

Supplemental Information for:

Transient use of hemolymph for hydraulic wing expansion in cicadas

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Contributions

MKS and JJS designed the experiments. ASS, resident cicada expert, led collection of cicada nymphs. MKS, TEE, TW, and JL timed, dissected, and recorded wing data. MKS, TEE, JL, ASS analyzed the data and made figures. MKS, JJS, and MLM performed the statistical analyses. All co-authors contributed equally to the writing of the manuscript.

Data availability

Complete tables of cicada wing masses, span, chord, and area and associated summary statistics are freely available at the Socha Lab Github site. Link: <https://github.com/TheSochaLab/Transient-use-of-hemolymph-for-hydraulic-wing-expansion-in-cicadas>

Additional Discussion

Developing pressure in the body and wings

Wing expansion requires a suite of hormonal triggers and motor programs (Carlson 1977, Hughes 1980, I-IV) to ensure normal emergence from the exuviae (juvenile casing) of the head and all appendages, and adequate internal pressure into the wing (Truman 2005). Upon ecdysis, for hemimetabolous insects, the wing is pulled out of its wing pad (protective casing) and hemolymph is transported into the wing (Hughes 1980I, Elliott 1981). Vigorous ventilatory abdominal pumping and wing flexing suggests that inflation with hemolymph is a primary driver of wing expansion (Glaser and Vincent 1979), but air may also play a role. For winged insects, post-eclosion, the process of unfolding and expanding the wings is a critical bottleneck in achieving sexual maturity.

Much of our current understanding of the pressurization mechanisms behind wing expansion have been investigated in species within Orthoptera (locusts, grasshoppers, and crickets). Because expansion behavior is somewhat stereotyped, behavioral events for these orthopterans and other hemipterans tend to follow six main events laid out by Elliott (1981) and Hughes (1980, I), including: 1) Nymph ceases to feed, new adult cuticle is laid down; 2) Nymph selects site for emergence, slowly inflates gut; 3) Gut inflation occurs, old cuticle is split, head, thorax, legs emerge; 4) Metathoracic legs fold; 5) Wings expand; and 6) New cuticle strengthens, gut deflates.

Prior to phase 1, the cuticle begins to soften, likely triggered by bursicon, an important hormone in both controlling and initiating ecdysis (Elliott 1981, White and Ewer 2014). As the head emerges, followed by antennae and legs, old tracheal lining is pulled out of the thoracic and abdominal spiracles. Maximum pressures occur during the process of wriggling and emerging from the juvenile form (Elliott 1981), and waves of muscular contractions are necessary for splitting the cuticle of the exuviae. The insect also extracts itself from its exuvia using deep inspirations into the tracheal system, which occurs in 20-60 second bouts (Elliott 1981). The insect curves backwards, head first, gulping in air to inflate its gut. This causes internal pressure to be uniformly pushed outwards on the exoskeleton, stretching it as it undergoes changing stiffness. The highest pressures occur during phase 3, and steadily decline into phase 5 (Elliott 1981). This process lasts from 15–20 min, depending on the insect species. Hanging from the tip of the exuviae for several minutes (depending on insect size), the nymph flips forward and begins wing expansion. During phase 5 (wing expansion), compression bouts of the expansional motor program result in a pulling of the wings laterally (Hughes 1980, I-III), which appears as a strong flex of the flight muscles.

Developing the necessary pressures within the insect body to extract itself from its juvenile form, to inflate its entire exoskeleton, and to push hemolymph into the wings, requires a complex sequence of timed events. Eclosion can fail at any point (White *et al.*, 1979), resulting in the insect's head and/or appendages remaining within exuviae (White and Ewer 2014), causing wing expansion to completely cease. Additionally, if the wing hinges are damaged and leaking hemolymph, the wing fails to extend to its full length (personal observation, MKS). In the last

stages of wing expansion, specifically for Orthoptera, if multiple appendages have been lost, the insect may not be able to fold its newly expanded adult wings into their proper adult configuration.

Despite this knowledge, overall our understanding of wing expansion and the role of circulation across insect phylogeny is piecemeal. Circulation in insect wings was described by Arnold (1964) across 14 insect orders and 100 species, but without connection to wing structure and physiology (Salcedo and Socha 2020). Physiology of thoracic wing hearts was described across holometabolous and hemimetabolous insects, but quantitative measurement of hemolymph flows within the wing or relation to wing expansion is lacking (Krenn and Pass, 1994, 1995). Lastly, previous studies of ecdysis have measured muscular coordination and behavior, without connection to the changing wing pressures, structure, or mass changes. Overall, our data on cicadas broaden our understanding of wing expansion, quantifying a feature of wing expansion (mass change in the wings) that has not previously been understood.

Why cicadas? An ecological perspective

Investigating non-model systems like cicadas can lend insight into broader issues such as climate change. Periodical cicadas lay eggs in tree branches; these eggs hatch, and the nymphs drop to the ground, where they feed on root xylem for the next 13-17 years. With increased urbanization, habitat loss causes a population decrease. It is already clear that higher temperatures are causing brood extinction (White *et al.*, 1979, Cooley *et al.*, 2013), and with climate change, effects on insect development, such as early emergence, are already seen in periodical cicadas under warmer conditions (Heath 1968, Moriyama and Numata, 2019). At our experiment's locale, cicadas largely emerged from trees lining the street, where pavement warmth and daylight exposure cued cicadas to emerge 2-3 weeks before those in forested areas. For periodical cicadas, synchronicity is a leading survival mechanism known as 'predator satiation' (Koenig and Liebhold, 2013), whereby the emergence of individuals at different intervals of time could weaken their advantage in numbers.

SPAN	Nparm	DF	DF Den	F Ratio	Prob > F	Note
Sex	1	1	138.7	0.9488	0.3317	
Time (min)	24	24	140.2	102.5122	<.0001	
Whole mass (g)	1	1	148.3	0.4828	0.4882	
Wing mass (g)	1	1	571.2	221.8368	<.0001	
Wing side (L/R)	1	1	445	0.0421	0.8374	
Wing type (Fore/hind)	1	1	559.6	750.2265	<.0001	
Time (min)*Wing type (Fore/hind)	24	24	448	63.2596	<.0001	Interaction
CHORD	Nparm	DF	DF Den	F Ratio	Prob > F	
Sex	1	1	127.6	2.1668	0.1435	
Time (min)	24	24	128	76.4007	<.0001	
Whole mass (g)	1	1	134	4.8148	0.0299	
Wing mass (g)	1	1	554.3	100.1525	<.0001	
Wing side (L/R)	1	1	446.9	0.0771	0.7814	
Wing type (Fore/hind)	1	1	526.8	12.72	<.0001	
AREA	Nparm	DF	DF Den	F Ratio	Prob > F	
Sex	1	1	129.3	2.6177	0.1081	
Time (min)	24	24	130.8	76.2139	<.0001	
Whole mass (g)	1	1	135.9	4.283	0.0404	
Wing mass (g)	1	1	524.8	170.8598	<.0001	
Wing side (L/R)	1	1	418.6	1.5096	0.2199	
Wing type (Fore/hind)	1	1	512	356.7712	<.0001	
Time (min)*Wing type (Fore/hind)	24	24	421	69.7361	<.0001	Interaction
WING MASS	Nparm	DF	DF Den	F Ratio	Prob > F	
Sex	1	1	142.1	4.4040	0.0376	
Time (min)	24	24	141.4	37.9776	<.0001	
Whole mass (g)	1	1	141.9	36.1532	<.0001	
Wing side (L/R)	1	1	500.5	17.6235	<.0001	
Wing type (Fore/hind)	1	1	500.9	8120.234	<.0001	

Supp. Table 1. Statistics from mixed-effects model testing four dependent variables: span, chord, area, and mass. *Terms:* Nparm - number of parameters, DF - degrees of freedom, DF Den - degrees of freedom density, F Ratio, Prob > F - P Value

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