long before sunrise. While aspirating my first or second live specimen, I saw another from the corner of my eye, slowly rising up, the lure of my light sheet losing hold to the ever brightening sky. Having but few samples and limited options, I attempted to aspirate the circa 1 mm insect directly out of the air. However, since this elenchid was airborne, things proceeded less fortuitously for me. As it adroitly dodged the suction hose a second, then third time, I quickly realized strepsipteran sensory capacities are underappreciated. (As we will see, Strepsiptera have a most definite deficit of ommatidia—the typically single-pixel providing sub-eyes that comprise a compound eye.) There are anecdotes of the elegance and apparent leisure with which Strepsiptera fly: "The little animals [*Stylops thwaitesi*] are exceedingly graceful in their flight, taking long sweeps, as if carried along by a gentle breeze..." (Thwaites 1841). But also striking reports of remarkable speed: "Its [*Xenos pallidus*?] life as an active imago cannot be longer than fifteen or twenty minutes, if as long, and during this time it exhibits fiery energy, and flies so rapidly that the eye can hardly follow it." (Hubbard 1892). And also ostensibly contradictory accounts of its poor flight ability: "[I]ts [*Stylops perkinsi*] flight is different to anything else I have ever seen; a very peculiar flight, … what I should call an uncomfortable flight, up and down, this way and that way, in fact at all angles, not keeping in one direction more than a few inches [≈ 8 cm]…" (Enock 1875). However, via applied photography, direct interaction, and careful reflection, I have reconciled these divergent views.

Strepsiptera (Fig. iii.1) is the only entirely obligate endoparasitic insect order.[2] No female Strepsiptera can fly, and in all but the most basal extant lineage, adult females cannot see, walk, or exit their hosts. As a consequence they cannot oviposit. Instead, their live young hatch internally and consume their mother—most of which is in the form of fat stores—from the inside (hemocoelous viviparity (Hagan 1948)). Later, the host is relied upon to inadvertently help disperse those offspring in areas frequented

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[2] Siphonaptera (fleas) are not parasitic as larvae and are ectoparasites as adults.

Fig. iii.1. Live but moribund adult male *Triozocera texana*: Clumsily placed legs and drooped antennae indicate he is dying. Although pharate[3] adult males survive much longer, upon emergence, males die within about 4 h. Like true flies, Strepsiptera also have halteres (flap-mediated gyroscopic sensors) but unlike Diptera, whose halteres are modified hindwings, strepsipteran halteres are modified forewings, reflecting that they are most closely related to beetles (Coleoptera). Like flies, however, airborne Strepsiptera are agile and also capable of hovering. Male *Triozocera texana* are 2–3 mm long. Both sexes parasitize the burrowing bug *Pangaeus bilineatus*, but as with most other strepsipteran taxa, adult female *T. texana* have no wings, eyes, antennae, or even legs, so after finding a host as a larva, they never leave. How their minute larvae—which cannot dig—arrive at larval hosts that spend most of their time underground is unknown, but is certainly related to maternal care, which is well-documented in the family Cydnidae, to which *P. bilineatus* belongs. The inset shows an expired male *T. texana* (upper right) laid alongside a penny for size comparison. U.S. pennies are 19.05 mm (0.75 in) in diameter.

by new potential hosts. If a stylopized[4] host dies when its Strepsiptera is already a pupa or adult (neither of which feed), then the parasites may yet survive, particularly if it is male (S.S. Saunders 1853; Dury 1902; Kirkpatrick 1937b), but perhaps also if female (Dury 1902; Kirkpatrick 1937b). However, diurnal pharate adult males will only emerge if exposed to light (S.S. Saunders 1853); exposure to darkness is likely necessary for nocturnal males to emerge from dead hosts. Thus, if a host (diurnal) dies, it must do so at a serendipitous site. Additionally, adult female Strepsiptera can apparently only persist if the body of the host remains moist (Kirkpatrick 1937b). However, even if so, their lifespans are significantly curtailed (Kirkpatrick 1937b). Therefore, a female that was able to call (i.e., to release sex pheromone)

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[3] Every new arthropod developmental stage (instar) begins while the animal is still enclosed in the exoskeletal husk of its previous instar. This part of the new stage is known as pharate + the name of the new stage (Hinton 1971). Development may continue unabated using the old cuticle as a protective layer. Thus, a pharate adult has separated from its pupal cuticle, but has not (yet) cast it off.

[4] Insects parasitized by Strepsiptera are called "stylopized" after the early identification of adult male Xenidae in wasps and Stylopidae in bees (the two families were initially united).

from within a dead host might still potentially mate (Kirkpatrick 1937b), but would not live long enough for her offspring to mature. Offspring of a parturient female embedded in a dead host are able to eclose if the body remains moist, but can only travel a very limited distance on their own—apparently much less than a foot (Dury 1902; Kirkpatrick 1937b). So, if the mother's host dies in a poor location, her offspring will be extremely unlikely to secure a host of their own.

Thus, unlike parasitoids, which are free to kill the host in the course of their own development—or even benefit from doing so—Strepsiptera have become true parasites. Virgin females call chemically, their sex pheromone summons conspecifics that engage in an airborne duel with dwindling time. Within a few hours, energy-depleted males may literally fall from the sky and twitch on the ground as they slowly die; figuratively: every parasite strepsipterous, a Siren or an Icarus. Even so, another corps of pharate males awaits in their puparia, half-extruded from their own hosts, their potentially pre-expanded wings partially pleated like poorly closed fans. Each male is equipped to fly upon (Hrabar et al. 2014) or promptly after (Hassan 1939; Smith & Kathirithamby 1984) using his highly protractile abdomen to push free of his puparium (Ulrich 1933), whereas most insects must pause to expand and dry their wings—a process often lasting hours; time Strepsiptera simply do not have to spare: Their hosts move as they will, sometimes more, sometimes less burdened by their passengers, but always without overt interference from them.

Despite my determination that morning in the salt marsh, I was unable to capture the fleeing specimen. In short order he flew just beyond the upper reach of my aspirator hose, and then a little further on, before settling back down among the grass blades. Gone… I was also unable to modify the insect rig quickly enough to capture photographs of flying *E. koebelei*, but I have done better since. Herein I present the first photographs of Strepsiptera in flight—free or otherwise—of which I am aware. My analyses of these images of flying *Triozocera texana* (Cook 2019), other interactions with *T. texana*, and historical accounts of other flying Strepsiptera have led me to conclude that the best way to characterize strepsipteran flight is by means of wing and power loading. Strepsiptera have low wing loading (highly maneuverable, but generally slow flight), but also low power loading (a large amount of power compared to their overall body weight, allowing for great acceleration). The interplay of low wing loading and low power loading while tracing sex pheromones accounts for the wide variance in observed strepsipteran flight characteristics.

Materials and Methods

Equipment used

Most of the photographs were taken with a Canon EOS RP (Canon Inc.; Ota City, Tokyo, Japan). Before receiving it, I used a Canon T3i (aka EOS 600D). I also briefly rented a Canon 5DS and 90D. After obtaining a Laowa 100mm F2.8 CA-Dreamer Macro 2X lens (Venus Optics; Hefei, Anhui, China), I used it exclusively. All images were collected in August of 2019–2021 from several backyards in two neighborhoods in Norman and Noble, Oklahoma. The photos were analyzed to provide insight into strepsipteran flight. Images were mainly processed using Affinity Photo version 1.10, and later version 2.5.3 for wing area calculations. Some angle measurements and clearly labeled accompanying graphics were provided using the open source vector graphics program, Inkscape 1.2.2.

Obtaining photographs of flying Triozocera texana (née *T. mexicana*)

Shepard (1979) identified the time of year and duration of the mating season of *Triozocera mexicana* (Strepsiptera: Corioxenidae), which I refer to as T. *texana*, in alignment with Cook (2019). I verified their continued local presence by investigating the Norman Mosquito Authority (NMA) by-catch. The NMA later allowed me to accompany them to the sites of several of their Strepsiptera-productive light traps, from which I inferred preferable host habitat. I used a New Jersey light trap (without a killing agent) to determine that male *T. texana* fly from about midnight to shy of 7 AM on each weather permissive night of the mating season. I checked the first and a subsequently added (and differentially located) second trap hourly, so several males could still fly when I recovered them. After acquiring this information, I set about capturing photographs of free-flying Strepsiptera in the field.

Macrophotography (i.e., photography at magnifications of at least 1:1) of insects in flight remains largely confined to studio work, even though—as I show—current technology allows it to be extended into the field, where it is well-suited to recalcitrant insects, and especially to nocturnal species. Unfortunately, a camera's built-in shutter is much too slow to capture clear images of quickly flying or tiny insects (the necessary magnification increasing effective flight velocity). *Triozocera* are nocturnal, however; so because darkness can act as a shutter (McIlleron & de Moor 2011), I avoided the nuisance of an external high-speed shutter. I also knew mate-seeking males would be drawn to ultraviolet light (James & Strong 2018). Thus, the camera was placed in Bulb mode, so that its built-in shutter could remain open for up to a couple of minutes before noise buildup became too great. Whenever the system was triggered by an insect flying through one or both pairs of crossed red laser beams[5], flashes were fired to provide light, and the shutter was closed immediately thereafter to prevent multiple exposures.[6] Over the course of three years, I increasingly modified a Cognisys insect rig (Cognisys Inc.; Traverse City, MI, USA) to improve the quality of the photographs it took of tiny flying insects. (Incidentally, no permanent changes were made to the stock rig; the modifications are all entirely and easily reversible.)

Wingstroke amplitude

Because strepsipteran halteres beat in antiphase to their wings (Pix et al. 1993), their wingstroke amplitudes can be ascertained from single images when the body axis is agreeable, and either the halteres or the wings are extended directly above the thorax. Two photographs were captured in which both criteria

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[5] Red being invisible to most insects. The intensity of the lasers was also weak, so that even the occasional moth that can see red would also not be deterred.

[6] This is known as the open flash technique (Morris & Crissey 1953), and was initially the only way a flash could be effectively used at all (Richter 2020).

were fulfilled. I used them to measure the angular offset from the central axis of the juxtaposed halteres to the leading edge spar of the outwardly opening wing (Fig. iii.5), thus obtaining the wingstroke amplitude from still images of *T. texana* in free flight.

Wing loading data sets

Wing loading is body weight divided by wing area. It is important in measuring maneuverability versus speed of flight. I combined two data sets containing measured insect wing loadings: Byrne et al. (1988) and Tercel et al. (2018). Byrne et al. (1988) were particularly concerned with Aphididae (aphids) and Aleyrodidae (whiteflies), both tiny insects of order Hemiptera, and thus not well-represented in previous data sets. They therefore amalgamated their own data with information collected from preexisting studies. Tercel et al. (2018) were primarily interested in wingbeat frequencies, which in former meta-analyses had been collected at different temperatures using multiple methodologies. To address this, they compiled their own data set under standardized conditions, but subsequently discovered that order-level phylogenetic patterns were consistent with previous studies (Tercel et al. 2018). Consequently, I combined their data set with the meta data set of Byrne et al. (1988) to provide more complete coverage. Byrne et al. (1988) had already averaged multiple readings from singular species, when they had been collected by the same research team. I expanded that to cover all entries, except when sexual dimorphism explicitly factored in or appeared to have done so[7]. Therefore, apart from sexual dimorphism and three other exceptions, each species is represented only once in the final compilation. Together, the combined data set comprises 11 insect orders and 234 entries (231 species). However, four of the orders—(1) Blattodea (roaches and termites), (2) Ephemeroptera (mayflies), (3) Trichoptera (caddisflies), and (4) Mecoptera (scorpionflies)—are only represented by a single species each, and the

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[7] Once for *Dialeurodes citri* (Byrne et al., 1988); and ostensibly once for *Vespula germanica* (Magnan (1934) (most likely a female worker) vs. Sotavalta (1952) (possibly a male or gyne)), both reported in Byrne et al. (1988). However, neither *V. germanica* entry from Byrne et al. (1988) matched well with those from Tercel et al. (2018), so I kept three separate entries for this species.

first three, by a single specimen. Unfortunately (and predictably) Strepsiptera were not included at all.

Estimating strepsipteran wing loading

Calculating the wing loading of an insect requires its wing area and its wet weight.

Calculating wing areas

To obtain wing areas, I detached the wings of several *T. texana* specimens preserved in 70% ethanol, spread them out as flat as possible, and photographed them alongside a paper ruler to calibrate the camera's pixels when calculating areas. However, the wings were so delicate I only succeeded in doing so without complication twice, and in one such case I had beheaded the specimen (to prepare its eyes for electron microscopy). Without its body length, its wet weight could not be estimated (see below) for use in calculating its wing loading. In another instance, I digitally combined the two wings of the same specimen to provide a single complete fully opened wing. In two other cases, I effectively unfolded overlaps by twice summing areas darkened by pleating—once for the exposed surface area and again for the unexposed area that produced the darkened patch (Fig. iii.S1). Finally, in two additional cases, I resized and fit the upper portion of other wings, so that a small largely uniform area subject to ripping at the wing base could be traced onto the two torn wings. Due to the difficulty of working with strepsipteran wings, when calculating wing loading, the area of a single wing was doubled, rather than the areas of the two separate wings having been summed. Images of all these wings and bodies appear in Fig. iii.S1 of the Supplementary Material. Hereinafter, I only refer to the wing loading of the specimen whose body and measured wing were complete. Its wing area and the average wing area of the headless Strepsiptera and those calculated from the somewhat flawed wings of the remaining candidates were nearly equal. Furthermore, their combined data form a nearly perfectly linear relationship of wing area vs. thorax + head length (calculated for the headless specimen) (Fig. iii.S2).

Estimating wet weight

Sage (1982) produced several related models to predict the wet and dry weights of different groups of adult insects based on their body types. I used the Diptera-Hymenoptera model (R^2 = 0.94; certified for body lengths 2.5–21.8 mm (Sage 1982)) to estimate the wet weight of Strepsiptera because it ostensibly includes nematoceran flies, whose body types are similar to those of Strepsiptera. I submitted the only *Triozocera texana* for which I had its wing area and wholly intact body, even though its body length of 2.38 mm was 0.12 mm below the certified size range. However, the body length limitation did prevent me from also applying the model to estimate the wet weight of *Hylecthrus rubi*, whose range of body lengths was recorded as only 1.06–1.76 mm (S.S. Saunders 1850).

*Flight muscle ratio (*Hylecthrus rubi*)*

The flight muscle ratio (FMR) is the proportion of body mass a volant animal dedicates to flight muscle (Marden 1987). As such, it is related to power loading (weight divided by power; see below), but only applies to muscle-powered flight. Other attributes equal, insects with higher FMRs are capable of faster more forceful flight. In special cases, an insect's FMR can be correctly calculated without ever weighing its thorax or flight muscles, but to determine its power loading, one would still need to know or estimate its wet weight and the weight of what was lifted. The FMRs of several insect species are known, allowing the relative flight muscle and power outlays of different insects to be compared.

Hylecthrus rubi Strepsiptera infect solitary bees of the genus *Hylaeus*, which are more commonly known as "yellow-faced bees." Because I was unable to find any data specifying the wet weight of adult male Strepsiptera, I calculated the *H. rubi* FMR based on the average measured wet weight of a congeneric host bee, an estimation of the *H. rubi* wet weight, and approximations of its FMR based on the following account of one struggling to free itself from its puparium,

> "during their brief term of existence they are not so weak and helpless as may be supposed, on

> one occasion when three male *Hylecthri* were obtained alive from a dead *Hylaeus*, the first which exhibited itself commenced dragging the bee about behind him, together with his unemancipated comrades, until he succeeded eventually in effecting his escape from the pupa-case" (S.S. Saunders 1853).

Before presenting those calculations, it is necessary to establish a few important points.

First, although the means by which the bee was dragged is not stated, it must have been by the strength of the parasite's wings. Unlike *Xenos* (wasp-infecting Strepsiptera) (S.S. Saunders 1853), *Hylecthrus* are situated in the puparium with their wings facing out toward open air, and their legs facing the host's abdomen (S.S. Saunders 1853). This means that if they were to walk before exiting the puparium, they would do so along the abdomen of the host, rather than the ground or other substrate. However, with the potential exception of Corioxenidae, which during the process of emergence are double-over under the hemelytra of their hosts (Kirkpatrick 1937b; Esaki & Miyamoto 1958), adult male Strepsiptera do not use their legs to help them emerge. Once the legs are free, they vibrate or wriggle idly (Ulrich 1933; Linsley & MacSwain 1957). Instead, the abdomen (Ulrich 1933; Hassan 1939; Linsley & MacSwain 1957) is pushed against the distal end of the pupal case to force the insect out, accompanied by strong movements—ostensibly contractions—of the thorax (Linsley & MacSwain 1957). Furthermore, strepsipteran legs are far too feeble to drag a host around (Hubbard 1892; Pierce 1909; Linsley & MacSwain 1957). Finally, like all Stylopidia except some Corioxenidae (Pohl & Beutel 2008), *Hylecthrus* tarsi lack claws and are therefore ill-equipped for fastening to generalized substrates[8] (Pohl & Beutel 2008). In fact, the walking ability of Strepsiptera is so compromised that Linsley and MacSwain (1957) referred to the gait of *S. pacifica* as 'flying-walking' because the driving force appeared to be supplied entirely by the wings. In the course of emerging, Strepsiptera flap their wings once they can usefully do

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[8] Seeing as to how they are recurrent when escaping from under they hemelytra of their hosts, rather than a randomly preserved plesiomorphic character, the reason why some Corioxenidae have retained a pair of (reduced) claws may be because they assist in achieving release from the puparium by gripping the exoskeleton more securely (assuming these Strepsiptera do use their legs to reverse direction when emerging).

so as they seek escape. In the videos provided by the following links, note that if the specimens had been flipped over, they could have begun flapping their wings even before the bulk of the thorax had been worked free. Also note that the legs do not assist in emergence: 1 (Hrabar 2015b), 2 (McCann 2013), 3 (Schoonvaere 2020) (this specimen mates with adjacent calling female before flying off). For these reasons, it should be understood that the dead host was dragged solely by force of the parasite's wings.

Second, by dragging but not being able to lift its host into the air, the *H. rubi* displayed its maximum lift production (Marden 1987; Coelho 1997). That is, the weight of the host bee, plus its own weight, and that of the two other Strepsiptera still within their pupal cases was the maximum weight the *H. rubi* could (nearly) lift into the air. This is known as the *marginal* flight muscle ratio (mFMR). When a weight-bearing volant animal is just barely able to lift off the ground from a standing takeoff, the mFMR is also the ratio of flight muscle mass to total mass (Marden 1987). In this case, that is basically the weight of an adult *Hylaeus rubicola* bee, because each *Ha. rubicola* egg is laid in a cell containing all of the provisions the bee larva will ever have. Thus, whatever weight went into the Strepsiptera, came out of the bee, and all of that weight would still have been accounted for: The two pharate parasites would not have defecated, while the partially emerged one either had not defecated (which was perhaps why its abdomen was stuck) or had done so within its unevacuated puparium. The host bee *would* have defecated but before emerging from its nest cell (Torchio 1984), and thus before having been weighed (Tepedino 1980). However, these facts do not reveal the mass allocation between the parasites and their host.

Third, clap-and-fling is a lift-enhancing mechanism used especially by small insects (Weis-Fogh 1973; Wootton 1990), including Strepsiptera (see Fig. iii.2). When employed, the wings are struck together, generally at the top of a wingstroke, canceling the oppositely circulating air currents attendant to the wings, thus freeing the them to immediately adopt new circulations (Chin & Lentink 2016), rather than having to wait for the existing ones to separate from the wings. The wings are then flung or peeled apart rapidly, producing strong vortices at the leading edges of the wings as they separate (of opposite orientation so they sum to zero and thus observe the conservation of angular momentum) (Lehmann 2004). Therefore, lift is generated more quickly and over an extended range (Chin & Lentink 2016). This produces additional unsteady aerodynamic effects that result in nearly ¼ more lift than that of conventionally flapped wings (Marden 1987) (which are also "unsteady" in that they are flapped, rather than remaining essentially motionless, as occurs with fixed-wing aircraft). Additionally, some of the work done to bring the wings together is converted into thrust via vortex recapture (Chin & Lentink 2016; Lehmann et al. 2021), as well as through the "cupping" (Fig. iii.2F) of extremely flexible wings (Johansson & Henningsson 2021), thus offsetting some of the cost of having to decelerate and accelerate the wings at the ends of each wingstroke (Casey 1981).

While measuring mFMRs, Marden (1987) also learned that maximum weight loads scale as the 1.0 power of muscle mass: Therefore, no matter how much muscle a flying animal has, having a given amount more muscle allows it to do the same amount more work (although the cost of doing so could also increase; e.g., the (exo)skeleton may not be able to support the addition muscle, etc.).

Taking the ¼ increase in efficiency from clap-and-fling into account, Marden (1987) found that mFMRs are nearly constant for all flying animals. He also found that the average mFMR of damselflies (which employ clap-and-fling) is 0.116 (Marden 1987). Thus, the percentage of mass dedicated to flight muscle compared to the mass a damselfly can just barely lift into the air (including its own) is 11.6%. The smaller that value is, the more efficient the flight apparatus is. Considering the scaling of flight muscle mass, this also means that at least 11.6% of a damselfly's body mass must be dedicated to flight muscle for it to become airborne. Thus, an animal's mFMR is the minimal allotment of total body mass that must be dedicated to flight muscle for it to be able to fly. The lower the mFMR and the higher the FMR (i.e., the actual mass dedicated to flight muscle), the more mass an animal can carry in flight, and the faster the unladen animal can propel itself through the air (albeit attenuated by drag).

The strepsipteran marginal flight muscle ratio

Given this 25% disparity in the flight efficiency of insects employing clap-and-fling versus those using conventional wingbeats, it is not surprising that Marden (1987) found that the mFMRs differed between them. However, the FMR was found to occupy a fairly narrow range within both groups (Marden 1987). Thus, muscle efficiency is relatively constant among all (volant) animals (Marden 1987). To my knowledge, the mFMR has not been determined for any strepsipteran species. Therefore, a linear combination of the mFMR of damselflies and that of Lepidoptera—the two clap-and-fling groups Marden (1987) investigated—should provide a reasonable baseline estimate of the strepsipteran mFMR. The average clap-and-fling mFMR for damselflies was 0.116, and that of Lepidoptera employing clap-and-fling was 0.138 (Marden 1987). There is evidence that the extent of clap-and-fling wing contact influences the degree of lift enhancement (Marden 1987). In the *Triozocera texana* clap-and-fling variant, it appears that about ¾ of their wings come into contact (Fig iii.2F—note how the bottom of the wings fold outward). However, Marden (1987) did not provide data for the extent of wing contact in damselflies or Lepidoptera, just the final mFMR value. Given how broad and flexible strepsipteran wings are, it is almost certain that they actually employ the clap-and-fling variant known as clap-and-peel. With clap-and-peel, the wings do not rigidly rotate about their trailing edges, but are instead pulled apart more gently, like two pages of a spine-down book being separated along their distal edges (Ellington 1984). This smooth separation is substantially more effective at creating circulation around the wings than the fling is (Ellington 1984). Although lepidopteran wings are also flexible—and particularly those of butterflies (Johansson & Henningsson 2021)—they are not nearly as flexible as strepsipteran wings (Figs. iii.2 & iii.3), therefore the average mFMR value of 0.138 obtained for Lepidoptera likely underestimates the strepsipteran mFMR. However, because the mFMR for damselflies (0.116) arose from clap-and-fling acting on two pairs of (separated) wings, it is much lower than usual, so the average of 0.127 arising from it and the lepidopteran value is probably a good representation of the true strepsipteran mFMR.

Calculating flight muscle ratios

From Coelho's work on cicada killer wasps (Coelho 1997), I deduced that the mFMR times the total mass (including that of the flier) an animal can just lift into the air (L_m) divided by its body own mass (B_m) is the FMR. In equation form:

$$\frac{L_m \times mFMR}{B_m} = \frac{total\ mass \times \frac{flight\ muscle\ mass}{total\ mass}}{B_m} = \frac{flight\ muscle\ mass}{body\ mass} = FMR, \tag{1}$$

In Coelho (1997), the lifted mass was expressed in terms of the body mass of the cicada killer. In equation form, this amounts to:

$$\frac{(1+1.88) \times 875.1 \times 0.1447}{875.1} = 1.88 \times 0.1447 + 0.1447 = 2.88 \times 0.1447 = 0.417, \tag{2}$$

where 1+1.88 corresponds to what was lifted: the mass of the cicada killer itself, plus the mass of the cicada it carried (which was 88% greater than the mass of the cicada killer),

875.1 was the mean body mass of all the surveyed female cicada killers, and

0.1447 is the effective mFMR of a cicada killer,[9]

This is fortuitous in that it shows that the FMR is precisely how many times the mFMR fits into the maximum liftable mass when expressed in terms of body mass. Apart from round-off error, this equals the value of 0.416 given as the FMR for all female cicadas in Table 1 of Coelho (1997). Therefore, as long as the actual mFMR or a plausible approximation is available, a viable flight muscle ratio can be computed without ever measuring the flight muscle mass itself. In general, the body mass must still be known or approximated, but not the mass of the flight muscles.

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[9] The "effective mFMR" = flight muscle mass ÷ (wasp body mass + cicada body mass). Thus, it is as the mFMR, but applied to a mass the wasp could only manage controlled descents with, rather than actual lift-off. The effective mFMR and the lift-off method of approximating the mFMR that Marden (1987) employed, asymptotically approach the true mFMR from above (overestimation) and below (underestimation), respectively.

Results

Strepsipteran tarsi end in well-developed claws only in the basal family Mengenillidae. In all others, they are either reduced (Corioxenidae), or absent altogether (Pohl & Beutel 2008). Thus, in the following photographs, it is common for the tarsi to be tipped with what appear to be yellow or white balls. These are hairs that better equip their bearers to adhere to appropriate areas of the host (Pierce 1909; Pohl & Beutel 2005, 2008) and to each other when grasping, but perhaps little else (Linsley & MacSwain 1957). This, and the general weakness of adult male legs (Hubbard 1892; Muir 1906; Linsley & MacSwain 1957) indicate that strepsipteran walking is very much in the direct service of mating.

Strepsipteran flight versatility

Figure iii.2 is an overview of photographically captured *Triozocera texana* flight modalities, including level cruising flight (Fig. iii.2A), swept back wings (Fig. iii.2B–D), knife edge flight (Fig. iii.2C), dramatic directional changes, represented by a steep turn (Fig. iii.2D), hovering (Fig. iii.2E–H) (the closer to vertical, the slower the flight speed), clap-and-fling variants (Fig. iii.2F–I), powered diving (Fig. iii.2I&J), and controlled descent (Fig. iii.2K&L).

Chief among strepsipteran wing attributes is extreme flexibility (Fig. iii.3A–D), including positive and negative camber (Fig. iii.3A&B), their ability to limply fold over at the tip (Fig. iii.3C)(I am unaware of what flight characteristics this enhances), and even wing collapsibility (Fig. iii.3D), wherein the wings do not provide lift. Wing collapsibility may form a transition between flight modalities during emergencies or other unexpected events. The angle of attack can also be altered by tilting the wings back (Fig. iii.3E) or forward (Fig. iii.2H).

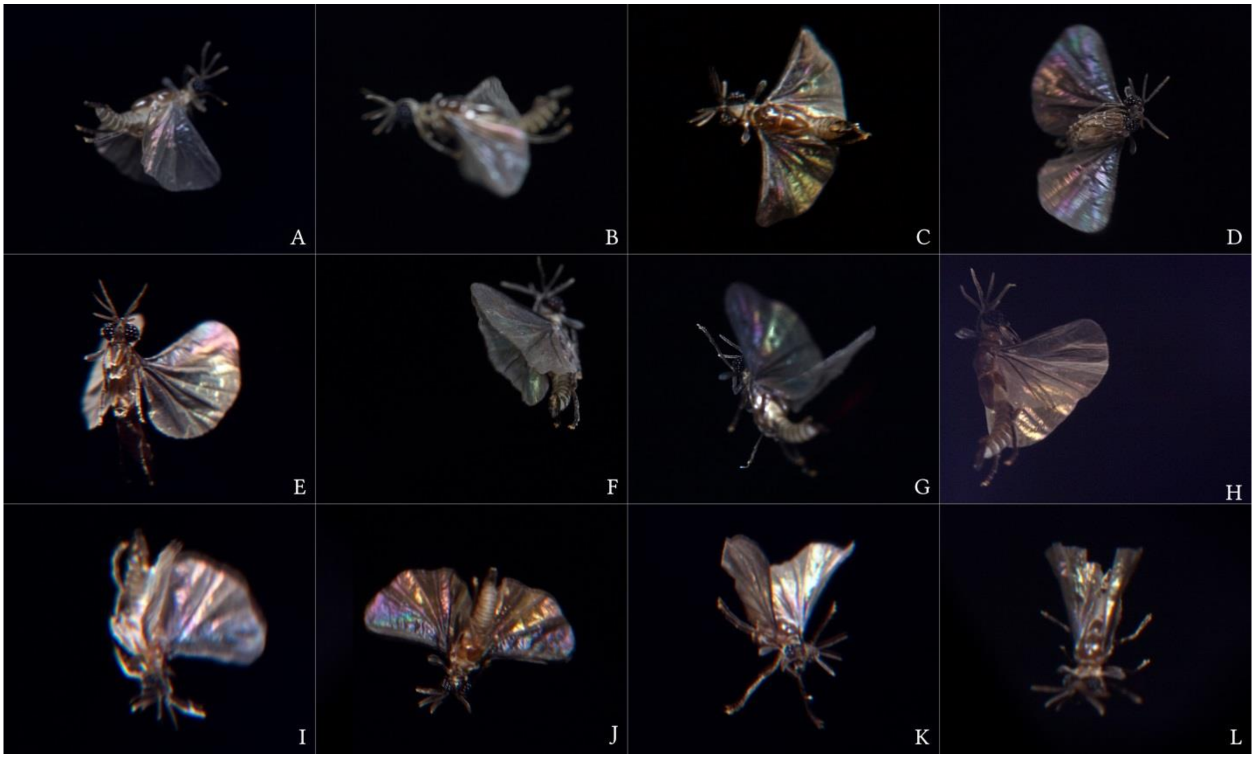

Fig. iii.2. Several strepsipteran flight modalities. In the depicted sub-images, the specimen was: (A) Flying near its cruise speed; its body is roughly horizontal and the leading edges of the wings are perpendicular to the long axis of the body. (B) Flying with its wings swept sharply back, thus reducing drag. (C) Flying at a nearly 90° angle, since both wings appear uniform with respect to one another (aka, a knife-edge maneuver). (D) Quickly changing its trajectory because the lower wing has greater camber and blur than the upper wing. (E) Hovering, because the body is nearly vertical. (F) Hovering and using a form of clap-and-fling to increase lift on the transition between the up- and downstroke. (G) Hovering and using clap-and-peel to enhance lift on the downstroke. (H) Hovering, but tilting the wings toward one another at the end of the downstroke, which should allow a near clap-and-fling to increase lift during the transition from downstroke to upstroke. (I) Most likely power diving because the wings are outstretched in a manner consistent with flapping, the legs are held close to the body, and the head is in alignment with the body. (J) Probably also power diving, but at a different point in the wingstroke than in *(I)*. (K) Controlled descent, as the legs are outstretched and the wings are fluttering, rather than actively modifying airflow. (L) Controlled descent; the legs are extended, and the wings are nearly folded, and the head is almost perpendicular to the body axis. All insects are presented at scale, with the possible exception of *(C)*, whose original magnification was estimated, rather than measured. All images are normalized to a magnification of 1.98×. *(I—K)* are blurred due to poor flash synchronization, not the speed of descent.

Fig. iii.3. A hallmark attribute of the strepsipteran wing is its pliability. (A&B) The wings can bend in both directions, allowing for both positive camber *(A)*, and negative camber *(B)*. (C) Despite the relative rigidity of the leading edge spar, the wingtips have gone flaccid. (D) The wings have been folded up like a fan, as if the insect was unexpectedly forced to transition between flight modes. (E) The angle of attack can be rotated back (as shown here), but also forward, as in Fig. iii.2H.

Reducing exposed wing area

In Fig. iii.2B–D, the wings are swept back—i.e., the wingtips have been temporarily displaced posteriorly, which reduces drag and increases wing loading. This is known to occur with increased flight speed in the hawk moth, *Manduca sexta* (Willmott & Ellington 1997). The strepsipteran wing is very broad, limiting flight speed. However, sweeping the wings back represents a viable mechanism by which Strepsiptera could produce and sustain higher flight speeds.

Passive and powered diving

While at rest or walking, at least some strepsipteran species can hold their wings outstretched horizontally (Fig. iii.4); however, it is not clear if any can do this while flying. Strepsiptera have asynchronous flight muscles (Smith & Kathirithamby 1984), so wing flapping is not under direct neural control. Instead, it is regulated by a partially self-sustaining process that relies on the inherent resonant frequency of the thorax (Josephson et al. 2000; Sane 2016), along with delayed stretch activation and shortening deactivation, both special characteristics of asynchronous flight muscles (Josephson et al. 2000). This, the fact that the position of the wings is different from that assumed by *E. koebelei* when they outstretch their wings horizontally (Fig. iii.4A), and that *T. texana* do not outstretch their wings horizontally at all when not in flight (personal obs.), all support that Fig. iii.2I is an example of a powered diving. Furthermore, the fact that the wings are touching at their distal ends is indicative of clap-and-peel, so the insect may have been flying with great urgency.

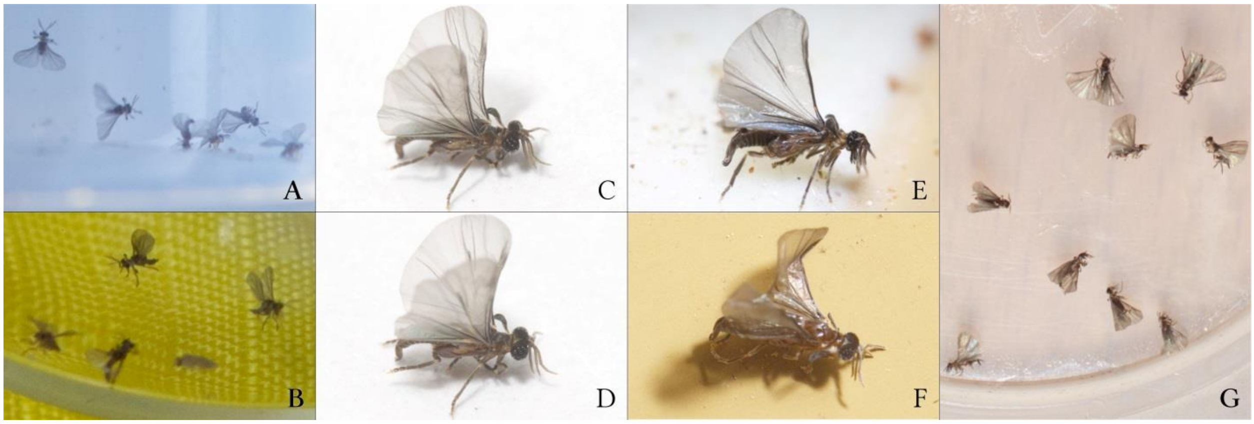

Fig. iii.4. Resting wing positions. *(A&B)* Are photos of *Elenchus koebelei*. Though most could no longer fly, all were still able to flap their wings at the time the photographs were taken. *(C–G)* are photos of *Triozocera texana*; (*C–E*) were given areas free of other insect species in which to walk. (A) *E. koebelei* wings are broadly uniformly placed when horizontally orientated. Also note that they are not pleated. (B) A pair of *E. koebelei* have their wings outstretched vertically; however, they are not juxtaposed like when they are no longer capable of flapping them. *(C&D)* are photos of the same *T. texana* taken at slightly different times. (C) The wings are held vertically, roughly perpendicular to the long axis of the body. (D) The wings are positioned vertically, but have been pulled forward and project laterally slightly. This movement suggests *T. texana* have no means of vertically locking their wings. (E) In this image, the wings are also held vertically, but the leading edge is leaning back slightly, again suggesting there is no vertical locking mechanism. *(F&G)* Unlike the others, these insects were dead by the time they were photographed. (F) This Strepsiptera expired on an arm of the insect rig itself. Its wings subsequently became incredibly misshaped, after having adopted the form of the edge of the insect rig on which it died. This is a strong indication that upon emergence strepsipteran wings—even if fully expanded—have not actually hardened. (G) Images of several specimens collected from a modified Mayfly (light) trap one evening. Their wings are oriented haphazardly: one horizontally, some vertically, several intermediate between those extremes—as if upon death, their thin wings dry completely in whatever position they occupied when the insect depleted the strength to move them. *(A–F)* are roughly drawn to scale.

Fig. iii.2J is also an example of power diving, only at a different point in the wingstroke than Fig. iii.2I was. Poorly synchronized flashes produced a double image at the trailing edge of the wing that is not also visible at the head of the wings, because there was more flutter toward the back of the wing than along its leading edge, which was more taut. Additionally, double exposure is most visible where contrast is greatest. Thus, unlike the trailing edge (which proceeded from black to partially lit), the interior of the wing appears blurred, rather than doubly exposed. Also, because the second flash was brighter than the first, it highlighted movement toward the back of the wings, while minimizing it at the leading edge. Finally, the wings appear to have been moving so as to cover a larger area of the camera sensor, thereby obscuring the outline produced by the first flash firing. Together, these processes provide further evidence that the wings were being flapped when the photograph was taken. In Fig. iii.2K, the wings are open wide enough to be flapped, but appear to be flaccid and tilted, rather than swept back, and so were probably disengaged to slow descent by increasing drag—strepsipteran wings have no fold lines, so although Strepsiptera can push their wings back, the wings themselves do not fold, and thus are always somewhat exposed. The legs are also outstretched, which further increases drag. The specimen also appears to have been turning out of the page (perhaps to change or stabilize orientation), with the forward parts of the body moving more than the rearward parts, such that its rotational axis of symmetry did not pass through the abdomen. In Fig. iii.2L, the wings appear to be drawn in too close for flapping, so the descent was likely also passive. Note that the blur in Fig. iii.2I–K was not due to the speed of flight, especially since the specimen in Fig. iii.2K was probably not actually flying. Instead, ghost images resulted from the substantial variant internal delays of one or both flashes, even though they were triggered simultaneously. Nonetheless, ostensibly due to the rapid alteration of direction and its unexpected course, diving in Strepsiptera may occur to avoid echolocating bats.

Wingstroke amplitude

The larger the angular extent of a wingstroke and the faster the wings flap, the more force is produced (and the greater the energy consumption). Greater force naturally translates into speed, but could also be used to hover or to rise more rapidly. I estimated the *T. texana* wingstroke amplitude to be about 154° (Fig. iii.5), using the average measurement taken from two photographs. Pix et al. (1993) measured a wingstroke amplitude of about 160° for *Xenos vesparum* using conventional high-speed videography. Their value agrees well with my determination, especially because 154° is probably an underestimate, due to the likelihood that the photographed wingstrokes were not at their absolute nadir. Given the inclination of its body, the specimen in Fig. iii.5A is likely flying at a reduced rate, however, it is also plausible that it is flying slightly upward, which would result in a larger measured wingstroke amplitude due to the greater lift requirements.

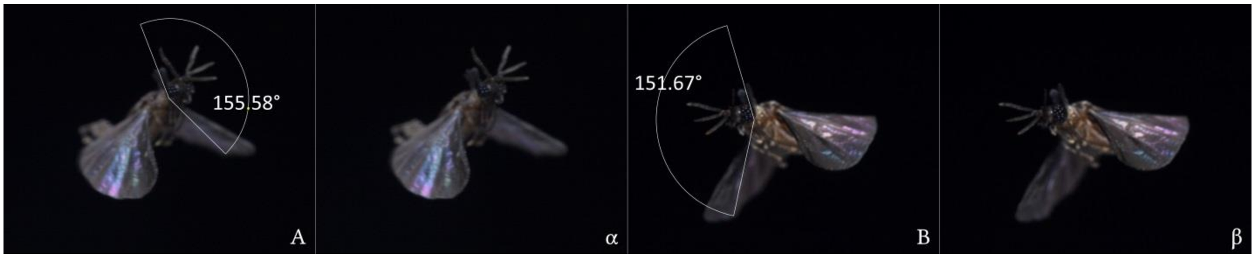

Fig. iii.5. Because the halteres and wings beat in antiphase, together they can be used to approximate the wingstroke amplitude, provided one or the other is at its apex, which is easily diagnosed. In the images with Latin letters, Strepsiptera have been drawn with the angular measurement between the intersection of the halteres and one of the wings included. For better landmark identification, the cells with Greek letters contain unadorned images of the same insects. In the Latin-lettered images, the leading spar of the (cambered) wing appears as a slight bulge along the inside edge of the rearward wing, marking the angular extent of the wings. (A) The halteres are straight above the abdomen, so the wings must be at near the nadir. The wingstroke amplitude was measured to be about 156°. (B) Here, the wingstroke amplitude was measured to be about 152°. Although the halteres nearly touch, the angle between their dorsal faces is substantial, whereas they are nearly parallel in *(A)*. This may due to the halteres being pronated at the end of their upstroke, which would emphasize their evolutionary origin as flight wings.

Wing loading

An insect can have larger or smaller wings compared to its body weight, an attribute called wing loading.

Wing loading is total weight divided by wing area.[10] For a given weight, the larger the wing area, the lower the wing loading. Low wing loading is associated with high lift, high maneuverability, and low power requirements, but also reduced speed (Cavagnaro 2019). High wing loading supports high sustained flight speeds and less perturbation by wind gusts, but requires more power, provides less maneuverability, and increases the difficulty of becoming airborne (Cavagnaro 2019). Tradeoffs are made between these traits depending on the phylogenetic heritage and ecological needs of different flying animals.

As discussed in the Materials and Methods section, the available test specimen was 0.12 mm below the lower range for which Sage (1982) verified his model. However, it is the largest Strepsiptera for which I know the wing area (0.03534 cm^2) and body length (2.38 mm), the parameters necessary for calculating the wing loading. Applying Sage (1982) to this specimen produced an estimated weight of 0.000956 g (9.56×10^{-4} g), and a wing loading for the surveyed *Triozocera texana* of 0.0271 g/cm^2. According to this result, on a scale of increasing wing loading from the combined data sets of Tercel et al. (2018) and Byrne et al. (1988), *T. texana* would be between 78 and 79 of 231 surveyed species, with wing loading just higher than an unidentified species of Tipulidae (crane fly) of the genus *Tipula*, and just lower than the southern darter dragonfly, *Sympetrum meridionale*.

Interestingly, Coleoptera were found to have higher wing loading than Diptera (Byrne et al. 1988; Tercel et al. 2018), despite the slow (but bumbling) flight of beetles, and the fast (but nimble) flight of flies. Thus, wing loading alone is insufficient to adequately characterize insect flight. These data sets also make evident that apart from Lepidoptera, the megadiverse insect orders all have high wing loading.

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[10] Wing and power loading are most consistently determined in terms of weight, *not* mass. There are high-force flight contexts in which the weight of a flying object would change—affecting flight performance—but its mass would not (Banner 2014). With respect to animals, this distinction is unimportant, however.

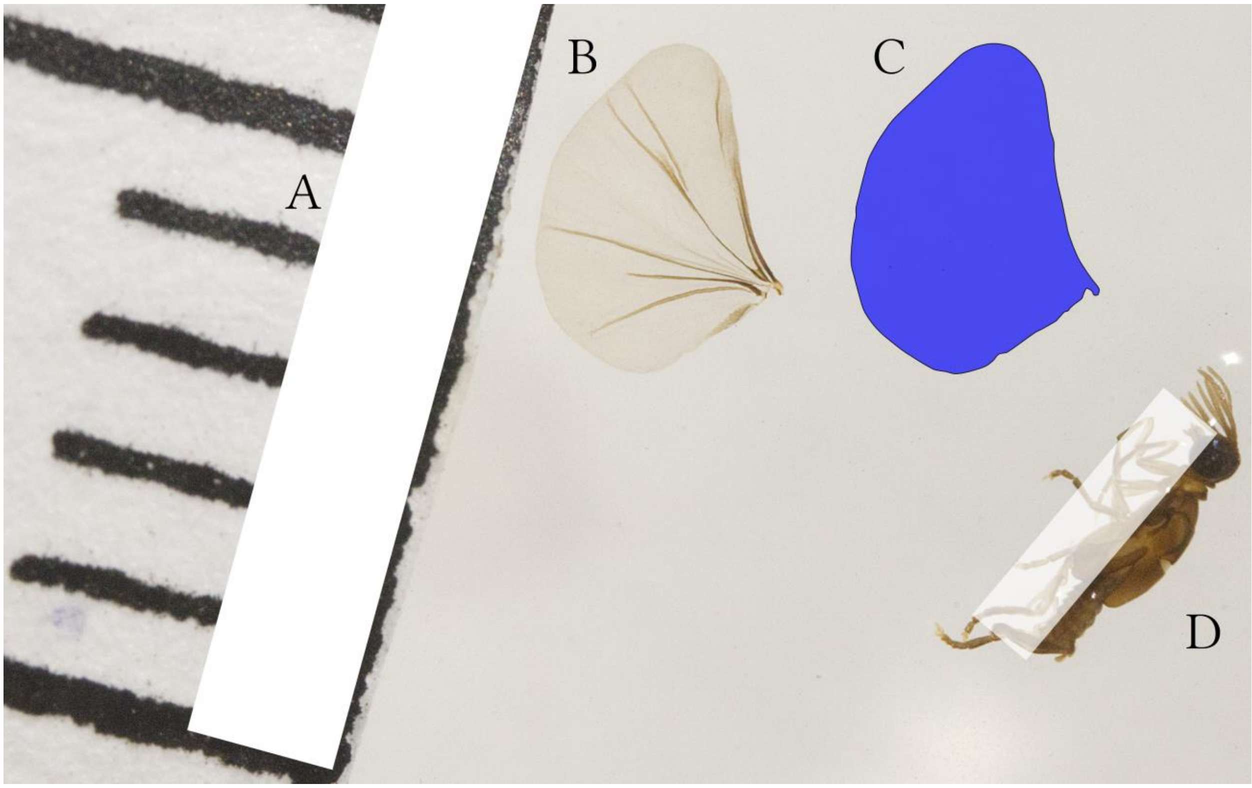

Fig. iii.6. A photograph of an excised wing and a ruler were used in the determination of each wing area. The body length of the donor Strepsiptera was also measured to estimate its wet weight for calculating the corresponding wing loading. (A) A rectangle spanning a 1 cm length of a paper ruler was used to establish the pixels per millimeter for determining wing area and body length. (B) The original strepsipteran wing. (C) Blue shaded outline of the wing indicating what was measured in calculating its area. (D) The body of the Strepsiptera from which *(B)* came. The translucent rectangle depicts what was measured as the body length of this individual.

Power loading

Power loading is weight divided by power—the rate at which energy is converted into useful work. Since all flight muscles output similar power per unit mass (Marden 1987), the FMR is an excellent stand-in for power loading when working with animals. However, power loading applies to both animals and constructed flying objects, so I have referred to power loading in general, and to FMR as a special case of power loading.

Recall that a *Hylecthrus rubi* strepsipteran was able to use its wings to drag 1) its own weight, 2) the weight of a first conspecific, 3) the weight of a second conspecific, and 4) the weight of the expired host bee. In the preliminary FMR estimate, I assumed the dead host had retained 80% of its full wet weight because it was still moist, but had died at some unknown previous point in time. If the parasites and the host bee all had the same wet weight, then the Strepsiptera would each contribute 0.127 to the total FMR, and the partially desiccated bee would contribute 0.127 × 0.8, for a sum of 0.4826, or about 48% of the *H. rubi* body weight being dedicated to flight muscle. This approach circumvents needing to know or estimate the *H. rubi* wet weight. It clearly demonstrates that *H. rubi* has a high FMR and strongly implies that it could engage in forward flight while carrying three other adult male conspecifics; that is, it could fly carrying three times its own body weight. Furthermore, even if the *H. rubi* mFMR were 0.116 (i.e., that of a damselfly, the lowest mFMR Marden (1987) documented), the *H. rubi* FMR would still equal at least 0.4408, so that about 44% of its body weight would be dedicated to flight muscle. That total just surpasses the most flight muscle-endowed sphinx moths (0.436) and is only known to be exceeded by the upper echelon of odonate FMRs (damsel- and dragonflies at 0.473 and 0.560–0.63, respectively) (Marden 1987, 2000). Additionally, the fact that the flight apparatus was already essentially that of extant lineages in all known strepsipteran stem groups (i.e., †*Protoxenos*, †*Cretostylops*, and †*Mengea*) is evidence that it developed early in strepsipteran evolution (Pohl & Beutel 2008). Furthermore, a Burmese amber (circa 100 myo) strepsipteran fossil, †*Cretostylops engeli*, has been found, providing strong evidence that the modern flight apparatus is at least that old (Grimaldi et al. 2005). Therefore in the absence of contrary information, it should be assumed that all extant Strepsiptera have high FMRs, and thus low power loading. Provided that *H. rubi* are not atypical Strepsiptera, 40% can be considered the lower-limit FMR for the order: Adult male Strepsiptera dedicate at least 40% of their body mass to

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† The dagger symbol indicates an extinct phylogenetic group. Furthermore, in this case, all three are also *stem* groups: they consist entirely of extinct organisms that display more of the morphological features of extant Strepsiptera than any other extant organisms. Thus, these extinct lineages had essentially the same flight apparatus as modern Strepsiptera, but evolved before the most recent common ancestor of all extant strepsipteran taxa.

flight muscle (Fig. iii.7).

In the Materials and Methods section, the strepsipteran mFMR was estimated to be 0.137. I was unable to find the wet weight of the host bee *Hylaeus rubicola* (*Ha. rubicola*). However, the body length of the voucher specimen was ⁵⁄₂₄ inch (5.29 mm)[11] (S.S. Saunders 1850), which corresponds well with the known body size range for females of the congeneric species *Ha. bisinuatus* (5–6 mm; assumed to be 5.5 mm long on average), for which the average wet weight is known for adult females just emerging from their nest cells (Tepedino 1980).[12] According to the wet weight estimation model proposed by Sage (1982) for Hymenoptera and Diptera ($R^2 = 0.94$), a body length of 5.29 mm corresponds to a wet weight of 0.004851 g (Sage 1982). The three Strepsiptera and the host bee were all assumed to have the same wet weight, but the bee was assumed to have lost 20% of its weight to desiccation, so the estimated total weight lifted was,

$$weight = 0.004851 \div 4 \times 3 + \ 0.004851 \div 4 \times 0.8 = 0.004608 \text{ g.} \tag{3}$$

If *Triozocera texana* can only lift the same weight as the much smaller *H. rubi* and the mFMR is correct for them both, then according to equation (2) the flight muscle ratio of *T. texana* is,

$$FMR = \frac{L_m \times mFMR}{B_m} = \frac{0.004608 \times 0.127}{0.000956} = 0.612. \tag{4}$$

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[11] The “unc.” appearing in S. S. Saunders (1850) is short for *uncia*, i.e., ¹⁄₁₂, or in this context, “inch” (being ¹⁄₁₂ of a (British Imperial) foot).

[12] Sage’s (1982) model predicts a mass of 5.392 mg for a 5.5 mm bee, only 2.4% more than the actual value of 5.262 mg (Tepedino, 1980). Thus, the model appears to work well for bee-shaped Hymenoptera within the verified size range.

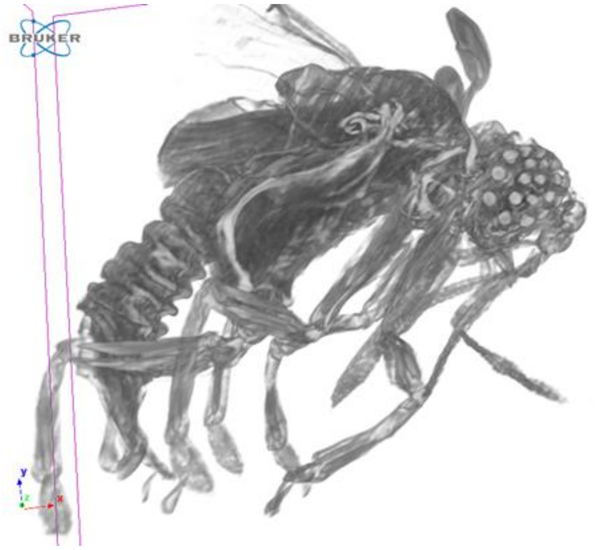

Fig. iii.7. Micro-computed tomography (microCT) image of an *Elenchus koebelei* showing the metathorax and an outline of its internal flight musculature. The prothorax is not clearly visible, but does not support the wings or halteres. The small hump behind the eyes is the mesothorax, from which strepsipteran halteres emanate. The strepsipteran metathorax supports the only pair of wings, and is greatly expanded. X-rays penetrate chitin, documenting that a very large portion of adult male strepsipteran anatomy is dedicated to primary flight muscles. The diagonal striations denote the dorsoventral (flight) muscles (DVM) and extend between the oversized metasternum (ventral sclerite of the metathorax) to the scutum, while the horizontal striations indicate the dorsal longitudinal (flight) muscles (DLM). Ancillary flight muscles that control wing orientation are also present in the metathorax, but are overshadowed by the exorbitant flight muscles. The protuberance extending from behind the notum (dorsum of the insect thorax, the scutum being the frontmost portion) is the leading edge of the left wing.

Haltere orientation

The dorsal and ventral surfaces of the relatively massive strepsipteran halteres (as compared to those of brachyceran flies) are distinct and exposed, making them more available for inspection than dipteran halteres. The orientation of the halteres in Fig. iii.8 is quite varied, even though the Strepsiptera all hovered in nearly still air. Fig. iii.5B also shows evidence of haltere orientation having been altered in the absence of an obvious causal external force. These are indications that like wings, strepsipteran halteres not only passively respond, but are also actively manipulated by the insect itself. Flies require a mechanism to prevent their own voluntarily flight initiatives from being overridden by gyroscopic inhibition (Chan et al. 1998). That could be achieved by periodically disabling haltere-based course correction (a prominent hypothesis), or by directing haltere movement such that changing bodily orientation in accordance with actively displaced halteres results in the desired unstable motion (another prominent hypothesis). The latter solution has the advantage of not temporarily disabling or dampening the safeguards fully functioning halteres provide. Subsequent research has provided support for actively directing haltere motion to override reflexive inhibition in Diptera (Dickerson et al. 2019). From the information presented in Fig. iii.8, Strepsiptera appear to have addressed the problem in the same manner.

Fig. iii.8. Haltere orientation. In the upper row, three photos of hovering Strepsiptera have been enlarged and rotated so that the body axes are roughly vertical. Even though the insects were hovering in nearly still air, the haltere orientations vary dramatically. This provides evidence that strepsipteran halteres are steered, just as the wings are. (A) The insect was rotated −17.6°, so the visible haltere was at an angle of −14.6° with respect to due north. (B) was not rotated. Its visible haltere was at an angle of about 32°. (C) was rotated 25.6°. Its foremost haltere was at an angle of about 52°. Blue lines indicate the approximate inclination of the haltere (from horizontal; measured along the dorsal face of the haltere). A straight yellow arrow indicates a horizontally flipped image. A curved yellow arrow indicates image rotation. Images in the upper row are at twice the magnification of those in the lower row. The images in the lower row show the insects in their original orientations, without haltere angle adornment.

Discussion

Flying Strepsiptera have been portrayed as slow (Friese 1883; Muir 1906; Kirkpatrick 1937b), remarkably quick (Hubbard 1892; Pierce 1909), peculiar (Thwaites 1841; Champion 1899), graceful (Thwaites 1841; Hrabar et al. 2014), erratic (Enock 1875; Kirkpatrick 1937b), powerful (S.S. Saunders 1853), and weak (E. Saunders 1888). What could account for all these ostensibly incongruous flight qualities?

Wing loading effects

A good way to characterize strepsipteran flight is via wing loading. Wing loading is total weight divided by wing area. Therefore, it is the average load each portion of a wing must support (Banner 2014).

Advantages of low wing loading (WL) (Taylor & Thomas 2014; Cavagnaro 2019) (i.e., having a large wing area compared to weight):

(1) High lift — enables low speed flight without stalling, and abrupt take-offs and landings

(2) Quick changes of direction

(3) Allows the flight muscles to be weaker

(4) Ability to soar (i.e., maintain or raise altitude or produce thrust by riding rising air currents)

  - Soaring may be entirely inaccessible to Strepsiptera, which may not be able to hold their wings steady in flight; at any rate, Strepsiptera follow pheromone plumes, so soaring would only be of any use to species that patrol

Disadvantages of low wing loading (Cavagnaro 2019):

(−1) Inefficient at high flight speeds (due to high drag)

(−2) Susceptible to wind gusts (due to expansive wing area)

The higher the wing loading (i.e., the smaller the wings), the harder an animal must work to remain aloft. But it is also generally true that the higher the wing loading, the faster an airborne animal is able to fly, especially for extended periods. Low wing loading optimizes for lower-speed, highly maneuverable flight. High wing loading optimizes for high-speed, predominately straight flight.

Power loading effects

Power is the rate at which energy is converted into useful work. Power loading is the ratio of weight to flight muscle (or engine) output. With increased power (i.e., *lower* power loading) insects can fly faster, so low power loading is often desirable. However, increased power also increases energy demand, because asynchronous flight muscles work most efficiently at a more or less constant resonant frequency (Josephson et al. 2000). Therefore, most insects usually produce roughly the same power output regardless of the actual demand, with any excess being wasted to allow for slower flight (generally by lowering the abdomen away from horizontal (David 1978)). As proposed above, the *Hylecthrus rubi* FMR is high, and by extension, that of the order Strepsiptera as well. Given that all volant animals have “nearly identical muscle mass-specific lift” (Marden 1987), low power loading can normally be appraised by simple visual inspection—and has been by several Strepsiptera researchers (Pierce 1909; Ulrich 1933; Pohl & Beutel 2008)—and further verified by histology (Smith & Kathirithamby 1984) or other high-resolution assessment (Fig. iii.7). In Strepsiptera, there is also little of the apparatus normally allocated to eating: the ventral mouthparts and their muscles are strongly reduced, the salivary glands, their receptacle, and the tentorium (attachment point for muscles that move the mouthparts) are all absent, and the midgut is strongly modified to hold air rather than food (Pohl & Beutel 2008), giving it the designation, “balloon gut” (Beutel & Pohl 2006). Accessory genital glands are also absent, and the Malpighian tubules are vestigial or strongly reduced (Pohl & Beutel 2008), all of which make for an expressly light frame, which lowers power loading more. Beutel and Pohl (2006) noted that the fat body is “well developed in the posteroventral head region,” but poorly developed overall (Pohl & Beutel 2008). It is interesting that the strepsipteran brain ostensibly has its own energy store, a situation likely linked to the lower energy demand of slow (nocturnal) eyes. Thus, the brain may tap a separate, more efficient energy store, reserving additional high turnover-rate energy for the flight muscles.

Advantages of low power loading (PL) (Banner 2014; Cavagnaro 2019; Hirschman 2020):

(1) Greater thrust (flight-enabling force)

(2) Faster climbing rate — reduces or removes the necessity of a pre-flight jump

(3) Rapid acceleration

(4) Higher cruise speed (i.e., speed at which flight is most efficient)

Disadvantages of low power loading (Cavagnaro 2019; Hirschman 2020):

(−1) Higher rate of energy consumption — poor for patrolling

- Flight range may not be reduced, however, because cruising speed is also increased

Strepsiptera that monitor the aggregate nesting sites of solitary bees (from which the patrolling males would also have emerged) may have higher power loading—i.e., *less* flight muscle mass and lower top flight speeds—than other Strepsiptera. However, given that Stylopidae (which includes all known patrolling Strepsiptera) branched later than Elenchidae (Pohl & Beutel 2008), whose enormous flight muscle outlay is represented here by *Elenchus tenuicornis* (Smith & Kathirithamby 1984) and *E. koebelei* (Fig. iii.7), wherein males are very likely to emerge near calling females (James & Strong 2018)—such that being relegated to slower, extremely maneuverable flight would be less disadvantageous—low power loading is a baseline feature of all extant Strepsiptera (Pohl & Beutel 2008).

Balloon gut functions, mysteries, and misconceptions

Especially considering that maturing males are confined to puparia embedded in the abdomens of other insects, the strepsipteran "balloon gut" almost certainly helps expand the thorax, abdomen, and wings of teneral pharate adults by forcing hemolymph into compressed areas, as occurs in other insects through fluid ingestion (Jousset-de-Bellesme 1877; Prell 1914; Fraenkel 1935). This increases the insect's volume before its adult cuticle hardens, thereby reserving area for enlarged flight muscles, etc. The balloon gut probably also provides thermal insulation, as thoracic air sacs do in some dragonfly species (Heinrich 1995). Finally, the capacity to perceive changes in barometric pressure has been found in various bark beetles (Bennett & Borden 1971; Lanier & Burns 1978). This sensitivity is linked to a large air bubble in the otherwise evacuated midgut (ventriculus) obtained by swallowing air prior to dispersive flight (Bennett & Borden 1971). It is hypothesized that atmospheric pressure-induced variation in the size of the bubble allows the beetles to avoid flying on days with unstable weather or gusty wind (Bennett & Borden 1971; Lanier & Burns 1978)—an ability especially important for smaller beetles (Lanier & Burns 1978). Thus, it is quite conceivable that the balloon gut also confers this ability to adult male Strepsiptera.[13]

One function the balloon gut certainly does not perform is reducing flight weight, as erroneously proposed in (Beutel & Pohl 2006) and (Pohl & Beutel 2008). When enveloped in an atmosphere, unpressurized air within a body weighs the same as the surrounding air, and thus has no effect on the weight of the animal. Actively ingested air, such as that sucked into the balloon gut by the strepsipteran air uptake apparatus (Beutel & Pohl 2006; Pohl & Beutel 2008), would be pressurized and would thus (v-e-r-y slightly) *increase* the insect's weight. Even if the balloon gut were to somehow produce a vacuum, its limited volume would not result in much weight reduction at all (Gunn 1931). Appeals to relative density (specific gravity) fare no better: despite a reduction in overall density due to ingested air, the insect would still weigh the same (actually, it would weigh somewhat more), so the same force would be required to lift it (Gunn 1931).

Although beetles have even higher wing loading (normally equated with faster flight) than flies, to account for their poor flight performance, Coleoptera must also have high power loading (relatively underpowered flight). It is likely elytra provide some lift enhancement (Le et al. 2014), allowing beetles to fly with even less power. In contrast, brachyceran Diptera—and some nematocerans, such as black flies—

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[13] It is not clear if female Stylopidia (Strepsiptera in which the female must remain within her host her entire adult life) have an organ analogous to the male balloon gut. However, at least *Mengenilla moldrzyki* and *Eoxenos laboulbenei* (species of Strepsiptera from the most basal extant family, Mengenillidae, wherein adult females are facultatively free-living) have a "mouthfield sclerite" (Tröger et al. 2023), which, in male Strepsiptera, functions as the opening to the balloon gut (Pohl & Beutel, 2008).

must have low power loading in addition to high wing loading to support their extremely nimble and rapid flight. Outstanding aerobatics despite high wing loading suggests that power output can be quickly and independently regulated between the two wings. This ability coupled with fast precise feedback from the halteres is likely what gives flies their great maneuverability. Although wing loading differs between the two orders, it is sensible to expect strepsipterans are capable of similar feats when flying at high speed (e.g., (Hubbard 1892); (Pierce 1909, p. 14)), at which times they ostensibly increase their wing loading by sweeping their wings back (Fig. iii.2B–D).

Consideration of diametric flight descriptions

Carried along by a gentle breeze

The "exceedingly graceful" flight observed by Thwaites (1841) is chiefly an expression of high lift and perhaps also high maneuverability, depending on the character of the gracefulness. Both are aspects of low wing loading (WL 1&2). Such flight is especially associated with males patrolling nest sites in temperate climates in late winter and early spring for *Andrena* (solitary ground-nesting bees) infected with female Strepsiptera (Friese 1883; Ulrich 1933).

Flight so rapid the eye can hardly follow

Based on the comparatively large volume of air strepsipteran wings displace with each wingbeat (Fig. iii.5), along with a respectable wingbeat frequency (150–160 Hz in tethered *X. vesparum* (Pix et al. 1993)), Strepsiptera are capable of quick acceleration. The whirlwind flight Hubbard (1892) observed stemmed in part from this (see PL 3), as well as the ability to abruptly change direction (WL 2). It is very likely Hubbard's *Xenos pallidus* also increased their wing loading by sweeping the wings back, thereby reducing drag (Fig. iii.2B–D). Even so, rapid flight exacts an energetic toll (see WL −1).

Not so weak and helpless

As quoted in Materials and Methods: *Flight muscle ratio (*Hylecthrus rubi*)*, an *H. rubi* used its wings to drag its dead host and two unemerged male conspecifics before freeing itself from its puparium. That display of strength was related to low power loading (PL 1). The normal strepsipteran wingstroke amplitude is likely about 160° (Pix et al. 1993); Fig. iii.5. Thus, some 20° may be reserved for responding to wing injury (Muijres et al. 2017), or for maximizing force or speed. Increasing wingstroke frequency within the rather tight confines available to asynchronous flight muscle also enhances force (Muijres et al. 2017), but at the cost of further increasing drag (WL −1) and most likely reducing the efficiency of the thorax-flight muscle resonance.

Flight reminiscent of a neuropteran

"Its [*Stylops melittae*] flight reminded me of that of the Neuropterous [*sic*] genus *Hemerobius*" (E. Saunders 1888). *Hemerobius* (brown lacewings) is a genus of the order Neuroptera, which easily had the lowest wing loading of the 11 insect orders represented in the combined Byrne et al. (1988) + Tercel et al. (2018) data set. Apart from the color difference, brown lacewings look like green lacewings with broader wings, which further reduce their wing loading. Often, low wing loading is synonymized with weak flight, meaning an insect has difficulty maintaining flight direction because it cannot overcome even minute air currents due to low flight speed (WL 1) or weak flight muscles (WL 3). Thus, weak flight tends to be associated with strepsipteran hovering when it is accompanied by abrupt changes in direction or orientation that are imposed by the environment (WL −2). Strepsiptera are light insects with broad wings, so this certainly occurs; however, being light is not the same as having weak flight. Weak flight is better characterized as the inability to vary air speed over an appreciable range (Krogh & Zeuthen 1941).

Very peculiar unsteady flight

When confined to small spaces, Strepsiptera often fly erratically (Kirkpatrick 1937b). This might be expected of insects frantically seeking escape; however, Strepsiptera have also been found to fly erratically in open air (Enock 1875), wherein it is energetically costly (WL −1) without an apparent rationale. Fortunately, however, observations of *Argyrotaenia velutinana* (red-banded leafroller moths) provide a very plausible explanation:

> Males were observed orienting toward the Pherocon 1C traps from as far away as 10 m downwind. Flight sometimes appeared to be erratic and of high velocity at first, but as males neared the trap edge, their forward progress slowed and the flight pattern was refined into small (10–20 cm) vertical or horizontal casting motions, which lasted as long as 17 sec before landing was attempted (Baker et al. 1976).

Analogous casting behavior seems be what Enock (1875) observed, although mediated by greater strepsipteran aerial agility, as evidenced by their possession of halteres and smaller size. Losing a strong scent plume (potentially indicating proximity) before establishing visual contact with a presumed odor source can result in much higher flight velocity (PL 1) and wide fluctuations in direction (Baker et al. 1976; Mafra-Neto & Cardé 1994) (WL 2). Such behavior would likely occur much nearer than 10 m downwind of a calling strepsipteran, however, considering how concentrated artificial pheromones from traps often are and that *A. velutinana* (5.5–8 mm) are significantly larger than most female Strepsiptera.

Elucidation of historical flight descriptions

Low wing loading and low power loading are the bases from which it is best to consider strepsipteran flight. As seen in the assessments above, all of the identified divergent strepsipteran flight descriptions can be explained by the application of these two factors. Due to low wing loading, all volant Strepsiptera can fly at low speeds (unlike, e.g., a locust) and are highly maneuverable (unlike most beetles). Due to low power loading, Strepsiptera can accelerate quickly and many species can attain high flight speeds, relative to their sizes. However, *maintaining* high flight speed comes at a great cost to endurance (Hubbard 1892). Lastly, the specifics of given wing planforms may allow some taxa to exhibit other prominent flight attributes, but these are versatile and foundational.

Strepsipteran pheromone pursuit

There are two known strategies that volant insects use to follow odor plumes (Dindonis & Miller 1980; Bursell 1984; Cardé & Willis 2008): Aim-then-shoot and optomotor anemotaxis. In aim-then-shoot, the current odor-bearing wind direction is assumed to be that of the odor emitter. That direction is determined before take-off and maintained while aloft, and backtracking or reassessment is only made if the odor is lost before its source is identified (Bursell 1984). Using this approach, if a potential odor source is not visually identified in a bout of flying, then an upwind-flying insect typically overshoots the odor emitter, lands, turns 180°, reassesses the wind direction, and then flies downwind, but not as far, lands, and repeats the process a few to several times, closing in on the target in a stepwise manner. Certain Diptera, such as tsetse flies (*Glossina pallidipes*) (Dindonis & Miller 1980; Bursell 1984; Cardé & Willis 2008) use this approach. Because insects employing aim-then-shoot do not seem to regulate their ground speed when surging forward, they probably use skylight polarization to ensure that they fly straight, regardless of altitude and any intermittent fluctuations in wind direction.[14] However, there is no indication that Strepsiptera have a dorsal rim area, so they are unlikely to be polarization sensitive. Furthermore, aim-then-shoot is only known from a few Diptera (Cardé & Willis 2008), none of which follow odorants emitted from individual insects. Aim-then-shoot also requires landing on potentially unknown substrates for course reassessment, which Strepsiptera are ill-equipped to do, because of their

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[14] It is surprising that skylight polarization has not been directly associated with optomotor anemotaxis (which might then be called "visual anemotaxis"). Perhaps sometimes it should be, but it is basically unavailable in the wind tunnels in which optomotor anemotaxis is tested.

specialized tarsi and poor walking ability.

In optomotor anemotaxis, an insect—e.g., a moth—follows odor plumes upwind (anemotaxis) using the speed and direction at which visual stimuli cross its eyes (optic flow) to evaluate its progress and to correct for deviations in its intended flight heading.[15] When a flying insect is moving straight ahead, visual stimuli proceed from front to back across its eyes. However, if its course is not directly forward, then the optic flow will have a transverse component (Cardé & Willis 2008). There are several flight patterns associated with pheromone-mediated optomotor anemotaxis, which can be used to determine if salient features of strepsipteran flight are congruous with it:

(1) If pheromone concentration is perceived to be roughly uniform but there is detectable air movement, moths will fly nearly directly upwind (Traynier 1968). This can occur if the concentration actually *is* uniform (Cardé & Willis 2008), or if separate odor filaments are encountered quickly enough (Mafra-Neto & Cardé 1994; Cardé & Willis 2008). (Almost like an 'odor filament fusion frequency.')

(2) Once distinct odor filaments have been detected, moths zigzag across the plume to increase the chance of remaining in contact with it, alternating between positive and negative deviations from directly upwind upon reaching the lateral extremes of the plume (David et al. 1982).

(3) When contact with a plume is lost, forward progress is halted and moths engage in often elongating flights perpendicular to the wind direction until either reengaging with the odor plume and resuming a zigzagged upwind approach, or abandoning the pursuit (David et al. 1982; Kennedy 1983), at which point a moth may elect to fly downwind to start again (Traynier 1968).

(4) While hovering near a pheromone emitter (Baker et al. 1976; Murlis & Bettany 1977), moths make visual assessments and depart if something appears to be amiss (Murlis & Bettany 1977).

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[15] Flying in wind is akin to swimming in current: you cannot assess your progress without reference to the shore (Noldus 2013).

(5) Finally, moths may land and approach on foot while wing fanning and making any displays that precede a mating attempt (Baker et al. 1976).

First of all, strepsipterans follow single-source (as opposed to aggregate) sex pheromones (Cvačka et al. 2012; Tolasch et al. 2012; Zhai et al. 2016), further reducing the likelihood of their employing aim-then-shoot to locate pheromone emitters.

Pattern 1 prototype

Because Strepsiptera are tiny, pattern (1) is difficult to document. Nonetheless, when Linsley and MacSwain (1957) placed calling females within a cage so that approaching males would be conspicuous, they were able to determine from distances of at least 200 ft (61 m) that incoming males flew directly upwind toward the cage at heights of 2–8 ft (0.6–2.4 m). The pair reported that “in general, their flight appeared weak, and yet they were able to reach the cages against winds of 5 to 10 miles per hour” (8–16 km h^{-1}) (Linsley & MacSwain 1957). Under calmer conditions, *S. pacifica* were able to land directly on the cage (Linsley & MacSwain 1957).

Pattern 2 prototype

Hrabar et al. (2014) found that laboratory-resident *Xenos peckii* males (*n* = 6) “approached calling females in a swaying flight with smooth turns.” Thwaites (1841) also reported that *Stylops thwaitesi* take long graceful sweeps in flight (see *Introduction*), as well as “occasionally… hovering at a few inches distant from the ground,” thus combining patterns (2) and (4 or 5). Its hosts, *Andrena wilkella* and *A. ovatula* (Cook 2019), build single or dispersed aggregations of ground nests.

Pattern 3 prototype

“Its [*Stylops melittae*] peculiar rapid hovering flight, make it a conspicuous object,” Champion (1899) and “they [*Corioxenos antestiae*] appear to hover among the [coffee bush] branches in a manner reminiscent of Pipunculid [*sic*] flies"[16] Kirkpatrick (1937), as well as Enock's observation of "uncomfortable" strepsipteran flight (*Introduction*), all elicit pattern (3) and possibly (4), albeit only when very near to the pheromone source. Also, when male *S. pacifica* overshoot a calling female, they allow themselves to be carried downwind—a maneuver that might itself be misconstrued as weak flight, à la E. Saunders (1888) (*Flight reminiscent of a neuropteran*)—and approach again (3), usually at lower altitude (Linsley & MacSwain 1957). However, in addition to reducing flight speed,[17] lowering the altitude may allow males to better discern landscape features (see *Vision in Strepsiptera* below).

Pattern 4 prototype

> They [*Elenchus tenuicornis*] hover very steadily around the sugar-cane stalks and along the leaves, seeking leaf hoppers bearing female *Elenchus*. Sight seems to be the chief sense by which they detect the presence of the leaf hoppers. (Muir 1906)

From this, it appears that after following a pheromone plume as near as possible to its source, elenchids attempt to find a match for a search image of a leafhopper host, in accordance with pattern (4).

Pattern 5 prototype

The following description clearly evokes pattern (5):

> If the hopper bears a mature female parasite[18], the male settles about half an inch away [13 mm, or 7–13 *E. tenuicornis* body lengths] and crawls towards the hopper, vibrating its wings all the time. This generally disturbs the hopper and it moves off, the *Elenchus* following till it gets a chance to spring upon its back and attach itself to the female. (Muir 1906)

This representative set of descriptions of strepsipteran flight coincides with the major paradigms of

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[16] Pipunculidae is a family of Diptera (true flies) that intersperse bouts of stationary hovering with strong saccades in which body orientation and position may change dramatically.

[17] Items closer to the eye pass by it more quickly, causing a flying insect to slow down to reestablish a reasonable optic flow.

[18] This would ostensibly be determined by scent, because it would usually be the planthopper that is detected visually: female elenchids may be entirely hidden by the host's opaque forewings.

moth sex-pheromone pursuit. Furthermore, apart from patrolling, all of the previously cited instances of incongruous strepsipteran flight correspond directly to different phases of pheromone following known from moths and other insects. This evidence demonstrates that strepsipteran flight accommodates pheromone-mediated optomotor anemotaxis. Therefore, it is fitting to assume adult male Strepsiptera use it to trace sex pheromones back to their sources.

In search of a search image

Once within a suitable range of a pheromone source, chafer beetles prioritize vision over olfaction (Fukaya et al. 2006; Oike et al. 2017), a state associated with odor-induced visual salience (Breugel & Dickinson 2014). Preferred candidate targets have appropriate coloration, and ostensibly also an appropriate size and shape, though neither of those attributes were tested explicitly. However, unlike chafer beetle females, all extant female Strepsiptera but those of the basal family Mengenillidae are cryptic and are sometimes completely concealed. For example, in *Triozocera*, the female is entirely hidden by the opaque hemelytra of her hemipteran host. In such cases, the search image, if any, is likely the host itself. Interestingly, *Corioxenos antestiae* Strepsiptera were found to reject hosts from which both pairs of wings had been removed. However, occasionally mating still occurred when only the fore- and hindwings that had covered the female parasite were removed but the adjacent pair was left intact (Kirkpatrick 1937b). Unfortunately, Kirkpatrick (1937) did not report having tested what occurs when the pair of wings covering the female parasite is left intact, but the opposite pair is removed. From the experiment, it is also unclear if mating was not pursued due to visual discordance, the absence or alteration of some tactile or chemical cue, or if wing amputation impeded the calling behavior of the female.

In Myrmecolacidae, females infect either crickets or mantids (species dependent), but males infect ants (Ogloblin 1939; Kathirithamby & Johnston 2004).[19] However, both sexes emerge from the same or a

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[19] Originally, both sexes probably infected crickets (an "old school" insect), but heavily infected hosts—particularly hosts that had also been infected by males, which produce a fungus-prone hole when they eclose—were subject to

related host species (if multiple species can host the female parasite) as 1st instar larvae, so adult males will have had at least some exposure to an appropriate target host. However, it is much more likely that strepsipteran search images are generally generic: The visual apparatus of 1st instars is limited, and even adult males of the diurnal species *Xenos peckii*—and by extension the entire order—are at best UV-green dichromatic (James et al. 2016).[20] Therefore, any insect-sized object in the expected vicinity of a calling female might be approached. Quite separate from any gait assistance it provides, mate localization may be the principal reason why wing fanning occurs, particularly in strepsipteran species in which potentially infected hosts cluster. Finally, the complete absence of a visible pheromone source does not discourage attracted Strepsiptera. Males will congregate and wing fan incessantly while traversing a cloth-covered cage containing an entirely obscured calling female until they all die of exhaustion (Linsley & MacSwain 1957). Thus, losing the scent is problematic, and sensing something incongruous may also be. But the host remaining entirely unseen and untouched is no deterrent, so long as the scent remains.

Why Strepsiptera fly as they do

Strepsipteran flight is tuned for tracking sex pheromones. Several other insect orders known to employ single-source sex pheromones, such as Lepidoptera (Greenfield 1981), Neuroptera (Aldrich & Zhang 2016), and Trichoptera (Löfstedt et al. 2008), also have low wing loading (Byrne et al. 1988; Tercel et al. 2018), as well as a high likelihood of being nocturnal or crepuscular (wind velocity usually decreases at night (Cardé & Willis 2008); aerial predation is also reduced, so greater pheromone-induced wandering can be tolerated). Very limited data are available on the power loading of these taxa, but from what can be ascertained from Table 6 of Marden (1987), power loading is surprisingly high among clap-and-fling employing Lepidoptera (basically, all Lepidoptera apart from Sphingidae (sphinx moths)); i.e., their flight

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a high rate of ant predation, whereupon they would be killed, taken back to the nest, and fed to larvae, which would promptly be infected by undetected 1st instar Strepsiptera. Initially, such females would also infect ants, but the comparative number of offspring they could produce was prohibitive, so they retained crickets as hosts.

[20] Kirkpatrick (1937a) concluded that 1st instars can perceive color, but he failed to control for light intensity.

muscles are unexpectedly weak. However, it appears that insects with low wing loading (i.e., smaller wings) are less apt to skimp on their FMRs, whether or not they use clap-and-fling. But even sphinx moths lack the enormous musculature dedicated to flight in Strepsiptera (Fig. iii.7), as well as their weight savings. These are necessary for eclosed adult male Strepsiptera because they are tiny, have very broad wings, and have extremely limited lifespans. The combination of low wing loading and low power loading can cause elements of strepsipteran flight to appear alarmingly jarring or even erratic, especially when juxtaposed against the smooth patient flight they are also capable of. Their conspicuously broad wings may also intensify any perceived randomness.

Why strepsipteran wings are as they are

The pursuit of point source pheromones apparently benefits from low wing loading. But why do Strepsiptera have such characteristic wings?

The insect wing begins as an outgrowth of the body wall between the dorsal and lateral sclerites (Clark-Hachtel & Tomoyasu 2016). As such, it is continuous with the hemocoel, the repository of hemolymph (insect blood). The dorsal and ventral wing surfaces are secreted by two thin sheets of cells that are separated from one another by columnar protuberances. This framework allows the life-supporting hemolymph (including hemocytes) to pass between the wing blades (Pass et al. 2015). The growing wing also contains tracheated veins, which transport oxygen, as well as hemolymph. In the following sections, *wing inflation* refers to flooding the space between the two wing blades in a manner akin to filling a balloon with air, and *wing extension* refers to fully elongating the wings both laterally (spread) and longitudinally (stretch). Wing inflation usually occurs en route to wing extension, whereupon the thin sheets of cells separate from each other (probably under hemolymph pressure), delaminate from the wing surfaces, and are drawn out of the space between the wing blades, along with the non-venous hemolymph. The two wing blades then fuse to form a single sheet, the *structurally* mature wing (tanning must occur before a wing is fully mature) (Pass et al. 2015). From that time on, hemolymph entering the wings is generally constrained to wing veins (Salcedo & Socha 2020). Thus, wing veins are the primary source of wing hydration.

To prepare for flight immediately after adult emergence, Strepsiptera appear to need to inflate and stretch—but not spread—their broad wings within the bounds of a pupal case confined to the abdomen of another insect. This provides little space and precludes any wing folding. Accordingly, strepsipteran wings have no crossveins (Pohl & Beutel 2005) or fold lines. As a rule, insect wings must be inflated, extended, and sclerotized (Prell 1914; Fraenkel 1935) before they are flightworthy. Strepsipteran wings are an exception to that rule, however. They are covered in fine closely-packed microtrichia (Fig. iii.9), which may enable them to fly immediately after adult emergence. Interestingly, *Triozocera texana*, which live in fields, have microtrichial densities similar to those of *Elenchus koebelei* (Fig. iii.9), which live in salt marshes. *E. koebelei* resist drowning (James & Strong 2018), ostensibly by means of their microtrichia trapping an air bubble around the body. However, because *E. koebelei* have no easy way to extract themselves from water were they to fall onto its surface, the hairs must serve other purposes. Like the microtrichia discussed in Polet et al. (2015), one of them is probably to reduce adhesion by resisting wetting in the high humidity of a sealed puparium. Well-hydrated wings are pliant and can be pressed against the body to reduce the risk of damage during emergence, but wings that are actually wet may stick to themselves or other surfaces. The underlying thinness of strepsipteran wings further increases flexibility, which probably improves flight performance directly (Mountcastle & Daniel 2009), and also reduces rotational inertia (Neville 1965) and wing weight, allowing for faster more efficient flapping. Unfurled strepsipteran wings are unbelievably fragile, however (a condition likely worsened by long-term storage in (70%) ethyl alcohol). Finally, after successful adult emergence, the disadvantages of wings that cannot be folded back are inconsequential: When not mating, or wing fanning in preparation for mating, mature male Strepsiptera basically fly non-stop until they are unable to remain airborne.

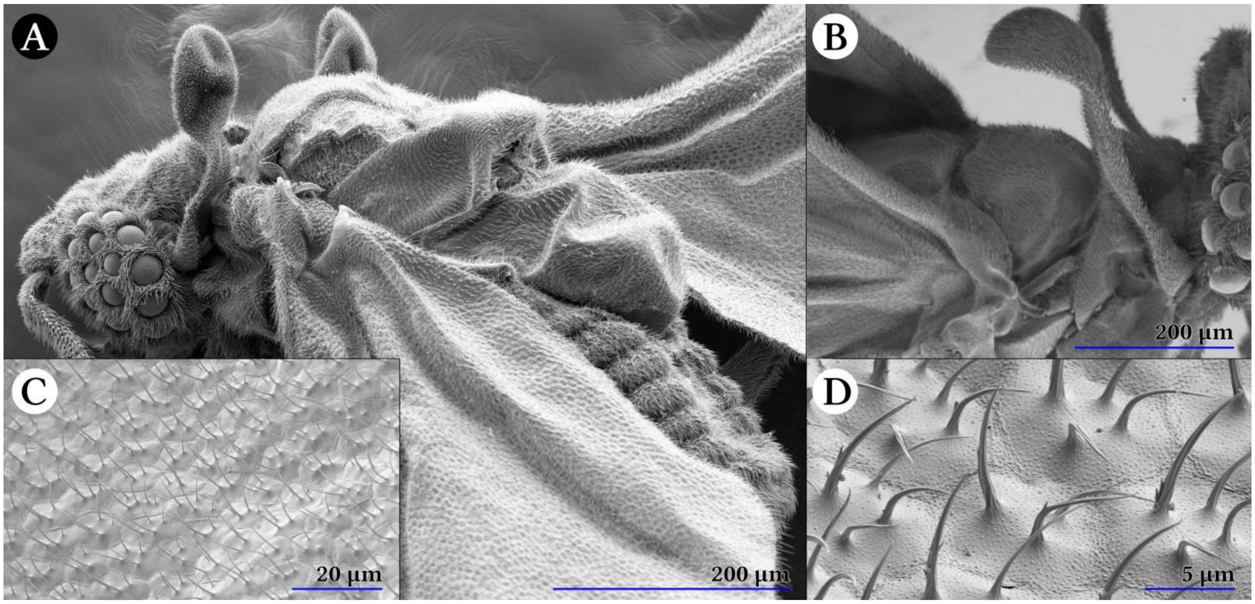

Fig. iii.9. Strepsipteran wing hairs as revealed by scanning electron microscopy (SEM). Strepsipteran bodies are covered with microtrichia, but those of their wings may be of particular importance in allowing them to be opened fully enough to expand in the puparium during development—or else to at least be expanded while emerging. (A) An SEM image of an *Elenchus koebelei* Strepsiptera at a magnification of 150×. Note the fine 'hairs' on the wings and most of the rest of the body. Also displayed is the enormity of the dorsal extent of the flight muscle-filled thorax (Smith & Kathirithamby 1984), as are the large and few ommatidia. (B) An SEM of a *Triozocera texana* Strepsiptera at 70×. The microtrichia, their density, and the underlying wing structure are all similar to that of *E. koebelei*, although this is less clear than would have been the case had the original scan been made at higher resolution. (C) The (dorsal) surface of a *T. texana* wing at 1000×. (D) The (dorsal) surface of the same wing at 4000×.

Wing desiccation

Although strepsipteran wings are inflated by or shortly after emergence, they are not sclerotized (Fig. iii.3C), which should greatly enhance their flexibility. It furthermore appears that the wings are not meant to harden during a male's active life as an adult. However, in both live *E. koebelei* and *T. texana* specimens that had been given a free area in which to walk, but had aged or starved past the point of incessant wing flapping, I noticed that the wings seemed to set; most often in a vertically extended position (see Fig iii.4). Thus, either the wings desiccate gradually as emerged adult males age, or more likely, they rapidly dry when hemolymph is not continuously pumped into them (Wootton 1992). Pumping could be assisted by wing flapping (Salcedo & Socha 2020), which would help explain why Strepsiptera keep their wings in almost constant motion for the entirety of their active adult lives, except while mating (Kirkpatrick 1937b; Linsley & MacSwain 1957), when wing flapping would likely interfere with retaining aedeagus attachment. Prior to emergence, the high humidity of the pupal chamber presumably prevents wing desiccation, as must also be true of other insects, such as butterflies. Whatever the case, although both beetles and Strepsiptera are generally able to fly immediately upon entering the open environment, the strepsipteran approach is utterly distinct from how Coleoptera typically fully extend, completely stiffen, and fold their wings before exiting their maturation chambers in wood or underground (Arakane et al. 2008).

Flying on immature wings?

The developmental status of strepsipteran wings has not been characterized, neither before emergence nor after it. However, there is simply insufficient room to spread the wings before emergence due to the tightly confined puparium, which is further restricted by being lodged in the body of the host. But as occurs in their sister group Coleoptera (beetles), strepsipteran wings could conceivably be stretched to their full length during emergence (though not also spread to their full width). In the red flour beetle, *Triobolium castaneum*, wing stretching is achieved in only about 2 min (Arakane et al. 2008). However, it is preceded by 3–8 rounds of strong “reverse-bending,” (lasting less than 1.5 min) in which the beetle’s somewhat convex body is repeatedly inverted until rendered more-or-less equally concave (Arakane et al. 2008). Kathirithamby (1983) noted that when an *E. tenuicornis* is ready to emerge, it “undergoes alternate inflations and deflations at regular intervals.” These *could* correspond to reverse bending. However, due to substantial differences between the two movements, they also might not. Moreover, a year later, Smith and Kathirithamby reported that,

> emergence of the adult has been observed in the laboratory by Varley and Kathirithamby (unpublished), who noted that *E. tenuicornis* is able to fly vertically from the puparial surface promptly on emergence, wing inflation apparently occurring before rupture of the puparium or immediately thereafter. (Smith & Kathirithamby 1984)

Therefore, it is also valid—though not necessarily correct—to assume strepsipteran wings are also not stretched or spread prior to adult emergence, and that the process of emergence itself achieves each of these.

As far as I am aware, there is no evidence that strepsipteran wings are inflated before adult emergence, only that Strepsiptera are at worst *usually* capable of flying immediately thereafter (Hubbard 1892; Kirkpatrick 1937b; Hrabar et al. 2014). To that point, Hassan (1939) reported that *E. tenuicornis*, "hangs for a short time to the host while its wings spread and get dry," and Linsley & MacSwain (1957) wrote that initial flight in *Stylops pacifica*, "occurred within a minute" after emergence.[21] Therefore, how far wing extension has proceeded by the end of emergence *may* depend on the host species, perhaps progressing further in Xenidae (wasp-infectors) (Hubbard 1892; Hrabar et al. 2014), than in Elenchidae (planthopper-infectors) (Hassan 1939), or Stylopidae (solitary bee-infectors) (Linsley & MacSwain 1957). However, Strepsiptera exiting potentially aggressive hosts may be motivated to fly the moment they emerge, regardless of the state of their wings. From both temporal and spatial standpoints, either scenario—wings that are stretched or inflated before *or* during emergence—could present great difficulty for clearing refuse (i.e., the cells that secreted the wing surfaces) from between the wing blades.

Wing circulatory organs

Insects have an open circulatory system, so their hemolymph is not transported as efficiently as it would be in a closed circulatory system. On the other hand, insect breathing is not dependent on hemolymph

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[21] Neither report indicated these species are incapable of immediate flight, just that they did not engage in it.

transport. However, the movement of nutrients, wastes, hormones, etc., is. Therefore, insects have evolved modifications to the dorsal vessel (the insect "heart"), as well as a number of accessory pulsatile organs (helper hearts) in the vicinity of appendages that require pronounced nutrient flow or waste removal. A set of such paired appendages are the wings. All volant insects with membranous wings that have been investigated to date have had at least one wing circulatory organ (Pass et al. 2015) per wing pair (Pass et al. 2006), and in some Diptera and some Lepidoptera, each wing has a single dedicated circulatory organ located at its base (Pass et al. 2006, 2015). Wing circulatory organs are informally known as "wing hearts." Only a few flying insects with fringed wings, such as thrips (Thysanoptera), are known to *not* have wing hearts (Pass et al. 2015). In Strepsiptera, their presence or absence is unknown (Pohl & Beutel 2008; Pass et al. 2015). However, given the expansive relative area of the wings and the enormity of the scutellum—which has been described as a pump enclosure for wing circulatory organs (Pass et al. 2015)—Strepsiptera are extremely likely to possess one or a pair of wing hearts.

Just after adult emergence, most insect wings are tightly compressed. They are subsequently inflated and extended, allowing the removal of debris that arose from the delamination of the cells that excreted the wing cuticle. One or more wing hearts then attempt to draw this waste material and the non-venous hemolymph from between the wing blades (Tögel et al. 2008). However, deviations from a flat wing surface severely compromise this process, often leading to areas in which cell debris pools. Thus, failing to completely extend the wings and to keep them extended during wing clearing and maturation presents an insurmountable problem for other winged insects, including bees, cicadas, dragonflies, and true flies. For example, improper extension results in butterfly wings that are crumpled or deformed. Interestingly, Strepsiptera are never able to extend their wings in their constricted puparia. How they manage to arrive at functional wings is undocumented. However, any wing heart able to clear debris in such circumstances should be easily identified—provided the right thing is sought: Although paired wing hearts are generally considered more capable, given the shared phylogeny of the two taxa, a singular wing circulatory organ as found in Coleoptera (Pass et al. 2015) might also be present in Strepsiptera.

From the perspectives of compactness (which would ease emergence) and ensuring wing uniformity (which improves flight dynamics), emerging with folded wings would be less problematic than emerging with wings that are inflated and stretched, but unfolded. But because the former is apparently not possible for Strepsiptera, and since terrestrial insects only seem to fully elongate their wings during adult ecdysis when they can also fully spread them (Prell 1914; Fraenkel 1935; Arakane et al. 2008; Salcedo et al. 2023), the process of emerging probably helps Strepsiptera to stretch their wings, as occurs in the locust *Schistocerca gregaria* (Elliott 1981). This may be why the diameter of the opening through which adult strepsipterans emerge is artificially narrow, especially in Elenchidae (see Fig. iii.3 of (Kathirithamby 1983)), for example, but also in Xenidae (see *Xenos peckii* dislodging pupal cap (Hrabar 2015a)). If this is the case, then it is also true that strepsipteran wings are not fully mature when they first take flight.

In an elegant experiment, Tögel et al. (2008) found that ablating a single wing-heart in *Drosophila* prevented cellular debris from being cleared from the corresponding wing. This resulted in a wing that was cloudy—like those of flies that have just eclosed (Tögel et al. 2008), but that never cleared. In all other respects, the wings appeared normal, though the upper and lower wing blades could not have fused. Such *Drosophila* were unable to fly, however, falling back to earth if tossed into the air (Tögel et al. 2008). This was probably due to the wing not having been stable. Because its wing epithelial cells had become delaminated but still remained between the wing blades (along with an increased volume of hemolymph), the material could slosh around in response to wing flapping. In fact, it is probably this clearing process that needs to progress further before less fortunate black flies, for example, are able to fly up from the water surface following their own emergence (Emery 1913; Hannay & Bond 1971). That is, having wings still overridden with epithelial waste is much more problematic than their being unsclerotized. Somewhat "flabby" wings can be dealt with as long as the leading edge is strong, but debris-laden wings cannot be used at all.

In Strepsiptera, wing inflation is at least partially achieved before or during the normal process of adult emergence—although apparently in some species it is not always completed by the end of emergence (Hassan 1939; Linsley & MacSwain 1957). Furthermore, strepsipteran wings are not always dry after emergence either (Hassan 1939), as might be expected of pre-expanded wings. If anything, with their extreme flexibility (Fig. iii.3C&D), the wings function as if teneral for the majority or totality of an adult male's active life (Fig. iii.4F). Thus, adult male Strepsiptera may fly on immature wings. Whatever the true condition of their flight-worthy wings, immediate sustained flight upon or very quickly after adult insect emergence is rare, and therefore should be examined and characterized carefully.

To have or lack halteres

If an insect has low wing loading it may seem superfluous for it to also have halteres: low wing loading with any degree of power already provides high maneuverability. So why do Strepsiptera have halteres? Ostensibly, they had no primordial need to avoid predation. Fossils and extant basal species strongly suggest Strepsiptera were always small and originally nocturnal (Pohl & Beutel 2008). There are still no nocturnal aerial visual predators, insect or otherwise, and according to existing fossil evidence, Strepsiptera had already evolved halteres long before the first appearance of bats (Simmons et al. 2008; Speakman 2008; Misof et al. 2014). Even presently, Strepsiptera have few natural enemies (Muir 1906; Kirkpatrick 1937b), and well-armed or potentially aggressive hosts—mantids, ants, wasps, and bees—were only first colonized relatively recently (Pohl & Beutel 2005). Furthermore, host aggression appears to only be problematic for males seeking to mate at the nest sites of social Hymenoptera (Hubbard 1892), which will seek to protect their larvae.

Unlike low wing loading, in addition to improved maneuverability, halteres provide direct feedback enabling insects equipped with them to quickly correct their orientation in response to perturbation—a particularly useful ability for a tiny, ancestrally nocturnal, ocelli-less taxon that is probably polarization insensitive, and so cannot use skylight polarization to establish or maintain straight flight. Halteres do not rely on vision to correct flight anomalies, so they maintain functionality even in conditions when the visual system provides limited spatial or temporal resolution. Due to their ability to counteract involuntary directional changes, halteres would also have allowed strepsipteran wings to broaden evolutionarily despite wind gusts. Thus, rather than duplicating functionality, halteres may increase the range of wind speeds in which low wing loading can be exploited by a tiny insect. Strepsipteran flight executed just after emergence may suffer from unequal or incomplete wing clearing (see *Wing circulatory organs*). An interesting way to combat that problem would be to sweep the wings back as needed, to mask some or all of the discrepancy. That in turn, would raise the wing loading, thus enhancing the role of the halteres.

Xenos vesparum have about 130 campaniform sensilla at the base of each haltere[22] (Ulrich 1930), compared with 130–141 (Dinges et al. 2021) for the 0.5 mm smaller fly, *Drosophila melanogaster*. Although the sensilla are far more precisely organized in *D. melanogaster*, the strepsipteran investment is large, and the information they provide is probably comparable.

Unlike andrenid bees, pheromone-following Strepsiptera do not pause their flight activity even when the sky is completely overcast (Ulrich 1933; Linsley & MacSwain 1957). Thus, being able to contend with wind gusts, substantially reduced nightly temperatures (as occur in arid regions), and potentially even low-light conditions[23], all acted as preadaptations for colonizing taxa that emerge in early spring in temperate zones. Likewise, halteres allow high maneuverability combined with fast flight, but apparently did not evolve in Strepsiptera to provide that ability.

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[22] Pix et al. (1993) reported that Ulrich (1930) stated the range of sensilla in both the dorsal and ventral fields [of *Stylops muelleri*] to be 70–100. Ulrich, however, was unable to count them explicitly, but estimated both have 50–100 sensilla, with the dorsal field containing somewhat fewer (Ulrich, 1930). I counted 72 sensilla in the ventral field of the *Xenos vesparum* haltere bulb photographed in Pix et al. (1993). An image of the dorsal field was not included, so I have estimated the total from both fields to be about 130.

[23] Although it is more likely that flight interruptions in *Stylops pacifica*, etc., would be due to their thoracic temperatures becoming too low when unassisted by incident solar radiation (Ulrich 1933; Krogh & Zeuthen 1941).

Vision in Strepsiptera

Strepsiptera are renowned for having few large ommatidia, which could complicate orientation and navigation. Although a thorough exploration of strepsipteran vision must wait, some appraisal of their visual capacity is in order because optomotor anemotaxis requires that a flying insect discern enough environmental features to determine that its direction has not changed (based on *how* visual features stream past its eyes), and the rate at which it is moving with respect to its surroundings (based on *how fast* visual features stream past its eyes). This requires that sufficient visual features are detected, which may not be the case considering the extremely low number of ommatidia Strepsiptera have. For example, the eyes of adult male *Elenchus koebelei* contain 19 statically-positioned ommatidia (Fig. iii.S4). That is staggeringly low resolution for the whole of a hemispheric eye. In particular, it remains to be seen how elenchids manage “to spring” (wing-assisted) onto the abdomens of planthoppers to access calling female conspecifics (Muir 1906) using vision limited to only some 40 ommatidia for the entirety of two eyes, while Salticidae (jumping spiders) have such remarkably good eyesight precisely to enable jump-mediated predation (Land 1969; Williams & McIntyre 1980; Harland & Jackson 2000). However, each strepsipteran ommatidium contains its own potentially image-forming mini-retina (Buschbeck et al. 1999), rather than a rhabdom containing only 8 or 9 photoreceptors, as is typical for trichromatic insects. Furthermore, Strepsiptera are at best dichromatic (James et al. 2016), which *could* provide better resolution at the cost of reduced color perception. However, insect are able to perceive colors at much lower light levels than vertebrates (Fain et al. 2010); furthermore, due to their use of rhabdoms, color perception is less likely to impinge upon resolution. but if instead of only resolving about 40 points total as typical ommatidia would, each ommatidium (literally “little eye”) were to act as an independent image-forming simple eye (an “eyelet” (Buschbeck et al. 1999)), the outputs of which were subsequently unified into a single whole, then much greater acuity could be obtained. Each strepsipteran ommatidium has an inordinate number of photoreceptors, about 100 in the case of *Xenos peckii* (Buschbeck et al.

1999). This may not simply be a holdover from their nocturnal ancestry: Losing traits is comparatively easy, and Xenidae and other day-active clades have been diurnal for millions of years. Thus, either there is weak selection pressure to improve vision in the diurnal clades, or the "excess" photoreceptors are likely being used to enhance functionality. I have envisioned two modes in which such an eye could be used. One would be enhancing temporal resolution while using "slow" photoreceptors and only seeing at a spatial resolution of one pixel per ommatidium. Then, having so many slow photoreceptors per ommatidium could ensure enough of them fire during considerably shorter time frames, so as to allow the eye to function much more quickly at high light levels, while saving energy (and not needing to evolve fast photoreceptors). However, Strepsiptera have a global (i.e., integrated across all photoreceptors) flicker fusion rate of just 35 Hz (Buschbeck et al. 2003) (for contrast, many flies have rates that are 6× faster), so this possibility is not tenable.

The other would be to enhance spatial resolution. According to Buschbeck et al. (2003), a diurnal species such as *X. peckii* may have the capacity to resolve 6–35 points per ommatidium. At 20 points per ommatidium, jump-oriented "attacks" may become viable for *Elenchus koebelei*.[24] Super-ommatidial resolution could also use slow photoreceptors because of inherent delays associated with stitching together images from separate eyelets. Each eyelet would have fixed boundaries with its neighbors, but differences in light levels, resolving blind spots, and removing seam artifacts that could be confused with actual visual features would still need to be worked out. Finally, the more detailed visual mode could be automatically selected on the basis of odor-induced visual salience, in which pheromone following insects become sensitized to tiny objects once they are within the vicinity of the pheromone source (Baker et al. 2018). Odor-induced visual salience could also explain the results from a study in which *X. vesparum* only responded to environmental movement as if its acuity was limited to its actual number

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[24] Jumping spiders have the additional task of needing to discriminate between potential prey and potential mates (Williams & McIntyre 1980). Also, some prey species are capable of counter-attacking (Harland & Jackson 2000), so the visual demands of jumping spiders are significantly greater.

of ommatidia (Pix et al. 2000). In that study, no sex pheromone was administered; however, releasing pheromone from a nearby emitter might have produced a different result. Even so, in a careful observational study, vision appeared to play little to no role in male *X. peckii* recognition of calling females (Hrabar et al. 2014). Instead, the insects relied heavily on touch and chemical recognition. Although it was still necessary for males to land oriented in the correct direction and positioned near the proximal end of the wasp abdomen, that did not appear to require super-ommatidial resolution: A male would simply fly past the calling female (from behind), land on the proximal end of the host's abdomen, and then walk backward until his middle legs came into contact with the calling female, whereupon he would immediately grasp her protruding cephalothorax, and begin mating (Hrabar et al. 2014).

As for optomotor anemotaxis, normal strepsipteran ommatidial vision should usually suffice (the principal optical mode must be optimized with respect to something). However, when a target is accidentally overshot, both moths (Traynier 1968; Murlis & Bettany 1977) and at least *S. pacifica* Strepsiptera (Linsley & MacSwain 1957) fly or allow themselves to be carried downwind and approach the pheromone source again. However, *S. pacifica* often does so at a lower altitude (Linsley & MacSwain 1957), which could allow more detailed super-ommatidial resolution imagery to be extracted, although enough detail may be obtained from simply being closer to the source, while using the eyes normally. *E. koebelei* either fly very close to the tops of salt marsh grasses in the first place, or sometimes apparently even fly within the canopy itself (James & Strong 2018). Although at closer distances images move past an eye more quickly, insects using optomotor anemotaxis fly more slowly as they approach a pheromone source (Farkas et al. 1974; Baker et al. 1976; Murlis et al. 1982).

A new visual paradigm?

In the most robust nocturnally innovated optics (e.g., superposition vision and neural superposition vision), taxa that inherit them benefit from the new visual approach even in lineages that adapt to diurnal light regimes. However, Pix et al. (2000) note that in adapting to a diurnal lifestyle, Strepsiptera invariably produce more ommatidia. Although it is not clear what also transpired at the retinal level (since no comparative studies of crepuscular or nocturnal strepsipteran retinas have been completed), the larger number of ommatidia indicates a need for more sampling at the level of the ommatidia (Pix et al. 2000). Furthermore, that is the only sampling level known to exist: There is experimental evidence for sampling at the ommatidial level (Pix et al. 2000; Maksimovic et al. 2007); however, as of yet, there is no direct evidence of super-ommatidial vision in Strepsiptera. However, there would almost certainly be a speed penalty associated with it, and having more ommatidia may be a way to avoid it for 'ordinary' vision. Incidentally, there is no indication that the number of ommatidia continues to grow in diurnal species, suggesting that there may be some advantage to having a high number of photoreceptors in each ommatidium, or at least that there is no overt disadvantage to it. However, it does appear that the proposed strepsipteran visual approach would not be very generalizable, given that the anticipated integration times most likely associated with building super-resolution mosaics would increase with increasing numbers of ommatidia. Therefore, in diurnal and perhaps also crepuscular optical environments, super-ommatidial resolution might only provide useful visual enhancement to smaller species (which are themselves more difficult to see, and could therefore tolerate being less responsive), while to avoid predation, larger species would increasingly need to sacrifice super-ommatidial resolution in favor of the faster but less improved spatial acuity provided by a greater number of ommatidia.

But perhaps the entire situation has been approached backwards by first considering strepsipteran vision from a diurnal standpoint. Whatever advantages the strepsipteran eye design has should be evident in the nocturnal species for which it ostensibly initially evolved. Therefore, as employed by nocturnal species, the strepsipteran eye likely has considerably more adaptive value (either in terms of signal-to-noise ratio or resolution) than a nocturnal apposition eye would, despite its normal visual mode (i.e., sans super-ommatidial resolution)—which was like its *only* visual mode, at least at some point—having considerably less resolution than a diurnal apposition eye. Then the situation in larger diurnal Strepsiptera could be compromised by an evolutionary history dominated by nocturnality. Thus, in well-lit environments, even the super-resolution visual capacity Strepsiptera may possess could be inferior to that produced by faster and vastly simpler apposition optics. It may be telling that in the founding analyses, the authors compared the eye of *X. peckii* to that of *Drosophila melanogaster*, with the intent to match its acuity, rather than to soundly surpass it (Buschbeck et al. 1999, 2003), as would be expected of a new visual paradigm. In fact, if arthropod eye architecture is flexible enough to repurpose insect optic neuropiles to support "chunk" vision (Buschbeck et al. 1999), then it should also be able to reconstitute apposition optics—a profoundly more direct task—rather than simply being stuck with subpar diurnal vision, especially since diurnal Strepsiptera are currently documented as the most specious by far.

Yet whatever the case concerning strepsipteran optics, it is impressive that despite potential aerial predation, Strepsiptera manage to follow sex pheromone plumes in broad daylight. However they are achieving this success is noteworthy.

Can't see the meadow (f)or the grass

> In taking flight from the ground in the midst of grass or other vegetation, males will frequently "fly-walk" up the stems, catapult into the air, collide with other stems, and fall to the ground a number of times before finally completing a successful take-off. (Linsley & MacSwain 1957)

This is odd behavior for Strepsiptera, particularly specimens that have not just emerged. One would expect the stems to be seen and avoided. It is also not immediately clear why *Stylops pacifica* would find it advantageous to (use their abdomens to) catapult themselves into the air when they already have low wing loading and low power loading. But note that wind gusts are frequent during the *S. pacifica* mating season in early spring, and that stiff breezes arise intermittently, as well (Linsley & MacSwain 1957). Due to extremely low mass but substantial power, like *Lymantria dispar* (gypsy/spongy moths) (Murlis et al.

1982), *S. pacifica*, can likely orient and fly in wind of considerably higher speed than it can successfully takeoff in. Abdomen-catapulting would then be used to increase initial flight speed, because the legs are too weak to do so (Hubbard 1892; Muir 1906; Linsley & MacSwain 1957), and stem-climbing would relax initial lift requirements, allowing less wing area to be exposed.

Live fast, die young

A disorienting aspect of strepsipteran flight is the brief period for which emerged adult males are able to engage in it. However, considering that Strepsiptera cannot feed as adults, a flight endurance of 2 h is not unusual. A well-fed, fully mature *Drosophila melanogaster* has a flight endurance of 4–5 h (Wigglesworth 1949). If *D. melanogaster* were incapable of feeding as adults, death would follow soon after. Male honey bees whose lives are not terminated by mating (lethal for drones) have average flight durations of 32.6 ± 22.5 min, with a maximum just shy of 160 min (Witherell, 1971). Witherell (1971) found that drones that were confined at a drone congregation area for longer than 10 min either became weak and unable to fly, or literally starved to death. Similarly, if unable to consume additional carbohydrates, a foraging honey bee (*Apis mellifera*) typically has a flight endurance of about an hour[25] (Park 1928; Miller 2017). (Note that (adult) bees can only use carbohydrates as energy sources (Keller-Kitzinger 1935; Beutler 1936); they do not and cannot metabolize protein (Keller-Kitzinger 1935), and thus starve with all of their protein "stores" in place.)

Thus, strepsipteran endurance is somewhere between that of flies and that of bees. Compared to similarly-sized *Drosophila*, Strepsiptera pay a sizable endurance penalty, but are also stronger more versatile fliers than they are. Moreover, under normal circumstances Strepsiptera are able to fly much longer than bees can without additional feeding. Surprisingly, emerged Strepsiptera prevented from flying

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[25] Because the crops of honey bees foraging for nectar are used to store both their fuel and the bounty of their labors (which will eventually become honey), initially the crop should normally not ever be even half-filled with nectar or honey. However, an hour is about how long a bee is typically provisioned for a single foraging bout.

die within the same time period as those allowed to fly (pers. obs.). It thus appears that emerged Strepsiptera expend as much energy keeping their flight muscles primed for flight as they do actually flying.

That the Strepsiptera Hubbard (1892) observed became exhausted after only 15 min of flight *is* unusual. However, co-confinement to a tiny area surrounding a nest the resident wasps were obliged to defend, coupled with the emanation of sex pheromone from multiple calling females into a small volume of air absent any direction-providing wind (David et al. 1983; Kennedy 1983) would have provided impetus for exorbitant maximization of flight speed, thus consuming a large amount of energy in a very short amount of time.

Friese having observed an emerged *Stylops aterrimus* survive at least 62 h is exceeding unlikely (Friese 1883; Pierce 1909)—or would be, if the animal had not been forcibly removed from its puparium (Friese 1883) before having reached full maturity. Oddly, his account contained nothing concerning the insect's flight or other activities despite the remarkable length of time it would have spanned without pause. Friese (1883) writes, 'despite its forcibly promoted pupal hatching' [translated from German]; however, its longevity was not *despite*, but *because* of that: Friese did not record the first "free-flying" Strepsiptera (indirectly indicating that his specimen did not fly) until February 26, about 1.5 months after having actively removed this specimen (Friese 1883). Therefore, Friese's Strepsiptera must have been teneral. It is remarkably improbable that a tiny insect, especially a Strepsiptera (recall Hubbard (1892)), could fly continuously for 2.5 d without feeding—and adult Strepsiptera *cannot* feed (Pohl & Beutel 2008)—particularly without wind assistance (the insect was indoors and Strepsiptera probably cannot soar), or that such incredible flight activity would have gone without direct comment.

Further support is found in Bohart (1941), in which he removed the pupal cap from a *Paraxenos lugubris* [formerly *Pseudoxenos lugubris*] that then survived 26 h (Bohart 1941). It was unable to remove itself from its puparium, however, and never flew or even walked, "The male continued to twitch its legs, mouthparts, and antennae, but not its undeveloped wings" (Bohart 1941).

Whatever Strepsiptera do to prime their flight physiology is apparently inaccessible if their bodies are too teneral. It is incorrect to consider all males confined to pupal cases pharate adults, because they may also be immature. Thus, one should assume they will liberate themselves at the first best opportunity and normally leave them to it. Caution should be exercised when contemplating accounts of prematurely released Strepsiptera. Strepsipteran self-liberation may constitute a beneficial warmup similar to moths shivering before flight in cool weather. Insufficiently warm flight muscles could be why some Strepsiptera do not fly immediately after quitting the puparium, even when they executed the entire procedure autonomously (Linsley & MacSwain 1957). Furthermore, as noted above, delayed initial takeoff may also be due to the wings not having been fully inflated and stretched by the end of adult emergence: "It [a male *Elenchus tenuicornis*] hangs for a short time to the host while its wings spread and get dry" (Hassan 1939). Hassan (1939) worked with *E. tenuicornis*, the European counterpart of the North American *E. koebelei*. Depending on the host species, both species may reside in marshes (Hassan 1939; James & Strong 2018), where colliding with water would probably be fatal—either from being consumed by fish, or from energy depletion or eventually drowning from failing to become airborne again. Interestingly, I independently deduced a delay between the expected time of first arrival of *E. koebelei* and their actual time of appearance at the light sheet (James & Strong 2018). It could be that normally in nature, elenchids expand their wings completely before taking flight (thereby improving their initial flight competency) because the environment is more problematic than their former host. The opposite may be true for Strepsiptera that parasitize social wasps and inadvertently eclose on the nest. Although the wasp's orientation gives some indication of whether or not it is resting on the nest (for paper wasps, upside down = likely at the nest site), more leisurely wing expansion may not be worth the risk. This could explain the poor flight of this just-emerged *Xenos peckii* (Hrabar 2015b). Finally, perhaps rather than overt danger, any chance of interference from the former host could prompt a hasty maiden flight. At any rate, the Strepsiptera I have encountered in the field (i.e., both *E. koebelei* and *T. texana*, but not *X. peckii*) have all flown skillfully.

Synopsis & future directions

This effort was fueled by the desire to be the first to photograph Strepsiptera in flight, as well as the frustration of having seen Strepsiptera fly in the wild, knowing that they can be very proficient at it—and really must be—but being repeatedly told otherwise by people who have never seen them alive at all. How could an entire order of insect (albeit a tiny one) that is dependent on sex pheromones not be proficient at flight?

An obvious next step is to obtain the actual wet weight of a Strepsiptera. Knowing the wet weight of any strepsipteran would be of excellent informational value (along with the corresponding body length and thorax width, and wing area), however, it would be preferable to have that of *Hylecthrus rubi* (in which case it would be even more compelling to obtain the wing area of one or more specimens), *Triozocera texana*, or a species of similar size to either of those. A related follow-on effort would be to obtain the wet weight of the thorax and if feasible, that of the thorax muscles themselves.

Increasing camera sensitivity, resolution, and dynamic range are of imminent interest, as is improving the light quality, especially in terms of synchronizing flash pulses or splitting a single light source to remove the possibility of any light-related synchronization issues, and increasing light intensity or placement without increasing pulse length. A less pressing but useful feature would be enabling burst image capture. A more remote improvement would be to fashion the rig with an automatic insect recognition system. In this way, encountered specimens of certain species could be cataloged without retaining their images, which could free space for less common insects, or those of greater interest. It would also be nice to lower the weight of the system and to try using it to help document insect diversity.

It would also be very interesting to test (emerged) adult male Strepsiptera for auditory and vibrational sensitivity—not just for the potential to detect bat echolocation, but also as a means of avoiding interception in general. Augmenting vision with other sense modalities would help compensate for any reduced visual capacity.

Investigating mysteries of strepsipteran wings

It would be informative to determine if Strepsiptera have wing circulatory organs, and if so, to ascertain if they are attached to the dorsal vessel, separate from it, or separate from it and paired. If Strepsiptera are found to not have wing hearts, then the mechanism they do use to prepare their wings for flight should be discovered and documented. Using electron microscopy to investigate strepsipteran wings for the presence of epithelial cell layers (to determine if the cells that secreted the wings delaminate at all) and measuring the thickness of "strep" wings compared to those of similarly-sized insects and insects with wings of similar area could help characterize the expansion state of pharate adult wings, as well as the nature of the wings of emerged adults. A more nuanced evaluation of the status of wing expansion could potentially be obtained by fixing puparium-bound specimens of different developmental stages—including some in the act of emerging—and examining them via dissection and various forms of microscopy. Measuring the thorax temperature of just emerged adult Strepsiptera would also be informative.

Strepsiptera are so recalcitrant that I am presently more interested in collecting less complex data (such as wingbeat frequency and flight speed) in the field than in the lab. It is simply an additional and generally unnecessary challenge to rear them in the lab, or to collect unemerged specimens and coax them forth in the lab. Working in the field also provides informative context. So little is known about strepsipteran life histories that constructing optimal experiments remains difficult. Adult male Strepsiptera are probably really only performing at their finest when responding to a pheromone plume or to imminent danger. Thus, it would be outstanding to obtain video footage of a species in untethered flight. With such data and environmental information pertaining to host species and expanses between known stylopized populations, one could begin to identify new areas that may already contain strepsipteran populations, or are most suitable for colonization. In many cases, unfavorable winds must contribute enormously to the patchy population distributions Strepsiptera often present, whether they act predominately on the host or the parasite. Such data would be especially interesting when collected from sympatric strepsipteran species that are not cryptic.

Laboratory experimentation

It should not be forgotten that laboratory experiments are models, and as such, simplifications of the systems in which animals evolved and live. However, laboratory-based experiments are often desirable due to their repeatability and the ability to isolate aspects of interest. In studies of Strepsiptera these pluses are tempered by the difficulty of obtaining and transporting material, the general necessity to keep both parasite and host alive, the brevity of emerged adult male life, and the small size of specimens. Furthermore, emerged adult males have very limited concerns, which are less compatible with known or widely available laboratory techniques. Therefore, it must be borne in mind that certain natural behaviors may fail to present entirely or produce subpar results if convincing stimuli are not attendant. Thus, it may be advisable to bring certain laboratory procedures into the field, rather than bringing Strepsiptera into the laboratory.

Immobilizing specimens

When pursuing laboratory studies, the ability to temporarily halt insect activity is very useful. Currently, refrigeration is often administered to just-emerged Strepsiptera to reduce their activity. However, the fact that doing so does not elongate their lifespans as emerged adults suggests that Strepsiptera attempt to compensate for reduced thoracic temperatures in a manner that negatively impacts their energy reserves. Physical performance of recently cold-treated specimens may also be altered as they return to normal body temperature.

Another common means of immobilizing insects is to administer CO_2. Although Strepsiptera *are* immobilized after only a few seconds of exposure to pure CO_2, in my experience, treated specimens never regain the power of flight. In fact, they seem to be impaired in general thereafter, as Kirkpatrick (1937) documented independently, "none so treated recovered properly from any anaesthetic used."

Alternatively, specimens can be immobilized by means of physical constraint. Overall, it may be most effective to temporarily immobilize Strepsiptera by slipping a microscope slide over emerging specimens and gently sliding it along to expose the portion of the body requiring preparation (Ulrich 1933). While doing so, it may also be useful to employ a deep red light source in an otherwise unlit room (James et al. 2016).

Finally, Ulrich (1933) also explained that adult males emerge by using their abdomens to push-off against the base of the puparium. This can be exploited by exposing the base of an occupied puparium (which requires killing the host and breaking its abdomen open) and cutting it off (Ulrich 1933). In pharate adult males, the puparium is translucent and its base contains discarded pupal integument, so the specimen should not be harmed by its removal. Subsequently holding a stiff flat surface against the open puparium base will allow the resumption of emergence (Ulrich 1933). Ulrich (1933) did not expect this approach to be practical, and a male struggling to escape would certainly expend energy, but in some circumstances it could still be useful.

Other considerations

The value for FMR proposed for *Triozocera texana* in equation (4) is remarkably high, but may not be an overestimate considering that Strepsiptera do not feed as adults and also have a sizable "balloon gut" (Beutel & Pohl 2006) which is filled with air, rather than tissue, as is much of the adult male abdomen (Hofeneder 1924). However, since *T. texana* can certainly lift more into the air than the substantially smaller *Hylecthrus rubi*, the efficiency of strepsipteran wings due to their extreme flexibility or their implementation of clap-and-peel may be better than estimated here—particularly in patrolling species, though the wing loading of patrollers is also expected to be lower. Although lightweight is often mistaken for weak, I hope it is clear from this work that the strepsipteran FMR is not low by any standard, despite their diminutive mass. The total weight of an animal is one parameter, but the type and distribution of that weight is another, and both are important.

Finally, the times when flying Strepsiptera are most likely to be encountered are just after emergence, when in the near vicinity of a calling female, and just before or just after mating. However, none of those occasions are likely to showcase their finest flight ability.

Parting shots

The order Strepsiptera ('twisted wings') was given its name on account of the deformity of the "forewings" of adult males (i.e., their "elytra," which are in fact halteres), and not that of their true wings: "*Strepsiptera*† is the term I propose by which to designate the order, which name I have given it on account of its distorted elytra" (Kirby 1813). Thus, the common name, "twisted wing parasite" would be more appropriately rechristened, "twisted haltere parasite."

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Supplementary Material

*Wing areas (*Triozocera texana*)*

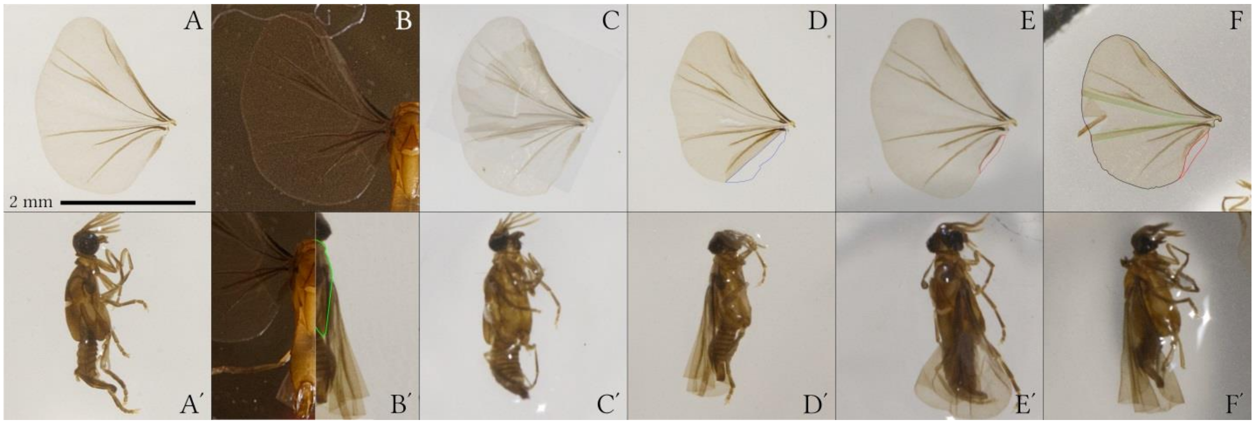

Fig. iii.S1. Wing areas and body lengths. Six *Triozocera texana* wings and the bodies from which there were removed are shown. The wing-body pairs are presented from least to most augmented. Given how extremely delicate strepsipteran wings are, in all but the first two *(A)* & *(B)*, some digital manipulation was necessary to arrive at the total wing area. However, because in *(B′)* the head was removed for analysis before the body length was measured, only *(A+A′)* was used to calculate the wing loading. Nonetheless, all of the wings are of similar shape. (A) An entirely detached fully unfurled *T. texana* wing. (B) Outstretched wing measured while still attached to the (headless) body. (B′) To avoid abdomen shrinkage issues while approximating the body length of this headless specimen, the thoraces of several headed specimens were resized to fit to that of the target insect, and the resultant average head length was added to that of the headless body. In this image, the headless body appears at left and one of the fitted bodies at right. Note that the ethanol-solution immersed abdomen of the target specimen has shrunk less than that of the dried specimen presented to the right. (C) The left and right wings of this specimen were torn, but because one tore at the bottom and the other at the top, they were fit together to form one complete wing. (D) This wing was fine except that it contains a darkened folded over portion at its base. It has been digitally traced, flipped outward, and its area (blue) added to that of the rest of the wing. (E) The proximal part of the wing base was apparently lost. I fit wing *(A)* to it and added the additional area outlined in red. (F) Many adjustments were made to this wing. Areas darkened due to overlap (outlined in green) were added twice. The ripped portion at the trailing edge of the wing was reconnected to complete the wing profile. Finally, a portion of the base of the wing that had been torn away was traced from another wing, resized and repositioned to closely match the target wing, and added to its base (in red). Note that if the green areas were expanded, the leading edge spar of the wing would move into a position similar to that of the other five wings. All images are drawn to scale.

*Body length vs. wing area (*Triozocera texana*)*

In the single instance in which it was used to estimate body mass, body length was measured from the base of the antennae (i.e., the outer edge of the eye) to the tip of the aedeagus (Fig. iii.6). In general, however, body lengths of adult male Strepsiptera are unreliable due to abdominal shrinkage subsequent to death. Shrinkage is most pronounced in dried Strepsiptera, but is also often present in specimens preserved in diluted ethanol, as well. Therefore, to compare wing length to wing area in Fig. iii.S2, only the head and thorax were used to compute "body" length. The resultant curve fits are very strong, indicating that the wing area calculations involving folded over areas, small peripheral tears, and reconstructions from partial wings are very likely to be highly accurate, although those data were not actually used.

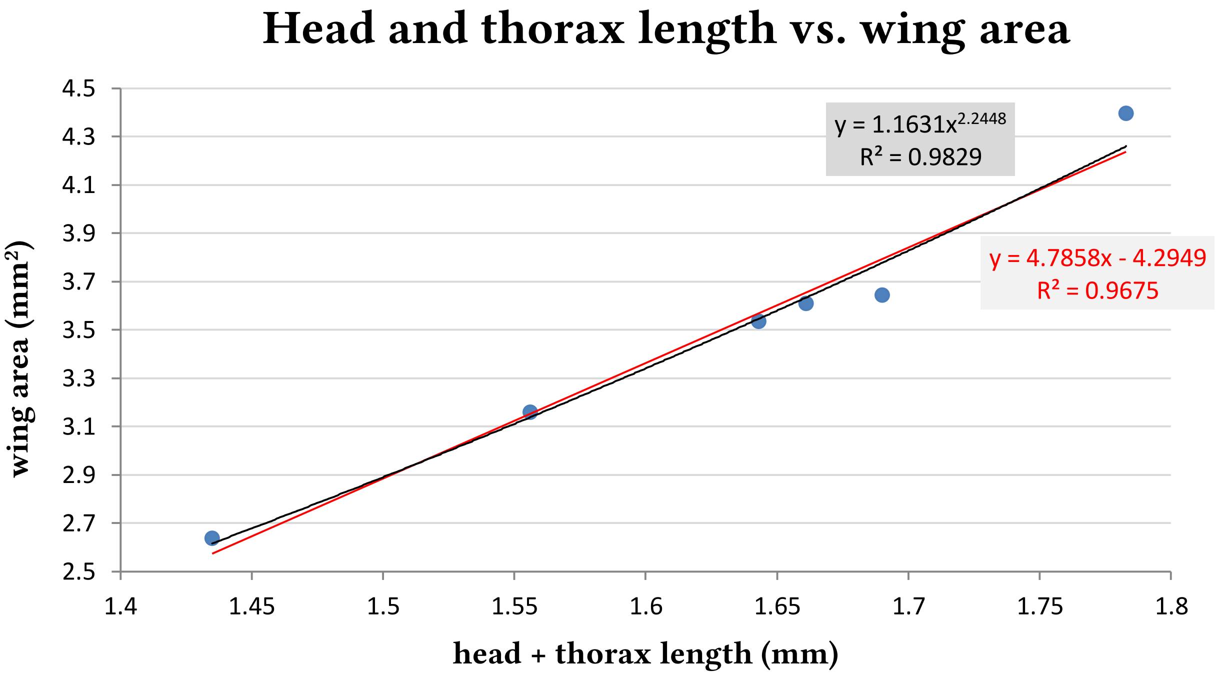

Fig. iii.S2. Head and thorax length vs. wing area. The head and thorax length of the *Triozocera texana* depicted in Fig. iii.S1 are here plotted against their wing areas, which were determined as described in Fig. iii.S1. A nearly linear relationship ($R^2 \approx 0.97$) between wing area and head + thorax length was found, which should also hold for the entire body length, provided the abdomens are relaxed (i.e., neither shrunken nor distended). Fitting to a (super quadratic) power curve produces a slightly better result ($R^2 \approx 0.98$). Thus, wing area appears to vary systematically with body length (and weight) in *T. texana* (and Strepsiptera at large).

The body length of Elenchus koebelei

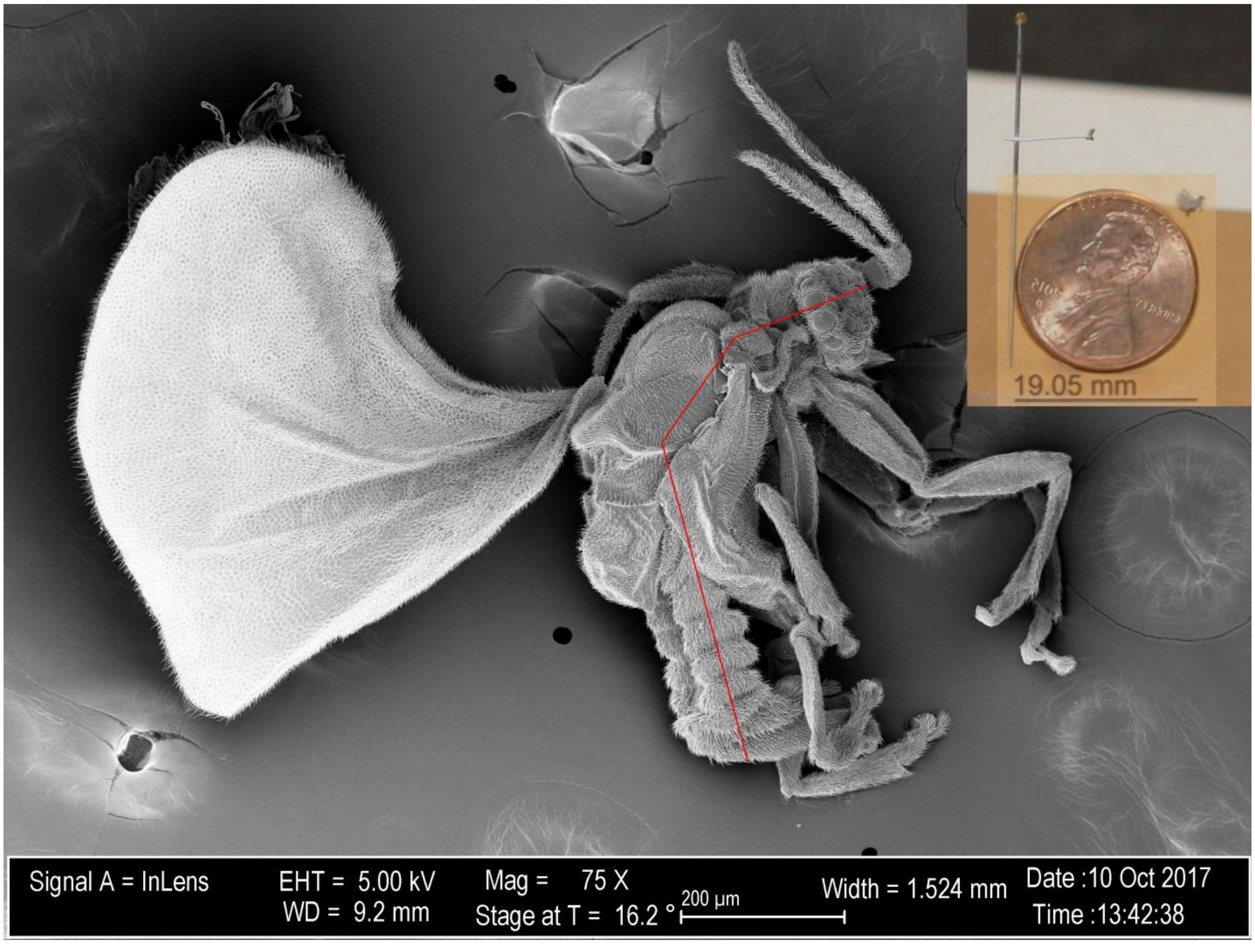

Fig. iii.S3. *Elenchus koebelei* body length. From this scanning electron micrograph (SEM), I determined the body length of an *E. koebelei* from which the frontmost wing had been removed. I used the included scale bar and a few line segments to model the body as if it were laid out straight. From this, I found that the body length was a mere 0.724 mm — about half what I had earlier assumed (< 1.5 mm). For further verification, I compared the size of a mounted *E. koebelei* with that of a penny (19.05 mm in diameter). I also superimposed that image onto the inset image from Fig. iii.1, fitting the diameter of the penny from the *E. koebelei* photo to the penny in the *Triozocera texana* photo. I thus compared both to the known diameter of a penny and fixed their scales relative to one another. Unfortunately, the images of the Strepsiptera, particularly that of the *E. koebelei*, were not captured at high resolution and are therefore somewhat pixelated. Even so, I found the body length of the second *E. koebelei* to be 0.8 mm and that of the *T. texana* to be 2.75 mm. The inset image is not drawn to scale with respect to the main *E. koebelei* image. Note that in the main image, the strepsipteran thorax extends both dorsally and ventrally, enabling it to house additional flight muscle. [Image courtesy of the Samuel Roberts Noble Microscopy Laboratory of the University of Oklahoma.]

The body lengths of most of the *Elenchus koebelei* I collected were apparently much shorter than I had earlier supposed. I realized this must be the case when looking back at a scanning electron micrograph (SEM) and noticing the width of the entire field was about the body length I had expected of *E. koebelei*. I therefore used the SEM image to calculate the body length of that specimen based on the scale bar included in the image. I found it to be 0.72 mm long (Fig. iii.S3). I then calculated the body length of the specimen in Fig. iii.7 using the same technique, and found it to be 0.736 mm long. Although its ultimate accuracy is limited by pixelation, in the inset image of Fig. iii.S3, a third *E. koebelei* was found to be about 0.8 mm long by comparing its body length of the diameter of a penny (19.05 mm) with which it had been photographed. Previously, I had only formally noted adult male *E. koebelei* to be < 1.5 mm long (James & Strong 2018), about twice their actual body length.

Strepsipteran ommatidial counts and configurations

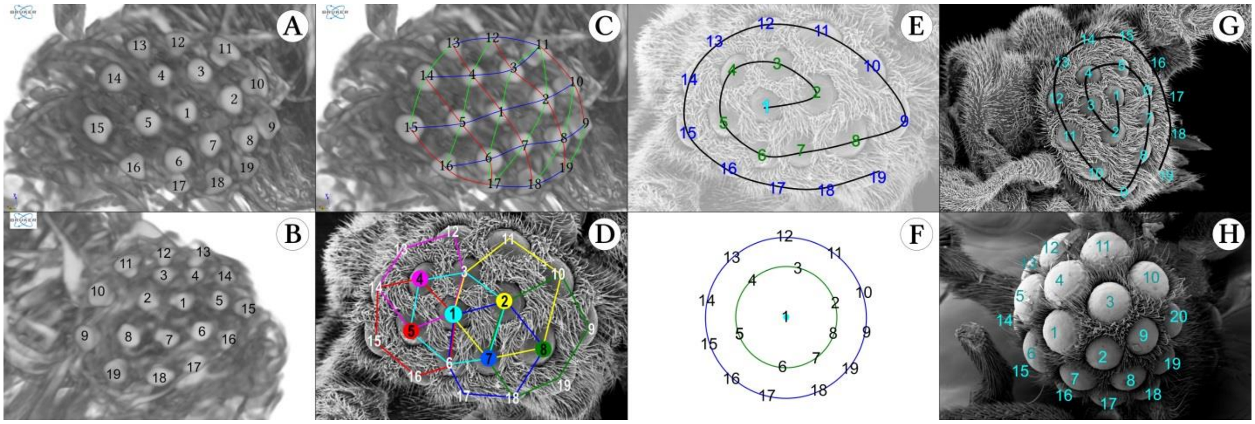

Fig. iii.S4. Strepsipteran ommatidial counts and configurations. In strepsipteran species where the maximum number of ommatidia is below around 25, the exact same number of ommatidia ostensibly appear in the left and right eyes of the same individual, as presented here for *Elenchus koebelei* and *Triozocera texana* eyes. (A−C) MicroCT scans naturally highlight insect lenses, a useful feature when counting ommatidia. These scans are of the same specimen. (A&B) In *Elenchus koebelei*, the number of ommatidia in the two eyes always appears to be equal, and furthermore, the number also appears to be constant for the entire species, at 19 ommatidia per eye. (A) The left eye of an *E. koebelei* specimen. (B) The right eye of the same individual. The number of ommatidia is the same. (C) To be useful in discerning direction, the strepsipteran eye must be unambiguously organized. This *E.*

koebelei is adorned with hypothetical lines of optic flow drawn along its left eye. There are three cardinal directions, as befits most insect compound eyes, because they are nearly always primarily hexagonally-configured.[1] (D) The hexagonal-dominant underpinning of the *E. koebelei* eye, and thus that of its putative optic flow. Only the interior ommatidia are capable of having a complete hexagonal surround, but two of the eight candidates (3 and 6) fail to do so anyway: their neighborhoods are pentagonal due to a stable irregularity in the ommatidial arrangement—the placement of ommatidium 8. (E) A spiral encapsulating *E. koebelei* ommatidial placement. Although the positions of the second and seventh ommatidia could be reversed, by (my) convention, the spiral rotates counterclockwise in the left eye and clockwise in the right eye. Thus, the eighth ommatidium follows the last node of the hexagon centered on the central ommatidium. (F) Stylized schematic of the two concentric rings of ommatidia that surround the central ommatidium. The eighth ommatidium alters the populations of the two rings from 6 and 12 ommatidia, which would produce a purely hexagonal ommatidial arrangement across the eye, to 7 and 11, which does not. (G) Another specimen demonstrating that *E. koebelei* eyes have 19-ommatidia. Although the 17th ommatidium is completely hidden from view, its location is assured from the positions of the other 18 visible ommatidia, as well as the gap in the microtrichia adjacent to the 17th label. Due to the orientation of the body, the second ommatidium is downward from the first instead of behind it (note that the antennae are situated upward instead of nearly horizontal, as in the other sub-images of this figure). The spiral curves clockwise because this is an image of the right eye. (H) Right eye of a *Triozocera texana*, from which I removed an antenna for full exposure. Members of this species are significantly larger than *E. koebelei*, and have much bigger, more spherical eyes, with 16, 18, or 20 ommatidia in the left and right eyes of the same individual. The (two) eyes of this specimen have 20 ommatidia, although the 15th ommatidium is hidden from view.

When ommatidial counts are small (less than about 25), strepsipteran eyes appear to show a great deal of conserved structure. This is clearly true of *Elenchus koebelei* (Fig. iii.S4). Although I have not studied their ocular morphology as carefully, it also appears to be true of *Triozocera texana* (Fig. iii.S4H). In *E. koebelei*, the number of ommatidia was invariably found to be 19; furthermore, the ommatidia were arranged in a stereotyped array. So far in *T. texana*, the ommatidia count has always been found to be 16, 18, or 20—perhaps reflecting the greater range of body sizes *T. texana* exhibit. Additionally, both eyes of the same specimen have always had the same number of ommatidia (J. Cook, personal communica-

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[1] The slight indentation attendant to ommatidia 1 and 7 is an artifact of the eye having collapsed slightly during the process of preparing it for electron microscopy (i.e., “fixing” the eye).

tion, October 5, 2020). For reasons yet unknown to me but presumably linked to ocular organization, the *T. texana* ommatidial count always appears to be even. These findings are in stark contrast with reports on the eyes of large diurnal strepsipteran species, which have several to many more, ostensibly haphazardly arranged, ommatidia (Kinzelbach 1971; Pix et al. 2000).[2] I expect that when strepsipteran eyes contain very few ommatidia, their gross structure is genetically dictated, whereas in species with larger numbers of ommatidia it is not. This may reflect that the visual demands of diurnal flight require greater spatial resolution in a time-restricted manner. Whatever the case, well-ordered eyes are a hallmark of insects with superior vision (Beersma et al. 1977; Seidl & Kaiser 1981; Cagan & Ready 1989; Warrant et al. 1999). When present, such structure has so far been found at both morphologic and microscopic scales (compare (Ready et al. 1976) with (Chaĭka & Mazokhin-Porshnyakov 1988)). As yet, there is no indication of any such microscopic organization in the eyes of diurnal Strepsiptera. The eyes of smaller and non-diurnal strepsipteran species have yet to be investigated at light microscopic or higher spatial scales.

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[2] It should be noted that Kinzelbach (1971) is the sole source of most strepsipteran ommatidial counts. However, his ommatidial count ranges are problematic in that they do not differentiate between variation between the two eyes of an individual and between the eyes of different specimens. They also provide no indication of how many specimens were investigated.

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Combined Byrne (1988) and Tercel (2018) data set

	Order	Family	Genus	Species	Mass (g)	Wing area (cm^2)	Wing loading (g/cm^2)	Wingbeat (Hz)	Average wing loading for Order (g/cm^2)	Citation
1	Hemiptera	Aleyrodidae	*Dialeurodes*	*Dialeurodes citri* (male)	0.000036	0.0207	0.00174	–	0.03220	Byrne et al., 1988
2	Hemiptera	Aleyrodidae	*Trialeurodes*	*Trialeurodes vaporariorum*	0.000035	0.0165	0.00212	180.0	0.03220	Byrne et al., 1988
3	Hemiptera	Aleyrodidae	*Bemisia*	*Bemisia tabaci*	0.000033	0.0134	0.00245	168.6	0.03220	Byrne et al., 1988
4	Neuroptera	Chrysopidae	*Chrysoperla*	*Chrysoperla carnea*	0.0065	1.972	0.003	25.923	0.0051667	Tercel et al., 2018
5	Neuroptera	Hemerobiidae	*Micromus*	-	0.0003	0.106	0.003	94.413	0.0051667	Tercel et al., 2018
6	Hemiptera	Aleyrodidae	*Dialeurodes*	*Dialeurodes citri* (female)	0.000080	0.0264	0.00303	–	0.03220	Byrne et al., 1988
7	Hemiptera	Aleyrodidae	*Aleurothrixus*	*Aleurothrixus floccosus*	0.000065	0.194	0.00336	165.6	0.03220	Byrne et al., 1988
8	Lepidoptera	Pieridae	*Pieris*	*Pieris napi*	0.037	8.530	0.004	6	0.06651	Sotavalta, 1952
9	Lepidoptera	Geometridae	*Xanthorhoe*	*Xanthorhoe montanata*	0.0133	2.98	0.004	29.243	0.06651	Tercel et al., 2018
10	Lepidoptera	Nymphalidae	*Aphantopus*	*Aphantopus hyperantus*	0.0373	7.262	0.005	16.014	0.06651	Tercel et al., 2018
11	Hemiptera	Aleyrodidae	*Trialeurodes*	*Trialeurodes abutilonea*	0.000050	0.0096	0.00523	224.2	0.03220	Byrne et al., 1988
12	Diptera	Trichoceridae	*Trichocera*	- (2)	0.0012	0.200	0.00600	74	0.06765	Sotavalta, 1952
13	Lepidoptera	Geometridae	*Pseudopanthera*	*Pseudopanthera macularia*	0.021	3.400	0.006	25	0.06651	Magnan, 1934
14	Neuroptera	Hemerobiidae	*Wesmaelius*	*Wesmaelius subnebulosis*	0.0026	0.416	0.006	45.304	0.0051667	Tercel et al., 2018
15	Neuroptera	Hemerobiidae	*Hemerobius*	*Hemerobius humulinus*	0.0021	0.336	0.006	46.583	0.0051667	Tercel et al., 2018
16	Neuroptera	Hemerobiidae	*Micromus*	*Micromus angulatus*	0.0059	0.932	0.006	50	0.0051667	Tercel et al., 2018
17	Hemiptera	Aphididae	*Acyrthosiphon*	*Acyrthosiphon kondoi*	0.000702	0.1106	0.00633	81.1	0.03220	Byrne et al., 1988
18	Lepidoptera	Pieridae	*Pieris*	*Pieris brassicae* (3)	0.113	15.531	0.007	11.656	0.06651	Tercel et al., 2018; Magnan, 1934; Sotavalta, 1952
19	Neuroptera	Hemerobiidae	*Wesmaelius*	-	0.0033	0.444	0.007	54.583	0.0051667	Tercel et al., 2018
20	Hemiptera	Aphididae	*Aphis*	*Aphis nerii*	0.000467	0.0663	0.00750	118.1	0.03220	Byrne et al., 1988
21	Hemiptera	Aphididae	*Aphis*	*Aphis fabae*	0.000411	0.0526	0.00780	104.7	0.03220	Byrne et al., 1988
22	Lepidoptera	Papilionidae	*Iphiclides*	*Iphiclides podalirius*	0.300	36.000	0.008	10	0.06651	Magnan, 1934
23	Ephemeroptera	Baetidae	*Centroptilum*	*Centroptilum luteolum*	0.0027	0.306	0.009	75.045	0.009	Tercel et al., 2018
24	Hemiptera	Miridae	-	-	0.0011	0.122	0.009	127.872	0.03220	Tercel et al., 2018
25	Lepidoptera	Geometridae	*Pasiphila*	*Pasiphila rectangulata*	0.0107	1.132	0.009	41.358	0.06651	Tercel et al., 2018
26	Lepidoptera	Tortricidae	-	-	0.0055	0.612	0.009	52.214	0.06651	Tercel et al., 2018
27	Lepidoptera	Pieridae	*Gonepteryx*	*Gonepteryx rhamni*	0.107	12.00	0.0099	21	0.06651	Magnan, 1934
28	Lepidoptera	Nymphalidae	*Coenonympha*	*Coenonympha pamphilus*	0.046	4.80	0.010	22	0.06651	Magnan, 1934
29	Lepidoptera	Pterophoridae	*Pterophorus*	*Pterophorus pentadactyla*	0.0114	1.12	0.01	32.333	0.06651	Tercel et al., 2018
30	Lepidoptera	Erebidae	*Nyea*	*Nyea lurideola*	0.0258	2.542	0.01	33.095	0.06651	Tercel et al., 2018
31	Lepidoptera	Crambidae	-	-	0.0059	0.562	0.01	57.948	0.06651	Tercel et al., 2018
32	Lepidoptera	Geometridae	*Geometra*	*Geometra papilionaria*	0.1071	10.194	0.011	22.023	0.06651	Tercel et al., 2018
33	Odonata	Calopterygidae	*Calopteryx*	*Calopteryx splendens*	0.1092	9.76	0.011	19.318	0.02854	Tercel et al., 2018
34	Hemiptera	Aphididae	*Aphis*	*Aphis gossypii*	0.000114	0.0103	0.01106	123.4	0.03220	Byrne et al., 1988
35	Diptera	Tipulidae	-	-	0.002	0.168	0.012	94.606	0.06765	Tercel et al., 2018
36	Hemiptera	-	-	-	0.0014	0.114	0.012	152.247	0.03220	Tercel et al., 2018
37	Lepidoptera	Saturniidae	*Samia*	*Samia cynthia*	0.605	50.000	0.012	8	0.06651	Magnan, 1934
38	Lepidoptera	Nymphalidae	*Vanessa*	*Vanessa alalanta*	0.134	10.800	0.012	10	0.06651	Magnan, 1934

	Order	Family	Genus	Species	Mass (g)	Wing area (cm^2)	Wing loading (g/cm^2)	Wingbeat (Hz)	Average wing loading for Order (g/cm^2)	Citation
39	Lepidoptera	Crambidae	*Anania*	*Anania hortulata*	0.0293	2.41	0.012	40.996	0.06651	Tercel et al., 2018
40	Diptera	Psychodidae	-	-	0.0006	0.048	0.013	144.611	0.06765	Tercel et al., 2018
41	Hemiptera	Aphididae	*Uroleucon*	*Uroleucon cirsii*	0.0015	0.112	0.013	99.603	0.03220	Tercel et al., 2018
42	Lepidoptera	Erebidae	*Hypena*	*Hypena proboscidalis*	0.0565	4.496	0.013	30.587	0.06651	Tercel et al., 2018
43	Lepidoptera	Geometridae	*Idaea*	*Idaea aversata*	0.0303	2.42	0.013	32.088	0.06651	Tercel et al., 2018
44	Lepidoptera	Tortricidae	*Pandemis*	*Pandemis cerasana*	0.0124	0.89	0.014	54.184	0.06651	Tercel et al., 2018
45	Lepidoptera	Tortricidae	*Pseudargyrotoza*	*Pseudargyrotoza conwagana*	0.0044	0.318	0.014	64.246	0.06651	Tercel et al., 2018
46	Lepidoptera	Tortricidae	*Lobesia*	*Lobesia fuligana*	0.0076	0.526	0.014	64.566	0.06651	Tercel et al., 2018
47	Odonata	Calopterygidae	*Calopteryx*	*Calopteryx virgo*	0.1457	10.466	0.014	17.847	0.02854	Tercel et al., 2018
48	Hemiptera	Aphididae	*Mysus*	*Mysus persicae*	0.000334	0.0237	0.01412	90.9	0.03220	Byrne et al., 1988
49	Lepidoptera	Nymphalidae	*Aglais*	*Aglais io*	0.195	14.000	0.0147	18	0.06651	Magnan, 1934
50	Lepidoptera	Nymphalidae	*Argunnis*	*Argynnis pandora*	0.278	18.00	0.015	10	0.06651	Magnan, 1934
51	Lepidoptera	Nymphalidae	*Polygonia*	*Polygonia c-album*	0.1145	7.696	0.015	27.501	0.06651	Tercel et al., 2018
52	Odonata	Coenagrionidae	*Coenagrion*	*Coenagrion puella* (3)	0.0322	2.075	0.015	37.008	0.02854	Tercel et al., 2018
53	Lepidoptera	Saturniidae	*Saturnia*	*Saturnia pyri*	1.890	120.000	0.016	8	0.06651	Magnan, 1934
54	Odonata	Libellulidae	*Perithemis*	*Perithemis tenera*	0.061	3.812	0.016	39.4	0.02854	May, 1981
55	Lepidoptera	Nymphalidae	*Vanessa*	*Vanessa cardui*	0.173	10.40	0.017	20	0.06651	Magnan, 1934
56	Diptera	-	-	-	0.0009	0.05	0.018	204.355	0.06765	Tercel et al., 2018
57	Hemiptera	Miridae	-	-	0.0048	0.25	0.019	120.832	0.03220	Tercel et al., 2018
58	Odonata	Libellulidae	*Libellula*	*Libellula depressa*	0.245	13.200	0.019	20	0.02854	Magnan, 1934
59	Lepidoptera	Erebidae	*Lymantria*	*Lymantria dispar*	0.101	5.39	0.02	27	0.06651	Casey, 1981
60	Odonata	Libellulidae	*Pantala*	*Pantala flavescens*	0.308	15.400	0.020	22.9	0.02854	May, 1981
61	Odonata	Libellulidae	*Tramea*	*Tramea carolina*	0.358	17.900	0.020	23.6	0.02854	May, 1981
62	Hemiptera	-	-	-	0.0104	0.49	0.021	116.865	0.03220	Tercel et al., 2018
63	Hymenoptera	Ichneumonidae	*Ophion*	*Ophion luteus*	0.033	1.5501	0.021	62	0.17692	Sotavalta, 1952
64	Lepidoptera	Erebidae	*Epicallia*	*Epicallia villica*	0.165	8.000	0.021	20	0.06651	Magnan, 1934
65	Odonata	Libellulidae	*Pachydiplax*	*Pachydiplax longipennis*	0.178	8.476	0.021	24.3	0.02854	May, 1981
66	Odonata	Libellulidae	*Tramea*	*Tramea lacerata*	0.382	18.190	0.021	26.8	0.02854	May, 1981
67	Odonata	Libellulidae	*Erythemis*	*Erythemis simplicicollis*	0.176	8.38	0.021	28	0.02854	May, 1981
68	Diptera	Chironomidae	-	- (2)	0.0015	0.067	0.0215	550.92	0.06765	Tercel et al., 2018
69	Hymenoptera	Braconidae	-	-	0.0029	0.134	0.022	136.261	0.17692	Tercel et al., 2018
70	Mecoptera	Panorpidae	*Panorpa*	*Panorpa communis* (2)	0.035	1.621	0.022	38.443	0.022	Magnan, 1934; Tercel et al., 2018
71	Odonata	Corduliidae	*Epitheca*	*Epitheca cynosura*	0.165	7.500	0.022	27.6	0.02854	May, 1981
72	Diptera	Syrphidae	*Syrphus*	-	0.0005	0.022	0.023	190.86	0.06765	Tercel et al., 2018
73	Odonata	Libellulidae	*Libellula*	*Libellula luctuosa*	0.318	13.826	0.023	19	0.02854	May, 1981
74	Odonata	Libellulidae	*Orthetrum*	*Orthetrum coerulescens*	0.248	10.800	0.023	20	0.02854	Magnan, 1934
75	Odonata	Libellulidae	*Sympetrum*	*Sympetrum striolatum*	0.1595	6.944	0.023	40.665	0.02854	Tercel et al., 2018
76	Hymenoptera	Torymidae	*Torymus*	-	0.0024	0.098	0.024	160.011	0.17692	Tercel et al., 2018
77	Odonata	Aeshnidae	*Nasiaeschua*	*Nasiaeschua pentacantha*	0.430	16.538	0.026	21.1	0.02854	May, 1981
78	Diptera	Tipulidae	*Tipula*	- (12)	0.030	1.110	0.027	52	0.06765	Sotavalta, 1952
79	Odonata	Libellulidae	*Sympetrum*	*Sympetrum meridionale*	0.281	10.000	0.028	21	0.02854	Magnan, 1934

	Order	Family	Genus	Species	Mass (g)	Wing area (cm^2)	Wing loading (g/cm^2)	Wingbeat (Hz)	Average wing loading for Order (g/cm^2)	Citation
80	Diptera	Tipulidae	*Nephrotoma*	*Nephrotoma flavescens*	0.0118	0.402	0.029	79.47	0.06765	Tercel et al., 2018
81	Odonata	Libellulidae	*Libellula*	*Libellula quadrimaculatum* (4)	0.307	10.600	0.029	21	0.02854	Magnan, 1934
82	Odonata	Corduliidae	*Somatochlora*	*Somatochlora tenebrosa*	0.352	12.138	0.029	25.8	0.02854	May, 1981
83	Diptera	Tipulidae	*Tipula*	*Tipula maxima*	0.069	2.260	0.030	48	0.06765	Magnan, 1934
84	Hemiptera	Miridae	-	-	0.0119	0.402	0.03	108.171	0.03220	Tercel et al., 2018
85	Lepidoptera	Erebidae	*Calliteara*	*Calliteara pudibunda*	0.237	8.000	0.030	28	0.06651	Magnan, 1934
86	Lepidoptera	Zygaenidae	*Zygaena*	-	0.0804	2.64	0.03	60.595	0.06651	Tercel et al., 2018
87	Odonata	Aeshnidae	*Aeshna*	*Aeshna isoceles*	0.611	17.800	0.03	20	0.02854	Magnan, 1934
88	Odonata	Libellulidae	*Orthetrum*	*Orthetrum cancellatum*	0.4176	13.96	0.03	38.577	0.02854	Tercel et al., 2018
89	Diptera	Culicidae	*Culex*	*Culex pipiens*	0.0049	0.158	0.031	334.037	0.06765	Tercel et al., 2018
90	Hemiptera	Miridae	*Macrolophus*	-	0.0076	0.244	0.031	139.717	0.03220	Tercel et al., 2018
91	Odonata	Libellulidae	*Libellula*	*Libellula pulchella*	0.508	16.387	0.031	26.2	0.02854	May, 1981
92	Coleoptera	Chrysomelidae	*Oulema*	*Oulema melanopus*	0.0061	0.19	0.032	123.398	0.13409	Tercel et al., 2018
93	Hemiptera	-	-	-	0.0383	1.164	0.033	90.222	0.03220	Tercel et al., 2018
94	Lepidoptera	Noctuidae	*Plusia*	*Plusia gamma*	0.144	4.400	0.033	48	0.06651	Magnan, 1934
95	Odonata	Gomphidae	*Ophiogomphus*	*Ophiogomphus cecilia*	0.312	9.400	0.033	42	0.02854	Magnan, 1934
96	Odonata	Libellulidae	*Plathemis*	*Plathemis lydia*	0.365	10.735	0.034	31.4	0.02854	May, 1981
97	Trichoptera	Phryganeidae	*Phryganea*	*Phryganea grandis*	0.159	4.738	0.034	27.515	0.034	Tercel et al., 2018
98	Diptera	Drosophilidae	*Drosophila*	*Drosophila virilis*	0.0020	0.0576	0.035[†]	195	0.06765	Vogel, 1966
99	Diptera	Syrphidae	-	-	0.0044	0.124	0.035	198.89	0.06765	Tercel et al., 2018
100	Lepidoptera	Lasiocampidae	*Poecilocampa*	*Poecilocampa populi*	0.112	3.170	0.035	55	0.06651	Sotavalta, 1952
101	Diptera	Culicidae	*Aedes*	*Aedes cantans*	0.0066	0.182	0.036	286.949	0.06765	Tercel et al., 2018
102	Hemiptera	-	-	-	0.0184	0.518	0.036	112.917	0.03220	Tercel et al., 2018
103	Diptera	Chloropidae	*Thaumatomyia*	*Thaumatomyia notata*	0.0014	0.038	0.037	269.741	0.06765	Tercel et al., 2018
104	Hymenoptera	Tenthredinidae	*Athalia*	*Athalia scuttelariae*	0.0132	0.352	0.038	87.129	0.17692	Tercel et al., 2018
105	Lepidoptera	Noctuidae	*Orthosia*	*Orthosia gothica*	0.1253	3.26	0.038	47.053	0.06651	Tercel et al., 2018
106	Diptera	Tipulidae	*Nephrotoma*	*Nephrotoma quadrifaria*	0.0181	0.464	0.039	67.36	0.06765	Tercel et al., 2018
107	Diptera	Culicidae	-	-	0.0058	0.150	0.039	277	0.06765	Sotavalta, 1952
108	Diptera	Culicidae	*Aedes*	*Aedes aegypti* (2)	0.0015	0.037	0.039	480	0.06765	Sotavalta, 1952
109	Lepidoptera	Saturniidae	*Automeris*	*Automeris jacunda* (4)	0.298	7.572	0.039	17.1	0.06651	Bartholomew & Casey, 1978
110	Odonata	Aeshnidae	*Anax*	*Anax junius*	0.820	21.02	0.039	20.5	0.02854	May, 1981
111	Odonata	Macromiidae	*Macromia*	*Macromia illinoiensis*	0.545	13.974	0.039	30.0	0.02854	May, 1981
112	Diptera	-	-	-	0.0039	0.098	0.04	195.996	0.06765	Tercel et al., 2018
113	Lepidoptera	Notodontidae	*Pheosia*	*Pheosia tremula*	0.201	5.000	0.040	22	0.06651	Magnan, 1934
114	Lepidoptera	Noctuidae	*Agrotis*	*Agrostis exclamationis*	0.133	3.20	0.04	41	0.06651	Magnan, 1934
115	Lepidoptera	Lasiocampidae	*Malacosoma*	*Malacosoma americanum*	0.088	2.36	0.04	58	0.06651	Casey, 1981
116	Odonata	Aeshnidae	*Aeshna*	*Aeshna mixta*	0.530	13.80	0.04	38	0.02854	Magnan, 1934
117	Diptera	Syrphidae	*Epistrophe*	*Epistrophe gryssulariae*	0.0200	0.480	0.042	114	0.06765	Weis-Fogh, 1973
118	Lepidoptera	Zygaenidae	*Zygaena*	*Zygaena filipendulae*	0.127	3.000	0.042	48	0.06651	Magnan, 1934
119	Diptera	Chloropidae	-	-	0.003	0.07	0.043	180.05	0.06765	Tercel et al., 2018
120	Hymenoptera	Ichneumonidae	-	-	0.0233	0.546	0.043	110.116	0.17692	Tercel et al., 2018

	Order	Family	Genus	Species	Mass (g)	Wing area (cm^2)	Wing loading (g/cm^2)	Wingbeat (Hz)	Average wing loading for Order (g/cm^2)	Citation
121	Coleoptera	Coccinellidae	*Harmonia*	*Harmonia axyridis*	0.0283	0.644	0.044	79	0.13409	Tercel et al., 2018
122	Diptera	Culicidae	*Culiseta*	*Culiseta annulata* (2)	0.0073	0.162	0.046	337.66	0.06765	Tercel et al., 2018
123	Lepidoptera	Bombycidae	*Macrothylacia*	*Macrothylacia rubi*	0.595	13.000	0.046	18	0.06651	Magnan, 1934
124	Odonata	Macromiidae	*Macromia*	*Macromia taeniolata*	0.930	20.217	0.046	25.5	0.02854	May, 1981
125	Odonata	Aeshnidae	*Brachytron*	*Brachytron pratense*	0.557	12.000	0.046	33	0.02854	Magnan, 1934
126	Hemiptera	Miridae	*Lygus*	*Lygus rugulipennis*	0.014	0.298	0.047	115.183	0.03220	Tercel et al., 2018
127	Coleoptera	Cantharidae	*Rhagonycha*	*Rhagonycha fulva*	0.0183	0.38	0.048	79.712	0.13409	Tercel et al., 2018
128	Diptera	Syrphidae	*Episyrphus*	*Episyrphus balteatus* (8)	0.0240	0.490	0.0486	141.51	0.06765	Weis-Fogh, 1973; Tercel et al., 2018
129	Lepidoptera	Sphingidae	*Laothoe*	*Laothoe populi*	0.8449	17.152	0.049	29.33	0.06651	Tercel et al., 2018
130	Coleoptera	Coccinellidae	*Propylea*	*Propylea 14-punctata*	0.0105	0.21	0.05	102.427	0.13409	Tercel et al., 2018
131	Lepidoptera	Saturniidae	*Automeris*	*Automeris zugana* (4)	0.420	8.393	0.050	18.6	0.06651	Bartholomew & Casey, 1978
132	Diptera	Anthomyiidae	*Fannia*	*Fannia scalaris*	0.010	0.196	0.051	210	0.06765	Magnan, 1934
133	Lepidoptera	Saturniidae	*Hyperchirica*	*Hyperchirica nausica* (2)	0.200	3.950	0.051	21.6	0.06651	Bartholomew & Casey, 1978
134	Odonata	Aeshnidae	*Anax*	*Anax imperator*	1.200	22.800	0.053	22	0.02854	Magnan, 1934
135	Odonata	Aeshnidae	*Aeshna*	*Aeshna grandis*	1.2296	22.784	0.054	31.214	0.02854	Tercel et al., 2018
136	Diptera	Syrphidae	*Platycheirus*	*Platycheirus peltatus*	0.0128	0.230	0.056	147	0.06765	Weis-Fogh, 1973
137	Coleoptera	Scarabaeidae	*Aphodius*	-	0.0327	0.56	0.058	93.054	0.13409	Tercel et al., 2018
138	Diptera	Tipulidae	*Tipula*	-	0.0676	1.17	0.058	59.567	0.06765	Tercel et al., 2018
139	Diptera	Stratiomyidae	*Chloromyia*	*Chloromyia formosa*	0.0183	0.312	0.059	156.043	0.06765	Tercel et al., 2018
140	Diptera	Culicidae	*Theobaldia*	*Theobaldia annulata*	0.0099	0.169	0.059	262	0.06765	Sotavalta, 1952
141	Diptera	Muscidae	*Musca*	*Musca domestica*	0.012	0.200	0.060	190	0.06765	Magnan, 1934
142	Diptera	Scathophagidae	*Scathophaga*	*Scathophaga stercoraria*	0.0224	0.366	0.061	104.015	0.06765	Tercel et al., 2018
143	Diptera	Tabanidae	*Haematopota*	*Haematopota pluvialis*	0.0183	0.302	0.061	151.568	0.06765	Tercel et al., 2018
144	Diptera	Syrphidae	*Eupeodes*	*Eupeodes corollae* (3)	0.0213	0.350	0.061	174	0.06765	Weis-Fogh, 1973
145	Lepidoptera	Saturniidae	*Automeris*	*Automeris belti*	0.665	10.960	0.061	14.4	0.06651	Bartholomew & Casey, 1978
146	Lepidoptera	Noctuidae	*Noctua*	*Noctua pronuba*	0.485	7.800	0.062	24	0.06651	Magnan, 1934
147	Hymenoptera	Vespidae: Eumeninae	-	-	0.0184	0.292	0.063	135.597	0.17692	Tercel et al., 2018
148	Diptera	Empididae	-	-	0.0193	0.288	0.067	151.321	0.06765	Tercel et al., 2018
149	Lepidoptera	Saturniidae	*Hylesia*	- (5)	0.168	2.334	0.072	32.4	0.06651	Bartholomew & Casey, 1978
150	Lepidoptera	Sphingidae	*Hemaris*	*Hemaris fuciformis*	0.189	2.620	0.072	80	0.06651	Magnan, 1934
151	Diptera	Syrphidae	*Eupeodes*	*Eupeodes nitens*	0.022	0.300	0.073	172	0.06765	Weis-Fogh, 1973
152	Diptera	Syrphidae	*Syrphus*	*Syrphus ribesii* (4)	0.346	0.465	0.0748	183.977	0.06765	Tercel et al., 2018; Weis-Fogh, 1973
153	Lepidoptera	Sphingidae	*Deilephila*	*Deilephila elpenor*	0.5281	6.792	0.078	53.715	0.06651	Tercel et al., 2018
154	Hymenoptera	Braconidae	-	-	0.003	0.038	0.079	164.443	0.17692	Tercel et al., 2018
155	Lepidoptera	Sphingidae	*Macroglossum*	*Macroglossum stellatarum* (2)	0.314	3.895	0.080	79	0.06651	Magnan, 1934; Sotavalta, 1952
156	Diptera	Syrphidae	*Syrphus*	*Syrphus vitripennis*	0.0385	0.480	0.080	196	0.06765	Weis-Fogh, 1973
157	Lepidoptera	Crambidae	*Chrysoteuchia*	*Chrysoteuchia culmella*	0.109	1.33	0.082	40.626	0.06651	Tercel et al., 2018
158	Diptera	Syrphidae	*Scaeva*	*Scaeva pyrastri*	0.034	0.400	0.085	190	0.06765	Magnan, 1934
159	Lepidoptera	Sphingidae	*Acherontia*	*Acherontia atropos* (2)	1.920	21.931	0.087	25.58	0.06651	Magnan, 1934; Tercel et al., 2018
160	Lepidoptera	Saturniidae	*Eacles*	*Eacles imperialis* (3)	1.105	12.600	0.088	17.9	0.06651	Bartholomew & Casey, 1978
161	Lepidoptera	Saturniidae	*Automerina*	*Automerina auletes*	0.720	8.090	0.089	23.4	0.06651	Bartholomew & Casey, 1978

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162	Coleoptera	Oedemeridae	*Oedemera*	*Oedemera nobilis*	0.021	0.232	0.091	112.656	0.13409	Tercel et al., 2018
163	Diptera	Calliphoridae	*Calliphora*	*Calliphora vicina* (13)	0.051	0.552	0.092	162	0.06765	Sotavalta, 1952 & Magnan, 1934
164	Coleoptera	Cantharidae	*Telephorus*	*Telephorus fuscus*	0.109	1.160	0.094	72	0.13409	Magnan, 1934
165	Diptera	Syrphidae	*Sphaerophoria*	*Sphaerophoria scripta* (3)	0.0193	0.200	0.094	308	0.06765	Weis-Fogh, 1973
166	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum arcuatum*	0.073	0.740	0.099	144	0.06765	Magnan, 1934
167	Hemiptera	Cicadidae	*Cicada*	-	0.752	7.64	0.10	42	0.03220	Ahmad, 1984
168	Diptera	Sarcophagidae	*Sarcophaga*	-	0.054	0.526	0.103	149.643	0.06765	Tercel et al., 2018
169	Lepidoptera	Saturniidae	*Automeris*	*Automeris hamata*	0.564	5.450	0.103	23.5	0.06651	Bartholomew & Casey, 1978
170	Coleoptera	Cerambycidae	-	-	0.142	1.33	0.107	80	0.13409	Sotavalta, 1952
171	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum vernale*	0.064	0.600	0.107	150	0.06765	Magnan, 1934
172	Hymenoptera	Apidae	*Andrena*	-	0.0376	0.352	0.107	213.815	0.17692	Tercel et al., 2018
173	Diptera	Syrphidae	*Chrysotoxum*	*Chrysotoxum bicinctum*	0.075	0.680	0.110	120	0.06765	Magnan, 1934
174	Hymenoptera	Sphecidae	*Ammophila*	*Ammophila sabulosa*	0.045	0.420	0.11	120	0.17692	Magnan, 1934
175	Hymenoptera	Apidae	*Apis*	-	0.0213	0.20	0.11	130	0.17692	Ahmad, 1984
176	Diptera	Syrphidae	-	-	0.0385	0.34	0.113	208.54	0.06765	Tercel et al., 2018
177	Lepidoptera	Sphingidae	*Manduca*	*Manduca lefeburii*	0.571	4.920	0.116	33.1	0.06651	Bartholomew & Casey, 1978
178	Diptera	Syrphidae	*Volucella*	*Volucella pellucens* (2)	0.117	0.966	0.117	127.09	0.06765	Magnan, 1934; Tercel et al., 2018
179	Hemiptera	Pentatomidae	*Pentatoma*	*Pentatoma rufipes*	0.1397	1.186	0.118	96.667	0.03220	Tercel et al., 2018
180	Coleoptera	Cerambicidae	*Leptura*	*Leptura quadrifasciata*	0.1173	0.982	0.119	93.768	0.13409	Tercel et al., 2018
181	Diptera	Calliphoridae	*Calliphora*	*Calliphora vomitoria*	0.0549	0.46	0.119	214.835	0.06765	Tercel et al., 2018
182	Hymenoptera	Vespidae	*Vespula*	*Vespula vulgaris* (3)	0.0891	0.755	0.121	153	0.17692	Sotavalta, 1952; Tercel et al., 2018
183	Diptera	Sarcophagidae	*Sarcophaga*	*Sarcophaga carnaria*	0.045	0.36	0.125	160	0.06765	Magnan, 1934
184	Coleoptera	Melolonthidae	*Amphimallon*	*Amphimallon solstitalis*	0.291	2.290	0.127	78	0.13409	Sotavalta, 1952
185	Hymenoptera	Apidae	*Andrena*	-	0.0453	0.346	0.131	172.581	0.17692	Tercel et al., 2018
186	Lepidoptera	Sphingidae	*Enyo*	*Enyo ocypete*	0.388	2.950	0.132	56.1	0.06651	Bartholomew & Casey, 1978
187	Coleoptera	Cerambicidae	*Rutpela*	*Rutpela maculata*	0.1026	0.768	0.134	86.84	0.13409	Tercel et al., 2018
188	Coleoptera	Scarabaeidae	*Aphodius*	- (2)	0.101	0.74	0.137	102.135	0.13409	Tercel et al., 2018
189	Hymenoptera	Vespidae	*Vespula*	*Vespula germanica* (3)	0.081	0.591	0.137	148	0.17692	Tercel et al., 2018
190	Diptera	Syrphidae	*Eristalis*	*Eristalis tenax* (8)	0.12	0.814	0.147	182.5	0.06765	Magnan, 1934; Weis-Fogh, 1973; Sotavalta, 1952
191	Lepidoptera	Sphingidae	*Pachygonidia*	*Pachygonidia drucei*	0.702	4.770	0.147	48.4	0.06651	Bartholomew & Casey, 1978
192	Hymenoptera	Crabronidae	*Ectemnius*	*Ectemnius cavifrons*	0.08	0.542	0.148	210.688	0.17692	Tercel et al., 2018
193	Blattodea	Blattidae	*Periplanela*	*Periplanela americana*	1.555	10.44	0.149	26	0.149	Ahmad, 1984
194	Diptera	Tabanidae	*Tabanus*	*Tabanus bovinus*	0.276	1.840	0.150	96	0.06765	Magnan, 1934
195	Lepidoptera	Saturniidae	*Adeloneivaia*	*Adeloneivaia boisduvalii* (3)	0.839	5.564	0.151	24.9	0.06651	Bartholomew & Casey, 1978
196	Diptera	Syrphidae	*Volucella*	*Volucella bombylans* (2)	0.1432	0.943	0.1515	126.539	0.06765	Magnan, 1934; Tercel et al., 2018
197	Diptera	Tabanidae	*Dasyrhamphis*	*Dasyrhamphis ater*	0.233	1.500	0.155	100	0.06765	Magnan, 1934
198	Hymenoptera	Apidae	*Apis*	*Apis mellifera* (7)	0.087	0.56	0.156	234.00	0.17692	Magnan, 1934; Sotavalta, 1952; Tercel et al., 2018
199	Lepidoptera	Sphingidae	*Xylophones*	*Xylophones libya*	0.559	3.560	0.157	48.5	0.06651	Bartholomew & Casey, 1978
200	Lepidoptera	Sphingidae	*Manduca*	*Manduca corallina* (4)	1.618	10.270	0.158	28.0	0.06651	Bartholomew & Casey, 1978
201	Lepidoptera	Saturniidae	*Syssphinx*	*Syssphinx molina*	1.630	9.700	0.168	22.9	0.06651	Bartholomew & Casey, 1978
202	Lepidoptera	Saturniidae	*Adeloneivaia*	*Adeloneivaia subungulata*	0.487	2.900	0.168	41.0	0.06651	Bartholomew & Casey, 1978

	Order	Family	Genus	Species	Mass (g)	Wing area (cm^2)	Wing loading (g/cm^2)	Wingbeat (Hz)	Average wing loading for Order (g/cm^2)	Citation
203	Diptera	Syrphidae	*Eristalis*	*Eristalis arbustorum*	0.0705	0.410	0.172	211	0.06765	Weis-Fogh, 1973
204	Hymenoptera	Apidae	*Bombus*	*Bombus hortorum*	0.159	0.900	0.177	135	0.17692	Magnan, 1934
205	Coleoptera	Scutelleridae	*Chrysocoris*	*Chrysocoris purpureus*	0.264	1.50	0.18	100	0.13409	Ahmad, 1984
206	Hymenoptera	Vespidae	*Vespula*	*Vespula germanica*	0.240	1.330	0.180	139	0.17692	Sotavalta, 1952 (potentially, a male)
207	Coleoptera	Melolonthidae	*Melolontha*	*Melolontha vulgaris* (2)	0.779	4.235	0.187	54	0.13409	Magnan, 1934; Sotavalta, 1952
208	Lepidoptera	Sphingidae	*Xylophones*	*Xylophones pluto* (2)	0.829	4.430	0.187	45.0	0.06651	Bartholomew & Casey, 1978
209	Hymenoptera	Apidae	*Exaerete*	*Exaerete frontalis* (4)	0.644	3.48	0.19	87	0.17692	Casey et al., 1985
210	Hymenoptera	Apidae	*Bombus*	*Bombus pascuorum*	0.1166	0.614	0.19	198.274	0.17692	Tercel et al., 2018
211	Hymenoptera	Vespidae	*Vespula*	*Vespula germanica*	0.187	0.980	0.191	110	0.17692	Magnan, 1934 (most likely a female worker)
212	Hymenoptera	Apidae	*Bombus*	*Bombus terrestris* (9)	0.216	0.128	0.194	158.507	0.17692	Magnan, 1934; Tercel et al., 2018
213	Hymenoptera	Apidae	*Euglossa*	*Euglossa mandibularis* (4)	0.090	0.44	0.20	209	0.17692	Casey et al., 1985
214	Hymenoptera	Apidae	*Euglossa*	*Euglossa saphirina*	0.071	0.35	0.20	265	0.17692	Casey, May & Morgan, 1985
215	Hymenoptera	Vespidae	*Vespa*	*Vespa crabro* (2)	0.582	2.820	0.207	102	0.17692	Magnan, 1934; Sotavalta, 1952
216	Hymenoptera	Apidae	*Englossa*	*Englossa imperialis* (6)	0.169	0.79	0.21	179	0.17692	Casey et al., 1985
217	Lepidoptera	Sphingidae	*Erinnyis*	*Erinnyis ello*	1.210	5.480	0.221	23.7	0.06651	Bartholomew & Casey, 1978
218	Hymenoptera	Apidae	*Euglossa*	*Euglossa dissimula*	0.104	0.45	0.23	220	0.17692	Casey et al., 1985
219	Hemiptera	Tessaratomidae	*Tesseratoma*	*Tesseratoma javanica*	0.926	3.88	0.239	66	0.03220	Ahmad, 1984
220	Hymenoptera	Apidae	*Eulaema*	*Eulaema nigrita* (2)	0.399	1.67	0.24	149	0.17692	Casey et al., 1985
221	Hymenoptera	Vespidae	*Polistes*	*Polistes gallicus*	0.115	0.460	0.250	220	0.17692	Magnan, 1934
222	Hymenoptera	Apidae	*Bombus*	*Bombus muscorum*	0.226	0.900	0.251	128	0.17692	Magnan, 1934
223	Lepidoptera	Sphingidae	*Manduca*	*Manduca rustica*	2.704	10.720	0.252	29.5	0.06651	Bartholomew & Casey, 1978
224	Lepidoptera	Sphingidae	*Perigonia*	*Perigonia lusca* (2)	0.638	2.470	0.258	62.9	0.06651	Bartholomew & Casey, 1978
225	Hymenoptera	Apidae	*Eulaema*	*Eulaema meriana* (7)	0.940	3.46	0.270	98	0.17692	Casey et al., 1985
226	Hymenoptera	Apidae	*Eulaema*	*Eulaema cingulata* (6)	0.547	2.03	0.27	128	0.17692	Casey et al., 1985
227	Lepidoptera	Sphingidae	*Oryba*	*Oryba achemenides* (2)	2.809	10.200	0.275	39.9	0.06651	Bartholomew & Casey, 1978
228	Hymenoptera	Apidae	*Bombus*	*Bombus lapidarius* (4)	0.446	1.472	0.29375	148.387	0.17692	Magnan, 1934; Sotavalta, 1952; Tercel et al., 2018
229	Coleoptera	Lucanidae	*Lucanus*	*Lucanus cervus*	2.600	8.000	0.325	33	0.13409	Magnan, 1934
230	Hymenoptera	Apidae	*Eufriesia*	*Eufriesia pulchra* (4)	0.425	1.27	0.33	170	0.17692	Casey et al., 1985
231	Hymenoptera	Xylocopidae	*Xylocopa*	*Xylocopa violacea*	0.614	1.720	0.357	130	0.17692	Magnan, 1934
232	Lepidoptera	Sphingidae	*Madoryx*	*Madoryx oeclus* (2)	1.699	4.715	0.360	41.8	0.06651	Bartholomew & Casey, 1978
233	Coleoptera	Cetoniidae	*Cetonia*	*Cetonia aurata*	0.537	1.300	0.413	86	0.13409	Magnan, 1934
234	Hymenoptera	Apidae	*Bombus*	-	1.600	3.50	0.46	125	0.17692	Ahmad, 1984

[†] Vogel (1966) actually reported the wing area to be 0.0576 cm^2, rather than 0.058 cm^2, as reported. This produces a corresponding wing loading of 0.035, rather than the otherwise calculated 0.034 g/cm2. I am not sure why the value was rounded up; four decimal digits were presented in several other instances. I also found that the mass and wing area data from Weis-Fogh (1972) for this same species (*Drosophila virilis*) is a copy of the data from Vogel (1966), with an error in the calculation of the wing loading (which should have come out to 0.034 according to their input values, rather than 0.04, as reported). The wingbeat frequency was also (correctly) modified, but that attribute was only of secondary interest to me, so I discarded that entry.

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Coda

Overview

The primary duty of the eyes of adult male Strepsiptera is to support nuptial flight. Flight is the only reliable way Strepsiptera are able to outcross. Parthenogenesis has not been unequivocally demonstrated in the order, but contrary evidence has been produced repeatedly (Hughes-Schrader 1924; Ulrich 1933; Kirkpatrick 1937). Additionally, in species with tiny hosts and those of the family Myrmecolacidae, in which males parasitize ants and females parasitize insects of another order altogether, flight is ostensibly the only way in which they are able to mate at all, as siblings could not inhabit the same host. Mature extant Strepsiptera do not feed, adult males have specializations for remaining attached to hosts and grasping females, rather than for walking, and eclosed adult males fly nearly continuously, generally pausing only to mate, until they are literally unable to remain (Hubbard 1892) or to become airborne[1]. Therefore, adult males primarily see so that they can fly. But when flying to a calling female, there are only a few things they need to use their eyes for: knowing when they are headed in a consistent direction, monitoring their approximate groundspeed, and avoiding collisions—including those with predators. None of these requirements rely on high acuity or high resolution. Strepsipteran eye morphology and ostensibly its attendant functionality, date back to the earliest evolution of the order (Pohl & Beutel 2008) and are closely tied to nocturnality (Buschbeck et al. 2003; Pohl & Beutel 2008). Given this, and the generally cryptic nature of calling conspecifics, it is highly unlikely that ancestral males ever identified females visually. Therefore, Strepsiptera evolved a mechanism of mate identification that did not depend on vision. I have no found compelling evidence that another mechanism has evolved to supersede the preexisting chemotactic one.

Hrabar et al. (2014) observed that the cephalothorax of female *Xenos peckii* is curled away from the

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[1] Although males that have landed may be waylaid indefinitely by a nearby pheromone source they are unable to locate.

surface of her host's abdomen when she releases sex pheromone. Although that increases her visibility, male *X. peckii* recognize females chemotactically (Hrabar et al. 2014), despite the fact that they are diurnal members of the most derived strepsipteran clade (Pohl & Beutel 2008). Moreover, they pay absolutely no attention to females that are not calling (Hughes-Schrader 1924)—as if they are literally unseen. Therefore, such posturing (in those species in which it occurs) apparently functions to provide a better surface for grasping, and/or to improve positioning during pheromone release, with the female counterpart of the male "balloon gut" (Richter et al. 2017) potentially being used to force fluid through the sex pheromone-producing gland[2]. Furthermore, in several Strepsiptera, such as the crepuscular species *Elenchus koebelei* (family Elenchidae), adult females may be obscured by either wing of their planthopper host, such that only a bulge may be seen at best. Even more striking, in the primarily nocturnal family Corioxenidae, the female is completely covered by her host's hemelytra, and is therefore never observed at all. Thus, vision often *cannot* be required for identifying a calling female in Corioxenidae, whether the species is nocturnal or not. This situation suggests that many strepsipteran groups lack the heightened visual ability apparently necessary to notice the cryptic (otherwise, other insects ought to detect them) calling females. Instead, it is identifying a potentially infected host that invariably relies on vision (Muir 1906; Kirkpatrick 1937)[3]. The exclusivity of this determination is apparently normally lax, thereby accommodating generalized host switching, and also requiring less robust vision. However, in special cases host identification *could* rely on super-ommatidial resolution and enhanced acuity.

Judging from the ocular prowess (Land 1969) and precise range-finding of jumping spiders (Nagata et al. 2012), one such case is when elenchids spring onto planthoppers infected with calling females (Muir 1906). In field observations made in Fiji, this preceded every incidence of *Elenchus tenuicornis*

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[2] Referred to as "Nassonow's glands" (Pohl and Beutel 2008), but not to be confused with the "Nasonov's gland" of honeybees, although both were first described by the same Russian (thus, Cyrillic alphabet) researcher.

[3] Interestingly, Kirkpatrick (1937) determined this, but failed to recognize that Strepsiptera employ pheromones to localize on receptive females, despite the enormous sensory-endowed antennae of adult males. Furthermore, Strepsiptera could scarcely invade large populations (the ones in which they are most commonly found) if they had to potentially check all adult coffee bugs (plus any accidental repeats) for an infected host.

mating (Muir 1906). Accordingly, *E. tenuicornis* also appear to routinely employ ‘odor-induced visual salience’ (Baker et al. 2018; Saxena et al. 2018) when in the vicinity of hosts potentially infected with calling females (Muir 1906). In fact, if enhanced adult male visual acumen exists at all, it is likely this element of odor plume resolving that necessitated and may have subsequently sustained it—even when the host is as enormous and easily identified as a typical bee or wasp (given their contrasting warning coloration, which in the case of colorblind or sufficiently color deficient animals presents as warning intensity variation). Alternatively, when females call from crowded flowers, isolating a single candidate Hymenoptera could also warrant sharper vision.

See to fly; fly to mate

In short, adult male Strepsiptera see so that they can fly, and fly so that they can mate. Therefore, any special eye adaptations should either assist them in flight (particularly anemotaxis), in identifying a potential pheromone source—whether while hovering or after having landed—or in securing a suitably infected host just subsequent to flight.

What would it take?

Having identified uses of the visual system most likely to depend on super-ommatidial resolution, it is fitting to consider how Strepsiptera might manage to supply the hypothesized ocular abilities. First, as pointed out in the Introduction, due to their diminutive size (Meyer-Rochow & Gál 2004), Strepsiptera are unable to employ optical superposition. They also could not have harnessed neural superposition ancestrally because it would not have provided enough sensitivity for nocturnality (Nilsson & Ro 1994); furthermore, open rhabdoms did not evolve in Strepsiptera or its progenitors. Thus, in Strepsiptera the ommatidia were dramatically enlarged in size but decreased in number; the number of photoreceptors within each ommatidium was also greatly increased, and perhaps the number of rhabdoms as well.[4]

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[4] The shape or even existence of multiple rhabdoms within each ommatidium has not been established, however.

These adaptations address the issue of light sensitivity at the cost of resolution and acuity. For most of a male's pheromone following journey, neither of those attributes is vital,[5] but very near to a pheromone source, where odor plumes may narrow to excessively fine filaments (Baker et al. 2018), use of vision is accentuated (Baker et al. 2018), and higher acuity or resolution may be required. Therein, exploiting the sometimes mentioned (Wachmann 1972; Pix et al. 2000; Buschbeck et al. 2003), but rarely described tiering of the strepsipteran retina (Wachmann 1972) could provide a way to gather visual information at higher magnification, resolution, and acuity, all without requiring the ability to adjust focus.

Thus, in its principal mode, an ommatidium would act as a massive light collector, contributing a single point to a very sensitive but low-resolution image. Light collected by a secondary tier would be further from the lens, and could therefore function like an extension tube attached to an interchangeable lens camera: only a narrow range of distance nearer to the eye would be in focus for this tier of photoreceptors, but at higher magnification and acuity within that range. Furthermore, if the images from several such "eyelets" were united (not necessarily, but most likely altogether to form a single unified image per eye), the resultant gain in resolution could be substantial. In this way, higher fidelity images could be produced at the cost of processing time and energy. When not acting under the ostensible influence of odor-mediated visual salience (Baker et al. 2018), the additional information would not be decoded and would simply be discarded as unnecessary.

A formidable challenge to the idea that Strepsiptera ever see with high acuity is that their ommatidial retinas so far investigated (all of which belong to diurnal species) are disorganized. Insect eyes for which vision is paramount are perhaps universally well-organized (e.g., (Beersma et al. 1977; Seidl & Kaiser 1981) vs (Chaĭka & Mazokhin-Porshnyakov 1988)), even when the number of ommatidia is lackluster, as in worker ants (62–96) (see ommatidia count data (Hunt et al. 2018)[6]). Furthermore, the tier-

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[5] Interestingly, the smaller the insect, the less well it can afford to see itself, due to how much more difficult it is for it to be seen.

[6] This is not an endorsement of Hunt et al. (2018), which I believe has serious problems with regard to the interpre-

ing so far documented is apparently unordered (Pix et al. 2000) [*Xenos vesparum*], without consistent photoreceptor length, orientation, number of tiers, or other uniformity (Wachmann 1972)[*Stylops* sp.], which does not accord with dual visual modes. However, in the case of *Stylops* and diurnal *Xenos*, the haphazard photoreceptor arrangement (Wachmann 1972; Buschbeck et al. 2003) may increase light sensitivity by randomizing photoreceptor orientation to compensate for their being too tiny to be twisted enough to cancel their inherent polarization sensitivity. Eleven micrometers (Buschbeck et al. 1999) is incredibly short for rhabdomeric light capture; so short it is plausible that even diurnal strepsipteran retinas are primarily tiered to enhance light capture by increasing effective path length.

Another challenge is that Pix et al. (2000) found no evidence of super-ommatidial resolution in their optomotor response experiments. That, however, could have been from there having been no odor-induced visual salience due to the absence of a sex pheromone plume from a calling female, or due to the distance to the projection screen having been too great, in the event of extension tube-style magnification. Finally, as Pix et al. (2000) found, in diurnal environments more ommatidia should be expected, rather than larger ommatidia containing additional photoreceptors, not only to provide better predator evasion via fast access to increased spatial resolution (Pix et al. 2000),[7] but also because most hosts attacked by diurnal Strepsiptera are large, and therefore require no additional (if any) inter-ommatidial acuity to be easily perceived. Thus, the greater number of ommatidia in these species may also allow hosts to be discerned without resort to super-ommatidial resolution, even if it is available.

Adult male Strepsiptera express two ocular opsins (James et al. 2016), but appear to be colorblind. Although the entire eye has not been scanned, none of their retinal axons have been found to proceed directly through the lamina to the medulla (Buschbeck et al. 2003), where both color vision and polarization sensitivity are processed. Thus, the UV opsin is likely to only help them distinguish ground from sky.

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tation of its results. However, the data themselves appear to be sound.

[7] Due to a lack of processing time, super-ommatidial resolution would not normally be useful for predator recognition anyway.

In fact, due to its usefulness, I posit that *all* volant insects are able to differentiate UV from long wavelength light, whether or not they can actually perceive color. Furthermore, in the case of *X. peckii*, the UV absorbance curve is quantitatively similar to the beta peak of its broadband green counterpart (James et al. 2016). This would make the pair poor for discerning colors, which is achieved by contrasting the amount of light simultaneously absorbed by different opsins in the same area of the eye.

Super-resolution is unlikely

Where does this leave us? In general, the most advanced insect eyes are those of taxa that returned to a diurnal lifestyle after a hiatus of nocturnality. This is true of dipteran neural superposition eyes (Zeil 1979; Hardie 1986), various diurnal superposition eyes, such as those of hawk moths (Warrant et al. 1999) and diurnal owlflies (Belušič et al. 2010), and the afocal apposition optics of butterfly eyes (Nilsson et al. 1984). As for diurnal Strepsiptera, however, this is apparently not the case. It does not seem that *Xenos peckii* or *X. vesparum* see very well, neither temporally—*X. peckii* (diurnal) was found to have an unbelievably low critical flicker fusion frequency of just 35 Hz (Buschbeck et al. 2003), nor spectrally (James et al. 2016), nor with respect to polarization, and perhaps not spatially either (Pix et al. 2000). Xenidae and Stylopidae, the most species-rich strepsipteran families, do not exhibit any of the hallmarks associated with superior insect vision: Lens placement is extensively variable, and lens count may—or may not—be wildly different between the left and right eyes of the same individual (Pix et al. 2000). Although ocular ultrastructure has only been at all investigated in diurnal species, the retinas of *Xenos* and *Stylops* are such disorganized jumbles (Wachmann 1972) that no one even knows what constitutes a strepsipteran rhabdom (Strohm 1910; Buschbeck et al. 2003). Such things simply do not occur in well-sighted flies (Beersma et al. 1977; Sukontason et al. 2008), bees (Seidl & Kaiser 1981), moths (Warrant et al. 1999), or even flightless nocturnal *Carabus* beetles (Talarico et al. 2007). Even in mating, the single aspect wherein super-ommatidial resolution might be sure to shine, *X. peckii* resorted to chemotactic recognition instead (Hrabar et al. 2014). It really should not be so difficult to ascertain a fundamental shift in visual functionality—if it is useful, then it should be used! Although it is odd for a diurnal insect that does not see well to have such enormous eyes, that may reflect photoreceptor overstocking, due to a lack of repair and replenishment during the adult pharate and eclosed stages. Additionally, in some species, the wide separation of the eyes could potentially assist in depth perception. In others, there simply may have been insufficient selective pressure to completely override the ancestral state.

Even so, with the current state of knowledge, it is not possible to definitively establish the extent of strepsipteran visual ability. To begin with, and as usual, it is not uniform across the order—that much is clearly demonstrated by differences in ommatidia count and size among species. Thus, whether or not the entirety of strepsipteran vision ever encompasses super-ommatidial resolution is unresolved. Considering that *Elenchus koebelei* only have 19 ommatidia per eye, it is hard to say how they manage to apprehend unruly planthoppers without it (but see *The last simple hope* below). Although I have never seen *Elenchus* mate, which might suggest alternatives, Muir's (1906) description leaves little room for doubt: not only were infected hosts seized, but the extension of a tiny thin planthopper antenna beyond the edge of a leaf appeared to be sufficient to alert eclosed males to the presence of infected hosts on its opposite side. Therefore, elenchids are good candidate species for further investigation. Interestingly, I have found that it is the smaller (≤ 2.5 mm) nocturnal and especially crepuscular species whose eyes are the most symmetric morphologically. All of the *E. koebelei* (crepuscular) whose ommatidia I counted, contained the same number (19) per eye, and the count of investigated *Triozocera texana* (nocturnal) ommatidia was 16, 18, or 20 (ostensibly depending on specimen size), with the same number in the left and right eyes (J. Cook, personal communication; October 5, 2020) (results obtained from random samples I provided). This regularity can also be seen in photographs of various specimens (Pohl & Beutel 2005), wherein Elenchidae and Myrmecolacidae (both crepuscular) show the most external order, followed by Corioxenidae (nocturnal), Halictophagidae (mixed), and even *Paraxenos* (diurnal)—a genus of the family Xenidae, which all display nearly or fully symmetrical external ocular morphology.

Test frameworks

A relatively easy potential way to obtain base level support for super-ommatidial resolution is to check ommatidia located in what would be the dorsal rim area (DRA). The smallest DRA I am aware of is that of the micro-wasp, *Megaphragma viggianii*. The *M. viggianii* eye contains 29 ommatidia, of which 10 are dedicated to the putatively functional DRA (Makarova et al. 2025). Therefore, one can take 10 ommatidia—or to be generous, 7 (since there were 3 conservatively identified "transitional" DRA ommatidia (Makarova et al. 2025))—to be the smallest number of ommatidia required to track skylight polarization. For comparison, the *Drosophila melanogaster* DRA contains about 40 ommatidia (Makarova et al. 2025) (of a total of 750–800 ommatidia), the *Sympetrum striolatum* (dragonfly) DRA has about 50 (Meyer & Labhart 1993) of at least 15,000 ommatidia, the *Apis mellifera* (honey bee) DRA has about 40 (Meyer & Labhart 1981) of 5375 (Streinzer et al. 2013), the *Melolontha melolontha* (cockchafer beetle) DRA has about 115 of 5500 ommatidia (Labhart et al. 1992), and the *Schistocerca gregaria* (desert locust) DRA contains about 400 ommatidia (Homberg & Paech 2002) of some 9400 total ommatidia (Shaw 1978). Because polarization is mediated by photoreceptor axons that pass directly through the lamina to the medulla (as is color perception) (Hardie 1984), one can histologically observe if the axons of any ostensible strepsipteran DRA ommatidia do so. If there are no occurrences, the ability to process skylight polarization can be dismissed, and there is also no support for super-ommatidial resolution. If there are from 1–6 occurrences, then there is support for super-ommatidial resolution and for perceiving skylight polarization (i.e., there are too few ommatidia with pass-through axons to support skylight polarization without super-ommatidial resolution, but the position of the ommatidia and their status of containing pass-through axons is consistent with polarization sensitivity). If there are at least 7–10 occurrences, then there is support of polarization sensitivity, but not for super-ommatidial resolution (there are enough ommatidia to support polarization sensitivity without super-ommatidial resolution).

I agree with Pix et al. (2000) that it would be very beneficial to know "how the tiered retinas are organised in detail," specifically, those of crepuscular and nocturnal species. To that end, I have fixed and embedded strepsipteran eyes I may yet find the means to process. Finally, unlike the vast majority of adult female Strepsiptera, which are eyeless, those of the most basal extant family, Mengenillidae (all of which are nocturnal), have eyes that resemble degenerate versions of those of adult males. That is, they are smaller and have many fewer ommatidia that are not separated by microtrichia. These females are not physically bound to their hosts and are thus capable of exiting their pupal cases and walking feebly. Their eyes are almost certain to only boost sensitivity, without forming high resolution images, since they would be unable to act on the additional information. The situation should be clear from histological as well as ultrastructural analysis. Insights gained could then be applied to the eyes of adult male Strepsiptera. Additionally, actually contrasting the ultrastructure (and other optical characters of) diurnal, crepuscular, and nocturnal adult male Strepsiptera would be illuminating, whether or not super-ommatidial resolution or high acuity is implicated. However Strepsiptera are achieving mating success is of interest.

The last simple hope

In my estimation, the last realistic hope for strepsipteran ommatidia acting as simple eyes rests with the tinier species, most notably, *Elenchus* spp., which have to 'jump' onto planthopper dorsa to mate (Muir 1906). There is little leeway around the proposition that jumping onto a motile target relies on precise vision. But another interesting matter concerning these animals is the wide expanse between their eyes. "*Stylops*" itself—the genus name, as well as the word "stylopized," used to describe specimens (visibly) infected with Strepsiptera—stem from the Greek words for pillar and eye, indicating that the eyes extend outward on stalks (Pierce 1909). From these considerations, it has only just occurred to me that strepsipteran eyes may protrude so widely to assist with depth perception, so as to provide the means to more accurately determine the distance to nearby hosts bearing calling females. Strepsiptera (*Stylops pacifica*) are thought to be nearsighted (MacCarthy 1991), which corresponds with Muir's (1906) observation of *E. tenuicornis* crawling to within half an inch (1.3 cm) of stylopized planthoppers before springing onto them to mate with embedded calling females. This ability may have been useful for better subduing Lepismatidae (silverfish and firebrats), probable hosts of early-branching Strepsiptera (Pohl & Beutel 2008). Could this be the capacity in which super-ommatidial resolution finally shows its face? Or perhaps a clever application of the ommatidia altogether eliminates any need for it…

To the latter point, although mantids (which include praying mantises) are the only insects known to exploit stereopsis, adult male Strepsiptera may achieve it in a manner similar to theirs. Unlike in typical stereopsis—described as spatial or static disparity—in which positional differences arising from the distinct vantage points of the eyes are assessed when binocularly overlapping objects are effectively stationary, mantid stereopsis operates on the basis of actively *changing* luminance within an eye, so-called kinetic disparity (Lee 1970). In both approaches, overlapping areas are mapped between the eyes, enabling distance calculations based on the known separation of the eyes and the projected matching points in space that comprise commonly viewed objects (Nityananda et al. 2018). The beauty of the mantid approach is that only areas expressing change are analyzed, greatly reducing complexity. Moreover, in animals with immobile eyes (whose relative positions therefore do not need to be determined), the speed and accuracy of these calculations are further improved (Collett 1996; Nityananda & Read 2017). Although there is no evidence that mantid stereopsis provides any information on stationary objects, it easily breaks camouflage (Nityananda et al. 2018), which could help it to support monochromatic vision. In fact, several species of mantid, including *Tenodera sinensis* (Sontag 1971), *T. australasiae* (Rossel 1979), and *Sphodromantis* sp. (Towner & Gärtner 1994), have been found to express a single opsin in their compound eyes, and are therefore colorblind.[8] It has even been suggested that mantid stereopsis *requires* monochromaticity (Nityananda & Read 2017). This is particularly interesting considering that adult male Strepsiptera are most likely colorblind, as well. Finally, Strepsiptera may be able to avoid having high spatial resolution while still pinpointing targets by only jumping when the contrast between ommatidia is greatest—when pursuing a calling female on foot, the host planthopper should maximally fill a single ommatidium of each eye—and then jumping the medial distance to the target.

Interestingly, poor resolution could even be turned on its head by being used to ensure a greater distance is placed between an approaching object and the strepsipteran observer. That is, overestimate the required clearance, and quickly move further away—something Strepsiptera should be able to do in part because they are so tiny, and thus have faster reaction times—all while saving energy from using slower photoreceptors. Even the inferior flicker fusion rate may be tolerable if nearsightedness tends to prevent evasive action from being taken until the last moment (but not beyond), wherein strepsipteran halteres and power loading could cause fast approaching predators to overshoot. Also, the hairs covering the bodies of adult male Strepsiptera likely respond to coordinated changes in airflow, which could help isolate approaching objects.

Tips for moving forward

Despite many odd independently evolved unifying characteristics (synapomorphies), it is important to remember that Strepsiptera is an entire order. Sometimes what is true of a particular taxon certainly will not apply to another because of that. Sometimes what is true of a strepsipteran taxon will certainly apply to all the others for the same reason. Therefore, choose study questions and organisms wisely. Another common issue is obtaining access to a reliable source of specimens. In this, try not to concern

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[8] Although only expressing a single opsin in their compound eyes, mantid ocelli express both a (separate) green and a UV opsin. This supports my expectation that all volant insects have a long wavelength and a UV band opsin. However, when present, ocelli are used for fast bodily orientation and do not normally form focused images at all.

yourself overmuch with species infecting paper wasps, unless you intend to raise your own stock; otherwise their locations and local populations are unstable from year to year. Strepsiptera infecting andrenid bees should not present this problem (if you must go diurnal), neither should other species whose nesting sites are fixed (including those of many solitary wasps), nor should species whose hosts reside on or within a stationary food source. Often, even the choice of which species to pursue has more to do with our vision than theirs; sometimes both literally and figuratively.

My goal in studying Strepsiptera was to understand their eyes. Perhaps I have actually achieved that; but, if so, I do not “know” that I have, so in that sense at least, I have failed. However, I have deduced deeply and I have hypothesized, if not correctly, then hopefully, well. I leave these approaches and testable ideas. Crush them empirically, if possible; provide evidence for them, if not.

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Glossary

•**accommodation** (eye): the ability to focus (literally, to become adjusted)

•**acuity**: acuteness of visual perception; sharpness. Acuity differs from resolution in representing high contrast between visual elements, rather than presenting a large number of visual elements

•**alloparenting/alloparental care** (ch3): any form of parental care provided to an immature individual that is not one's own offspring. Alloparenting may occur within or between species

•**airspeed**: the speed of a flying animal or aircraft relative to the surrounding air and therefore absent any wind assistance and thus, the true speed of the flying object (contrast with "groundspeed")

•**anemotaxis** (ch3): movement of an unbound organism in response to wind

•**apposition eye**/**apposition optics**: a form of compound eye in which the ommatidia abut one another, and the light collected by a facet only furnishes photons to its own underlying photoreceptors. Apposition optics is most common in diurnal animals where light availability is high

•**basement membrane**: a thin acellular layer providing a barrier between the eye and the hemocoel; it provides tissue support, acts as a barrier that prevents the entry of foreign substances, and blocks the diffusion of molecules

•**Beaufort scale** (ch2): a standardized descriptive scale that relates measured wind speeds to observed conditions; observations are then used to estimate wind speed in the absence of quantitative measuring devices

•**Bibionidae** (gl intro): family of nematoceran flies that evolved their own form of (complete) neural superposition, independent of that found in Brachycera. Bibionidae are also known as "March flies"

•**Brachycera**: a major (monophyletic) suborder of more recently derived Diptera containing some 120 families. Their most easily identified feature is reduced antennae (segmentation and size), but their ecology and behavior also differ. Brachycera also have a revised and consistent form of neural superposition. Contrast with Nematocera

•**calling** (ch 3): in this context, releasing sex pheromone (to attract male conspecifics for the purpose of mating); more generally any long-range signal (chemical, auditory, or vibratory) released to attract the opposite sex by expressing readiness and willingness to mate

•**compound eye**: one of two principal eye types, compound eyes consist of an array of self-similar independently-acting optical units called ommatidia (singular "ommatidium") that each contain their own lens—secreted by the ommatidium—and light-sensitive material (rhabdom/retinula) that provides a single point (or pixel) to a finalized optical image. Contrast with simple eye

•**Corioxenidae**: relatively early-branching (thus retaining several primitive traits), predominately nocturnal family of Strepsiptera. All known members infect true bugs, in which female Strepsiptera are entirely hidden from view by the hosts' hemelytra, the protective sheath the partially reinforced forewings form over the pair of hindwings and the abdomen when the wings are at rest (and thus folded). Corioxenidae includes the genus *Triozocera*, which is of great interest because it is one of the few nocturnal strepsipteran species known from the United States

•**Delphacidae** (**delphacid**) (ch2): a cosmopolitan family of planthoppers whose members are less than 4 mm in length, and have a large flattened movable spur (the calcar) on the apex of both hind tibiae; this spur is associated with increased jumping performance. Delphacids are hosts of several strepsipteran species

- **diffraction** (gl. intro): the spreading of light (or other wave systems) as it passes across an edge or through an aperture (an aperture is a hole through which light can pass). Focusing light always involves apertures and light itself is always a wave, so diffraction imposes a (wavelength-dependent) limit on the maximum resolution a system can achieve

- **dorsal vessel** (ch3): a tube that runs longitudinally along the inside of the dorsal body wall; it collects hemolymph in the abdomen and conducts it forward to the head. It is the major structural component of the insect circulatory system

- **DRA** (coda): Dorsal Rim Area, a special area found at the upper forward edge of many compound eyes, in which the natural polarization sensitivity of rhabdomeric photoreceptors is not canceled (cancellation usu. occurs by twisting a photoreceptor or fused rhabdom through ≈180°) as occurs in other areas of the eye, and furthermore, many polarization-enhancing modifications are found. Insects use polarization sensitivity to navigate

- **dynamic range** (ch3): the difference between the highest and lowest values a system can detect or represent

- **Elenchidae**: family of planthopper-infecting Strepsiptera; I am not aware of any nocturnal elenchids

- ***Elenchus koebelei***: a crepuscular species of Strepsiptera that infects *Prokelisia marginata* and *Prokelisia dolus* planthoppers (probably among others) in northern Florida and other coastal locations in North America

- ***Elenchus tenuicornis***: a European species of planthopper-infecting Strepsiptera; it is larger than *E. koebelei*, but similar to it because they both belong to the same genus

- **extension tube** (coda): a hollow tubular camera attachment inserted between a camera body and a lens, thereby moving the lens further from the focal plane and reducing the minimum focusing distance. Use of an extension tube increases magnification, reduces light availability, and reduces depth of field. Thus, objects are magnified, but a smaller range of distance is in focus at once

- **false colors** (gl. intro): spurious colors arising from a non-DRA rhabdom's natural polarization sensitivity not having been canceled, in which case a photoreceptor is sometimes able to absorb a photon because of its polarization angle, rather than its wavelength ("color"). However, because color-responsive photoreceptors only act as if color causes photon absorption (a photoreceptor does not know whether a photon's wavelength or its polarization caused it to be absorbed), then any photons absorbed because the polarization 'channel' was not sufficiently neutralized, are mistakenly interpreted as color stimuli, distorting the true perception of color. [Note that insofar as polarization is sensed in non-DRA ommatidia that can detect more than a single wavelength range/color, 'false polarizations' also arise in an analogous manner]

- **fixing/fixed** (ch3): halting biological processes and stabilizing tissue to preserve fine structure (known as ultrastructure). Fixation traditionally refers to chemical fixing; however cryofixation (abrupt freezing) is also possible

- **FMR**: Flight Muscle Ratio—the proportion of body mass a flying animal dedicates to flight muscle

- **fused rhabdom**: see rhabdom

- **groundspeed** (ch3): the horizontal component of the velocity of a flying animal or aircraft relative to the surface of the Earth. Groundspeed is also influenced by wind (contrast with "airspeed")

- **hawk moth/hawkmoth** (ch3): a member of the family Sphingidae, hawk moths are large, swiftly-flying moths, with stout bodies and thin, fairly long wings. Hawk moths superficially resemble hummingbirds (esp. the hummingbird hawk moth) and hover before nectar-bearing flowers in a similar manner. Hawk moths are typically nocturnal, but there are a few diurnal species, and some that begin their activities in the evening (i.e., they are vesperal), but before nightfall

•**hemelytra**: forewings that have been modified so that they are hardened—and thus darkened—toward and at the leading edge (the front of the wing) and membranous at the trailing end. This provides protection for the hindwings, while allowing for greater flight versatility than if the entire wing had been hardened

•**hemocytes** (ch3): blood cells, particularly those of invertebrates

•***Hylecthrus rubi*** (ch3): a species of Strepsiptera that infects solitary bees of the genus *Hylaeus* ("yellow-faced bees")

•**lamina**: the first optic neuropil of the insect eye, the lamina ganglionaris is instrumental for contrast enhancement

•**mantid**: a member of the insect order Mantodea

•**mantis**: a genus of mantid (thus, more properly, *Mantis*; technically, the only species that should be referred to as a praying mantis is *Mantis religiosa*)

•**medulla**: the second of three optic lobes (lamina, medulla, and lobula) in insects (and many other arthropods). Color is processed in the medulla (as opposed to simple wavelength sensitivity/recognition), as is localized motion (i.e., motion spanning only a few to several ommatidia). When processed at all, polarization is also processed in the medulla

•**mFMR** (ch3): the minimal proportion of flight muscle required for a standing takeoff (i.e., no jump or other assistance; takeoffs only powered by the wings); mFMR stands for "marginal flight muscle ratio," and refers to the animal's takeoff ability being marginal

•**Nassonow's glands** (coda): the sex-pheromone producing glands of adult female Strepsiptera

•**Nasonov gland/Nasonov's gland** (coda): a scent gland found on the dorsal side of the abdomen of worker honey bees that is used to orient workers to the nest, esp. after a some form of nest disturbance. It is also used to promote swarm cohesion and to recruit workers to water or to nectar with unusually high sugar content

•**Nematocera**: a paraphyletic suborder of Diptera, containing all true flies (i.e., Diptera) that are not in the (monophyletic) suborder Brachycera. Nematocerans have segmented antennae, and usually have elongated bodies and aquatic larvae.

•**neural pooling** (gl. intro): the pooling or combination of signals from the axons of photoreceptors. Neural pooling increases the intensity of pooled signals at the cost of spatial resolution

•**neural superposition**: physical redirection of the axons of the peripheral rhabdomeres, so that they are directed to the adjacent (Brachycera) or super-adjacent (Bibionidae) ommatidium for which they are directly on-axis. In order for a lens to focus light, either its shape or its optical power (gradient) has to change with position along the lens. This introduces a rim that is of insufficient optical power to focus the light centrally. Therefore, the light that falls onto or near it is more or less off-axis. Placing peripheral axons along this rim allows neural signals arising from slightly off-axis light that would otherwise not have been collected (and thus wasted), to be redirected to the central ommatidium for which the light would have been on-axis, had it struck the eye at a slightly different location. Neural superposition results in an intensity gain of 6×, with no loss of acuity. Furthermore, the peripheral rhabdomeres are completely furnished by off-axis light, enhancing acuity and color detection

  – incomplete neural superposition: neural superposition that is mixed with neural pooling

- **ocelli**: single-lens eye found in flying insects and apparently used to help stabilize flight, principally by quickly distinguishing sky (transmitted UV) from ground (low UV, high green content/signal); however, unlike simple eyes used for vision, nearly all ocelli are purposefully defocused so as to not form discernable images. Ocelli are also used to help regulate altitude

- **ommatidium**: one of the self-similar optical units that make up a compound eye. Ommatidia each consist of a lens, a rhabdom, and accessory cells, including the cells that secrete the lens, and almost always several pigment cells, which reduce the intensity of light and prevent light incident on one ommatidium from bleeding into adjacent cells

- **open circulatory system** (ch3): circulatory system in which "blood" is not restricted to vessels that directly conduct it throughout the body; blood instead flows freely through cavities, resulting in less efficient transferal of wastes and nutrients. However, note that insects (which have open circulator systems) breathe nearly directly to each cell of the body; therefore the hemolymph (insect "blood") does not carry oxygen

- **open rhabdom**: see rhabdom

- **parturient** (ch3): about to give birth

- **Pearson correlation coefficient** (ch2): a measure of the strength of the linear relationship between two statistical variables, ranging from −1 to 1. A measure of 1 indicates complete correspondence (when one goes up, the other goes up), a measure near 0 means no correlation, and a value of −1 means exact inverse (or opposite) correspondence (when one goes up, the other goes down). Measurements with values of 0.7 or higher are considered to have high correlation

- **photopsin** (ch1): informally, photopsins are visual pigments responsible for providing color vision. More formally, they are vertebrate visual pigments found in cone cells, and as such are used in bright light (photic) conditions; they differ from rhodopsins, which function in the rod cells of vertebrate retinas, and which are used for vision in low light (scotopic) conditions. Insects only have one kind of visual pigment, which can process color (and so is sometimes called a photopsin) but is also easily sensitive enough to also function at night. This is why several nocturnal insects have color vision

- **polarization**: a property of light and other transverse waves (i.e., waves in which the elements of the medium, usu. particles or photons, move perpendicular to the direction of energy propagation) that describes the geometrical (usu., but not always linear) orientation of the (transverse) oscillations. In terms of vision, the detection and deciphering of polarization shares many qualities of that of color discrimination, so much so that both are mediated by photoreceptors and translated in the same area of the insect brain (the medulla), generally by photoreceptor axons that pass directly through the lamina to the medulla without synapsing. Although objects could conceivably be (further) distinguished on the basis of polarization angles, rather than wavelength (i.e., color), this is not known to be done in nature. Instead, polarization, when used at all, is exploited for orientation or navigation (because of how light interacts with small atmospheric particles). Note that "unpolarized light" refers to photons en masse that together have no preferred orientation or orientations; however, every individual photon has a distinct (linear) polarization

- **power loading** (ch3): the ratio of a flying animal's weight to its flight muscle performance (since all flight muscles have very similar output per weight (Marden 1987), flight muscle performance is dominated by the *amount* of flight muscle, as well as any unsteady aerodynamic effects, such as clap-and-fling and vortex recapture)

- **Purkinje shift** (gl. intro): perceived shift in the color of visible objects toward blue, associated with the differences in peak sensitivity between the rods (low-light vision) and cones (bright-light vision) of human (and other vertebrate) retinas. Also called the Purkinje effect
- **resolution**: the maximum number of points that can be discerned, without regard to the contrast between them
- **reticular** (gl. intro): having a net-like structure. With respect to retinas, reticulation is problematic from the standpoint of acuity, because potentially separate visual cells are physically connected so that their signals are combined to a greater or lesser extent
- **retinotopic** (gl. intro): concordant spatial arrangement of the axons of photoreceptors in the retina and visual processing areas of the brain
- **retinula** (gl. abstract): (new Latin "little retina") either the combined photoreceptive portion of all (usu. 8) of the photoreceptors of a single ommatidium of a compound eye, or the entirety of all of the photoreceptive cells of a single ommatidium
- **rhabdom**: sum of the light receptive parts of the photoreceptors of a single ommatidium. A rhabdom usually consists of 8 rhabdomeres
    - **fused rhabdom** (gl. intro): a rhabdom in which all of the rhabdomeres are connected so that light can traverse from one rhabdomere to another. In the simplest form, no space exists between any of the rhabdomeres, so that they form a single filled-in pole; however, an annular rhabdom is also considered a fused rhabdom because a ring forms a single light guide
    - **open rhabdom**: a rhabdom in which one or more rhabdomeres or groups of rhabdomeres are separated from the others. In the most prevalent form, found in brachyceran flies, the peripheral rhabdomeres are all separate from one another and the two central rhabdomeres; however, the two central rhabdomeres (which are responsible for color perception) are tiered and so abut one another. An annular rhabdom with two central rhabdomeres that touch is also an open rhabdom because the ring and the central unit form separate wave guides
- **rhabdomere**: the photoreceptive part of a single photoreceptor cell of a rhabdom
- **simple eye**: an eye having a single lens that projects light onto numerous photoreceptive cells that form a (single) retina that produces an a singular united image. Due to the shared lens, the same input simultaneously acts upon the entire retina at once. Along with the compound eye, the simple eye is one of two principal eye types
- **Stylopidae**: a family of strictly bee-infecting Strepsiptera
- **Stylopidia** (ch3): a suborder of Strepsiptera, the Stylopidia include all clades in which the adult female cannot abandon her host, and instead becomes fused to it as an adult
- **stylopized**: said of an insect parasitized by a Strepsiptera. The term stylopized is often reserved for hosts that are visibly parasitized by a strepsipteran, which does not occur until the parasites mature. The term dates back to the discovery of the group, then called Stylopidae, and thought to be a family of Coleoptera (i.e., beetle)
- **superpose**: (1) to place on or above something else; (2) the combination of two or more things (such as waves) to form a new entity in accordance with the superposition principal (i.e., when two or more adherent physical qualities overlap, the resultant effect is the sum of the individual effects). Thus, superposed light is intensified
- **superposition eye**: a compound eye that uses superposition optics (also see "superpose")

- **superposition optics**: a form of compound eye optics found in insects and some other arthropods. In it, off-axis light from one ommatidia is optically redirected to other ommatidia for which it is absolutely or better on-axis, depending on the quality of the optics and the required sensitivity. Unlike neural superposition, in which the re-direction is done on *absorbed* light signals (i.e., photons), in superposition *optics* the redirection is done optically—either via refraction (the gradual/smooth bending of light) or reflection—rather than physically. This results in much higher sensitivity (typically 100–1000× more than otherwise similar apposition optics eyes (Meyer-Rochow & Gál 2004)). However, because light must be optically bent or reflected through a so-called clear-zone, a superposition eye must be of a certain minimum size. This size is much too large for them to be of use to Strepsiptera, so although superposition optics has evolved several times in insects (e.g., mayflies, Neuroptera (lacewings, mantispids, owlflies...), Trichoptera + Lepidoptera, beetles), it is not present in Strepsiptera, and apparently never has been

- **sympatric** (ch3): overlapping in geographic distribution (though perhaps not seasonally or temporally)

- **teneral**: a stage following ecdysis (molting; especially molting into the adult form), in which the body of an arthropod is pale and soft. During this stage, an insect's body is expanded to allow for internal growth before the exoskeleton hardens and prevents any further enlargement until the next molt—if available. (Note that all winged insects, except mayfly subadults/subimagoes have already reached their final molt; therefore they will never grow any larger.) It is also during this phase that many otherwise impregnable hosts can be and are colonized by 1st instar Strepsiptera

- **tiered**: said of ommatidia (the individual point-producing eyes of a compound eye) that have rhabdomeres that collect light exclusively or more effectively over a given range of the entire rhabdom length

- ***Triozocera texana***: a nocturnal species of Strepsiptera that infects the burrowing bug *Pangaeus bilineatus*. *T. texana* are the well-established nocturnal species of Strepsiptera in the United States, with the possible exception of some species present in sub-tropical Florida, and perhaps some parts of Texas. Additionally, *T. texana* infect a burrowing bug, complicating parasite propagation. However, the host *P. bilineatus* is a member of the family Cydnidae, which are known to exhibit maternal care, which could be exploited by 1st instar Strepsiptera to gain access to immature hosts, as long as the mother periodically leaves the 'nest' and returns from the surface with new nutrients (and perhaps strepsipteran stowaways)

- **twisted wing parasite** (ch3): common English name for Strepsiptera. Rather than referring to the hindwings, the name actually applies to the insect's halteres (Kirby 1813), before it was understood that they no longer function as wings at all

- **twilight** (ch2): the period between night and day when no sunlight reaches an observer directly, but sunlight redirected by the atmosphere still does (unlike at night). Morning twilight begins at dawn and ends at sunrise. (Evening twilight begins at sunset and ends at night. However, no *E. koebelei* were found to undertake nuptial flights at dusk.) Twilight is divided into three formalized stages—astronomical, nautical, and civil twilight; these are covered in Chapter 2

- **volant**: adjective describing an animal that has the ability to fly

- **vortex recapture** (ch3): when otherwise wasted energy in the form of eddies is absorbed by the wings and recycled, thus reducing overall energy expenditure and increasing flight efficiency (an analogous phenomenon occurs in water); vortex recapture usually relies on wings that are remarkably flexible

- **wing loading** (ch3): the ratio of the weight of a flying object to its wing area

•**Xenidae**: family of wasp-infecting Strepsiptera. Except for a species infecting *Apoica pallens*, all known “xenids” are diurnal, although ostensibly all are active after sunrise, several are matinal (occurring in or related to the morning) and could be considered (morning-only) crepuscular

•***Xenos peckii***: a species of Strepsiptera that infects paper wasps of the genus *Polistes*, *X. peckii* is the American counterpart of the European *X. vesparum*. *X. peckii* has about 10–15 fewer ommatidia per eye than *X. vesparum*

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