# Supra-resonant wingbeats in insects

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#### Abstract

Powering small-scale flapping flight is challenging, yet insects sustain exceptionally 4 fast wingbeats with ease. Since insects act as tiny biomechanical resonators, tuning their 5 wingbeat frequency to the resonant frequency of their springy thorax and wings could 6 make them more efficient fliers. But operating at resonance poses control problems and 7 potentially constrains wingbeat frequencies within and across species. Resonance may be 8 particularly limiting for the many orders of insects that power flight with specialized mus-9 cles that activate in response to mechanical stretch. Here, we test whether insects operate 10 at their resonant frequency. First, we extensively characterize bumblebees and find that 11 they surprisingly flap well above their resonant frequency via interactions between stretch-12 activation and mechanical resonance. Modeling and robophysical experiments then show 13 that resonance is actually a lower bound for rapid wingbeats in most insects because mus-14 cles only pull, not push. Supra-resonance emerges as a general principle of high-frequency 15 flight across five orders of insects from moths to flies. 16

Among the four evolutionary lineages of flying organisms, insects uniquely produce rapid, pow-17 erful wingbeats at the centimeter scale from the low whir of a giant silkmoth to the near-kHz 18 hum of a biting midge (Fig. 1a) (1, 2). Wingbeat frequency is a critical determinant of aerody-19 namic power production (3-6), but is only weakly predicted by body size at the species level. 20 For example, bumblebees flap at 180 Hz (7), but bee-mimicking hawkmoths, *Hemaris diffi*-21 *nis* (8), flap at 60 Hz despite being nearly the same size. Resonance is a popular explanation 22 for this many-to-one mapping between body size and wingbeat frequency. Most insects fly by 23 deforming an elastic exoskeleton with ultrafast flight muscles (9-15). Flapping at their resonant 24

frequency theoretically allows for the costs of rapid wing acceleration to be offset by elastic 25 energy storage, but at the expense of frequency modulation capacity. While wing-clipping ex-26 periments (3, 16) and models (9, 17) point to insect being resonant, it is an unresolved question 27 whether insects flap at resonance. Slow-flapping (<100 Hz), synchronous insects like moths 28 may not be overly restricted by resonance because their wingbeats are paced by time-periodic 29 neural signals, which can match or exceed the resonant frequency. However, resonance may be 30 particularly constraining for fast-flapping (typically >100 Hz) asynchronous insects that gen-31 erate self-excited wingbeats with specialized muscles that activate in response to mechanical 32 stretch (18-20) (Fig. 1b). By combining new measurements of insect muscle and exoskeleton 33 with models of 'spring-wing' dynamics, we investigate whether insects flap at resonance across 34 taxa and flight mode, and if not, what timescales set their wingbeat frequencies. 35

## **36 Bumblebees flap above their resonance frequency**

First, we focus our attention on the bumblebee, whose flight kinematics, morphology, and be-37 havior have been characterized in detail. Using materials testing in the context of a 'spring-38 wing' model of an insect's elastic thorax, and the inertial and aerodynamic forces acting on the 39 wing (8, 10, 11, 20), we demonstrate that asynchronous bumblebees flap above their resonant 40 frequency (Fig. 1c-d). We estimated the resonance frequency of *Bombus impatiens*, by com-41 bining measurements of bulk thoracic stiffness with estimates of wing inertia and wing-hinge 42 transmission ratio. The bumblebee's undamped resonant frequency  $(f_n)$  is a function of the 43 measured thorax stiffness (k), the wing hinge transmission ratio (T, the ratio of angular wing44 displacement to muscle displacement with units rad  $m^{-1}$ ), and the inertia of the wings and 45 added mass of air around the wing (I, see SI for extended description of all parameters), 46

$$f_n = \frac{1}{2\pi} \sqrt{\frac{k}{T^2 I}} \tag{1}$$

The elastic thorax and main flight power muscles (Fig. 1c) are in a parallel configuration that 47 drive indirect actuation of the wing. We ignore series elasticity of the wing hinge, which is likely 48 small and would widen but not alter the location of the undamped resonant frequency peak (13)40 (see Supplementary Discussion). We measured the isolated thorax stiffness of bumblebees using 50 vibrational testing and found it to be 4.1 kN/m (see SI section 2.1) Setting all parameters from 51 empirical measurements, we arrive at an undamped resonant frequency of  $f_n = 94.9$  Hz, which 52 is 44% lower than average wingbeat frequencies (180 Hz) (7) (Fig. 1d). Our measured thorax 53 elasticity does not take into account active stiffness contributed by the flight muscles. Since the 54 upstroke and downstroke muscles are antagonistic, when one contracts the other is stretched 55 under near-tetanic activation. We estimate, conservatively, that active muscle stiffness is equal 56 to the summed stiffness of both pairs of flight muscles, increasing the total thoracic stiffness to 57 6.4 kN/m and the resonant frequency to 102 Hz (21) when considering stiffness contributions 58 from exoskeleton and active muscle. Propagating error in the three parameters k, T, and I, we 59 find that supra-resonant bumblebee wingbeats are robust to reasonable measurement error in 60



Figure 1: a). The division between synchronous and asynchronous insects helps explain the wide variation in insect wingbeat frequency. Data replotted from (1, 2). b) Insects with synchronous muscle produce wingbeats at a frequency set by the neural drive to the flight muscles (blue dots). Insects with asynchronous muscle produce faster wingbeats that are decoupled in frequency from the underlying neural drive (red dots). c) Schematic of a bumblebee showing flight muscle anatomy on the left, and a biomechanical model on the right. d) Simulated displacement resonance curve (grey) and velocity resonance curve (black) of a bumblebee, assuming a sinusoidal forcing. Displacement resonant frequency ( $f_d$ ) and velocity resonant frequency ( $f_n$ ) are both below wingbeat frequency ( $f_{wb}$ ). Orange and yellow bars show 95% confidence intervals of the mean for velocity resonance and wingbeat frequencies respectively.

thorax or wing properties, resulting in resonant frequencies ranging from 76 to 124 Hz (Fig. 1d).

We can also reverse the analysis to ask what thorax stiffness is necessary for wingbeats to 63 be at the resonance frequency. This would require a stiffness of 21 kN/m, which is beyond 64 the largest stiffness that we measured and far exceeds any comparable stiffness measurements 65  $(\approx 2 \text{ kN/m})$  (15, 22). Incorporating the effects of aerodynamic force production or internal 66 thoracic damping into the resonance calculation can only further depress the estimated reso-67 nant frequency below wingbeat frequency (i.e. damped displacement resonance) (8, 11). More 68 complex resonant models could create nonlinear resonance at higher harmonics, but we are 69 interested here in the fundamental resonance from the exchange of inertial and elastic energy 70 during the wingstroke. Thus, in the absence of evidence that substantial elasticity is missing 71 from our measurements, we conclude that bumblebees are supra-resonant. 72

#### 73 Stretch-activated dynamics of asynchronous muscle

The discrepancy between bumblebee resonant and wingbeat frequencies motivated us to con-74 sider how the physiological process of stretch-activation in muscle can enable supra-resonant 75 flight in asynchronous insects. Asynchronous muscle generates active force in response to a 76 rapid stretch. This mechanical stretch-activation was measured previously by stretch-hold ex-77 periments (18–20, 23) (Fig. 2a-b). The force response of isolated insect flight muscle to a step 78 length change under tetanic activation has a shape that is composed of four phases (24) (Fig. 79 2c). The first two phases are fast, associated with the viscoelastic response of the muscle tissue. 80 The slower third and fourth phases comprise the delayed stretch activation (dSA) force, which 81 can be described with a single characteristic timescale,  $t_o$  (the time taken to achieve peak dSA 82 force after the end of stretch (20)), and a constant,  $\kappa$ , the ratio of the rates of force decay ( $r_4$ ) 83 to force rise  $(r_3)$  (Fig. 2d). An analogous process, delayed shortening de-activation (dSD) oc-84 curs following rapid shortening (Fig. 2e, g) and is the inverse of dSA. We hypothesized that an 85 asynchronous insect can flap above resonance if its  $t_o$  is sufficiently fast with respect to its nat-86 ural period, the reciprocal of natural frequency  $(T_n = f_n^{-1})$ . In this case the resulting wingbeat 87 frequency is set not just by the resonant mechanics, but by a combination of muscular  $(t_o)$  and 88 mechanical  $(T_n)$  timescales. 89

To measure a bumblebee's dSA timescale under typical flight conditions, we had to conduct 90 new stretch-hold experiments on isolated *B. impatiens* DLMs at the a realistic flight tempera-91 ture of  $40^{\circ}$  C (25, 26) (Fig. 2a-e). While some measurements of bumblebee stretch-activation 92 exist, we are the first to make them in intact whole muscle at a realistic body temperature. We 93 measured the bumblebee stretch-activation timescale,  $t_o$ , to be  $4.4 \pm 1.0$  ms, nearly the duration 94 of an entire wingbeat, which did not change depending on muscle length at the onset of stretch 95 (Fig. 2f-h). This value of  $t_o$  is somewhat faster than the only comparable characterizations in 96 bumblebees at lower temperatures ( $\approx 5 \text{ ms}$ ) (21, 27), which may be because dSA rate kinetics 97 are known to speed up with temperature (23). It is also substantially slower than the  $\approx 2.5$ 98 ms necessary for dSA alone to drive 180 Hz wingbeats in response to stretch (the duration of 99



Figure 2: a). Location of the DLM (downstroke) muscles in a bumblebee. b). Schematic of muscle physiology apparatus used to apply step strains along the line-of-action of the DLM under tetanic stimulation. c). Zoomed out cartoon of a single stretch-hold-release-hold trial with purple bar blown up in panel d) Phases three and four of the dSA response, with corresponding rates of force rise ( $r_3$ ) and force decay ( $r_4$ ) notated.  $t_o$  is the time until peak dSA force is reached, and  $\kappa$  is the ratio of  $r_4$  to  $r_3$ .  $\mu$  is proportional to the height of the dSA response. e) dSA characterization experiments from a single individual. A 1% stretch was applied under tetanic stimulation at multiple starting lengths. f) dSA phases 3 and 4 from a single trial from a single individual. Red line denotes a double-exponential fit. g). dSD phases 3 and 4 from a single trial from a single trial from a single individual. h)  $t_o$  computed across all individuals at all starting lengths. (n=10 individuals). i)  $\kappa$  computed across all individuals at all starting lengths. Closed and open circles correspond to dSA and dSD measurements respectively. Red line shows a linear regression with 95% confidence intervals.



Figure 3: a). Parameter space of emergent wingbeats over a wide set of  $t_o$  and  $T_n$  that encompasses the physiological range for most insects. For any fixed value of  $t_o$ , there exists a linear relationship between resonant and emergent frequencies. At large  $t_o$ , this linear relationship collapses onto the equivalency line. As  $t_o$  decreases, wingbeat frequencies become supra-resonant, and increasingly independent of resonant frequency. Every point in simulation has been normalized such that steady-state peak-to-peak stroke amplitudes match bumblebee invivo conditions. b). Phase lag between stress and strain as a function of normalized frequency. Black line shows phase for a synchronous insect and colored dots show predictions from the asynchronous model for varying  $t_o$ , with the same color scale as in panel a). c-e). Work loop in force-displacement space for operation above (c), at (d), and below (e) resonance. Dotted lines correspond to upstroke and solid lines correspond to downstroke. At resonance, no negative work is required of the flight muscle. Above and below resonance, negative work is done in the second half and first half of each half-stroke, respectively. f). Robophysical experiment where the sign of the dSA force is changed from positive to negative, causing oscillations to transition from supra-resonant to sub-resonant. g). Space of asynchronous wingbeats in a robophysical flapper, demonstrating that transitions between sub- and supra-resonance occur only when the dSA force sign changes (i.e. across the  $\mu = 0$  boundary). Parameter values corresponding to the transition in f). are shown by the arrow from i. to ii.

downstroke after being stretched during upstroke). The ratio of rates,  $\kappa$ , was weakly correlated with resting muscle length and on average equal to  $0.17\pm0.03$  (Fig. 2i). Thus, the muscular stretch response is faster than the timescale of mechanical resonance.

#### **Asynchronous insects flap at or above resonance**

To test our hypothesis that a sufficiently fast stretch-activation timescale (i.e.  $t_o$ ) can enable supra-resonant wingbeats, we developed a biophysical model of asynchronous muscle-driven resonant flight. We drive a 'spring-wing' model of the insect's thorax and wings (8, 10, 11) with a simplified description of stretch-activated muscle, rooted in our stretch-hold experiments in bumblebees (20, 30). Thus, we capture the essential dynamics of both stretch-activation and resonant mechanics.

The model consists of two coupled, second-order differential equations. The oscillatory 110 dynamics of the wingstroke angle,  $\phi(t)$  are parameterized by  $k_l$ , T, I, and an aerodynamic 111 damping coefficient  $\Gamma$  (Eq. 2). The stretch feedback-driven muscle forcing (Eq. 3) is parame-112 terized by  $t_o$  and  $\kappa$  (see Supplementary Methods section 1.6.2). There is one free parameter,  $\mu$ , 113 which can be set by matching the amplitude of the output wingbeats to free-flight bumblebee 114 wingstroke amplitude.  $\mu$  represents the strength of the dSA forcing and makes the dSA force 115 *in-vivo* proportional to the height of the two-phase response of the muscle to rapid stretch (Fig. 116 2c-d). Because the forcing is entirely state dependent and not prescribed exogenously (e.g. by 117 the nervous system) the system oscillates at an emergent frequency (See Supplementary Results 118 2.2). 119

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$$I\ddot{\phi} + \Gamma |\dot{\phi}|\dot{\phi} + \frac{k_l}{T^2}\phi = \frac{\mu}{T}f_{dSA}$$
<sup>(2)</sup>

$$\ddot{f}_{dSA} + \alpha_2(t_o,\kappa)\dot{f}_{dSA} + \alpha_3(t_o,\kappa)f_{dSA} = -\alpha_3(t_o,\kappa)\dot{\phi}$$
(3)

Simulating Eqs. 2-3 and evaluating the resulting  $\phi(t)$  wing stroke trajectories over a wide 121 range of muscular and mechanical timescales ( $t_o$  and  $T_n$ ) reveal that resonance is a lower bound 122 on emergent wingbeat frequency (Fig. 3a). There exists a large region of parameter space in 123 which asynchronous insects can flap significantly above resonance. Consistent with observa-124 tions from modifying the wing inertia of insects (3, 16, 31), the resonance frequency of the insect 125 increases as the emergent flapping frequency goes up, regardless of  $t_o$  (Fig. 3a). However, this 126 does not mean that the flapping frequency is at resonance. Fast stretch-activation, low  $t_o$ , results 127 in supra-resonant wingbeats, while slower  $t_o$  result in wingbeat frequencies that collapse onto 128 the wingbeat-resonant frequency equivalency line. Thus, stretch-activated resonant systems are 129 not constrained to resonance, and can oscillate supra-resonantly with the right combination of  $t_o$ 130 and  $T_n$ . Using our measured flight muscle stretch-activation timescale, we estimate a bumble-131 bee wingbeat frequency which exceeds resonance by 33%, in agreement with our experimental 132 characterization of supra-resonance using thorax materials testing. 133



Figure 4: a). Fraction of perfectly resonant stiffness contributed by muscle alone for three insect species: fruitfly, bumblebee, and hawkmoth (21, 28, 29). b). Emergent asynchronous frequency space as a function of muscle time constant  $(t_o)$  and natural period  $(T_n)$ . Both fruitfly and bumblebee achieve similar wingbeat frequencies with different combinations of  $t_o$  and  $T_n$ . c). Supra-resonant wingbeats across insects, compiling estimates from five insect orders. Markers - "×" (8), "+" (22), square (18), and diamond (17) - represent data from different studies. Dots show data from the current study. Dotted line shows equivalence of wingbeat and resonant frequencies. Orange bars show resonant frequency ranges assuming exoskeleton stiffness underestimates total thorax stiffness (exoskeleton + muscle) by up to a factor of two. Solid grey line shows a linear regression through all points, with dotted grey 95% CI.

#### <sup>134</sup> Muscle's asymmetry enforces supra-resonance

Why are asynchronous flappers apparently only able to flap at or above resonance? This con-135 straint emerges from muscle producing a positive force in response to stretch (i.e. always 136 'pulling' and never 'pushing'), such that increases in muscle stress will always lag positive 137 muscle strain (muscle extension) (Fig. 3b, inset). This phase lag is proportional to the stretch-138 activation timescale ( $t_o$ ), but can never be below a quarter cycle ( $\pi/2$  radians) because increas-139 ing  $t_o$  results in a frequency that asymptotes to resonance. As  $t_o$  increases,  $\delta$  approaches  $\pi/2$ ; 140 as  $t_o$  decreases,  $\delta$  approaches a maximum. Thus, stretch-activated wingbeats can never be sub-141 resonant, which would require  $\delta < \pi/2$  (Fig. 3b, colored points). This contrasts with a syn-142 chronous insect that has neurally activated muscle, which can theoretically achieve  $0 < \delta < \pi$ 143 by modulating the timing of neural signals to the flight muscles (Fig. 3b, black line). This 144 would be equivalent to activating the muscle such that it produces significant force in the half 145 cycle prior to lengthening (32-36). 146

The necessity of asynchronous insects to be supra-resonant can be visualized in the work-147 loop representation of muscle function, which visualizes muscle work output as the area en-148 closed in active force vs. displacement space (37-40). In this space, the effective storage mod-149 ulus, E', of the workloop represents the elastic component of the total muscle force necessary 150 to supply all energy requirements for flight (see Supplementary Results 2.3). E' = 0 represents 151 perfect exchange between wing kinetic energy and thoracic elastic energy (i.e.  $f_{wb} = f_n$ ) (Fig. 152 3d).  $E' \neq 0$  represents non-resonant conditions where the thorax is either too stiff or not stiff 153 enough with respect to the wingbeat frequency (Fig. 3c,e). The hysteresis of the loop is propor-154 tional to the phase lag  $\delta$  between force and displacement. The resulting area within the loop is 155 the effective loss modulus, E'', representing the mechancial energy generated by the muscle. 156

At steady-state, the muscle forcing  $f_{dSA}(t)$  will always follow a counter-clockwise ellipse 157 (negative loss modulus or positive net work) with a nonnegative storage modulus. This enforces 158 a phase lag,  $\delta$ , of  $\pi/2 < \delta < \pi$  in stress with respect to strain. If the insect is supra-resonant, its 159 muscle will produce negative work, dissipating energy, during the second half of each halfstroke 160 while the wing is decelerating (Fig. 3c, shaded area). Muscle assisting in the slowing of the 161 wing on each half cycle is only possible in a supra-resonant system, since the thoracic spring 162 will not be stiff enough to absorb all of the wing's kinetic energy before it reaches its extremal 163 positions. At resonance, the storage modulus of the muscle is identically zero and no negative 164 work is required at any point in the wingbeat (Fig. 3d). Below resonance, an overly stiff 165 thoracic spring would cause rapid wing acceleration that is counteracted by muscle dissipating 166 energy in the acceleration phase of each half-stroke (Fig. 3e, shaded area). Thus, a sub-resonant 167 asynchronous insect would have to generate negative work directly following stretch, which is 168 incompatible with the polarity of muscular dSA force response. Muscles only pull, they do not 169 push. 170

#### <sup>171</sup> Supra- and sub-resonance realized through a robophysical model

While the physiological limitations of biological muscle limit oscillations to at or above reso-172 nance, engineered actuators are not necessarily limited to this regime. In contrast, they can push 173 and pull. Following from the sub-resonant work loop (Fig. 3e), we predict that an engineered 174 system with muscle dSA-like actuators that pushed instead of pulled should be able to realize 175 sub-resonant asynchronous wingbeats. We demonstrate asynchronous sub-resonant oscillations 176 in a dynamically scaled robophysical flapping wing by controlling a brushless DC motor with a 177 velocity feedback-driven forcing analogous to dSA (Eqs. 2-3) (30). This system is also unable 178 to oscillate below its resonant frequency, when controlled with a muscle-like dSA forcing (Fig. 179 3f, i.). However, by changing the sign of the dSA force such that a negative (i.e. pushing) 180 force follows stretch, stable sub-resonant oscillations emerge that are bounded above by the 181 resonance frequency (Fig. 3f, ii.). By systematically changing  $\mu$  in the model that controls our 182 roboflapper motor, we see the boundary of switching between supra- and sub-resonant behavior 183 is at  $\mu = 0$ , where the sign of the dSA force flips (Fig. 3g). Thus, we demonstrate that the 184 physiology and arrangement of antagonistic stretch-activated muscles in asynchronous insect 185 thoraces constrain them to supra-resonant wingbeats. 186

Sub-resonance is realizable in some biological muscle-driven systems as well. For instance, 187 unlike asynchronous insects, synchronous insects can, in theory, be sub-resonant. They could 188 neurally activate their muscles at timings that would enable negative work production in the first 189 part of each half-stroke. Practically, this would require the downstroke muscle (or a combination 190 of muscles) to produce significant force during the beginning of upstroke, and vice versa. This 191 would require either multiple downstroke or upstroke muscles or a large degree of coactivation 192 which would likely reduce the production of useful work from the muscles. While we do not see 193 such activation patterns in insects, they are common in terrestrial locomotion especially where 194 multiple muscles operate in synergy at a joint or in a limb (33–36, 41). Indeed, some terrestrial 195 animals like kangaroos are sub-resonant (42), but do not have to contend with the asynchronous 196 muscle dynamics present in bumblebees or our robophysical flapper. 197

Supra- and sub-resonant systems also exhibit a key difference in how they modulate power 198 output outside of the steady state. In a supra-resonant system, acceleration is muscle-driven 199 and spring-assisted, with negative muscle work (dissipation) coinciding with the deceleration 200 phase of the wingstroke (Fig. 3c). The muscle assists the spring. This enables supra-resonant 201 systems to inject additional accelerative power via transient increases in agonist muscle force. 202 However, in sub-resonant systems, acceleration is driven by the spring and braked by the agonist 203 muscle (Fig. 3e). Positive power production is limited by the spring's capacity to return elastic 204 energy and additional agonist muscle force would only decelerate the wing faster. Thus, an 205 important benefit of supra-resonance for asynchronous insects may be to maintain the capacity 206 to transiently boost wing acceleration via positive muscle power production in the first part of 207 each half-stroke (Fig. 3c). 208

#### **Two timescales pace asynchronous wingbeats**

Having validated our model in bumblebees, we used recent characterizations of *Drosophila* 210 *melanogaster* muscle stiffness and flight mechanics to show that this widely-studied fruitfly is 211 also supra-resonant, although to a lesser degree than *B. impatiens*. *D. melanogaster* has more 212 than three orders of magnitude less mass than a bumblebee, but paradoxically has a wingbeat 213 frequency of around 200 Hz, which is very close to that of *B. impatiens* (43). Measurements 214 of fruitfly delayed stretch activation timescale,  $t_0$ , range from 5-8 ms which is slower than 215 our measured  $t_o$  for bumblebees (Fig. 2h) (44). We hypothesize that fruitflies have evolved a 216 similar wingbeat frequency to bees despite being much smaller in part by having relatively slow 217 stretch-activation in comparison to their natural period. Indeed, fruitfly wing inertia is roughly 218 four orders of magnitude smaller than that of a bumblebee, which in isolation would suggest a 219 resonant frequency far greater than  $\approx 100$  Hz we measured in bees. 220

To test this hypothesis, we first need an estimate of the bulk stiffness of the fruitfly thorax. 221 While exoskeletal stiffness values for fruitflies have not been measured, functional stiffness 222 in the fly thorax is thought to be dominated by active muscular elasticity, rather than parallel 223 thoracic stiffness. This is due to fruitflies' combination of small wing inertia compared to bees, 224 and asynchronous muscle which has much higher resting stiffness than synchronous muscle (19, 225 21, 28, 45). We quantify whether existing estimates of muscle elasticity alone are sufficient to 226 estimate resonance frequency, by deriving a new metric describing the contribution of muscular 227 elasticity to bulk thorax stiffness: the ratio of the active muscle stiffness  $k_{muscle}$  to the stiffness 228 that would be required to drive perfectly resonant wingbeats,  $k^* = (2\pi f_{wb})^2 T^2 I$  (Fig. 4a). 229 This expression depends on nonlinear interactions between morphological (wing inertia, I), 230 kinematic (wingbeat frequency,  $f_{wb}$ ), and biomechanical (transmission ratio, T) parameters. 231 It is not a simple function of body size. Bumblebees and moth thoraces are dominated by 232 exoskeletal stiffness, which is in excess of muscular stiffness by at least three-fold yet their 233 free flight frequencies are still above their undamped resonance. In contrast, *Drosophila* muscle 234 does supply nearly all the elasticity needed to drive wingbeats close to resonance (Fig. 4a). 235

Armed with a stiffness estimate for *Drosophila* we can then apply the same approach we 236 took with bumblebees to test if they operate at their resonant frequency. When we estimate 237 Drosophila resonant and wingbeat frequencies using Eqs. 1-3, we find a predicted emergent 238 wingbeat frequency (221 Hz) that is supra-resonant at 18% in excess of resonance (187 Hz) 239 (Fig. 4b). Supra-resonance arises from a combination of a much larger transmission ratio 240 (ratio of wingbeat angle change to muscle displacement) by virtue of small body size as well 241 as slower stretch-activation (longer  $t_o$ ). These frequencies are within the range of measured 242 free-flight wingbeat frequencies. While series-elastic effects in moths and bees are not large 243 enough to significantly impact our results (see Supplementary Discussion), high series-elastic 244 compliance in *Drosophila*-scale flies may widen their resonance curves to the point where they 245 can still achieve near-maximal resonant energy return while being supra-resonant (13). Thus, 246 our modeling demonstrates how dSA causes a single frequency that exceeds  $f_n$  to emerge from 247 a band of potentially equally efficient frequencies in insects with significant series compliance. 248

We find that bees and flies realize similar asynchronous wingbeat frequencies through different combinations of  $t_o$  and  $T_n$  while remaining slightly (flies) to significantly (bees) supra-resonant (Fig. 4b).

### <sup>252</sup> Supra-resonance as a general principle of insect flight

Our experimental and theoretical characterizations of resonance in bees and flies point to supra-253 resonance as a general principle of insect flight, but how broadly does it apply? Collating our 254 results with the only other comparable characterizations of resonance (17, 18, 22), we show that 255 supra-resonance applies to asynchronous bees, flies, and beetles, and synchronous moths. Even 256 the dragonfly, a synchronous insect with a direct flight muscle architecture appears to operate 257 above its resonance peak. This suggests that supra-resonance is not limited to insects with indi-258 rect flight muscles so long as there is some degree of elasticity in the muscles or wing hinge (17). 259 Thus, our results demonstrate a general pattern of supra-resonant wingbeats in insects, with all 260 insects included flapping at or above their resonant frequency. Insects generally fall on a line 261 with slope > 1 (slope = 1.32, p < 0.001,  $r^2 = 0.77$ ) but an intercept statistically indistinguish-262 able from 0. Thus across taxa, insects maintain a roughly constant ratio of wingbeat to resonant 263 frequency, with slower-flapping insects flapping extremely close to, but not below, resonance. 264

Interestingly, *Drosophila* lies closer to resonance than predicted by a line of best fit through 265 all other species (slope = 1.84, p < 0.001,  $r^2 = 0.80$ ), suggestive that higher frequency insects 266 may not continue to experience a divergence between resonant and wingbeat frequencies. This 267 makes sense in the context of its larger  $t_o$  compared to that of a bumblebee (44), despite being 268 orders of magnitude less massive. In addition, smaller insects achieve roughly the same am-269 plitude wingbeats with much smaller muscle displacements, resulting in a sharply increasing 270 transmission ratio (driving a decrease in  $T_n$ ) with size. Adaptations in wing hinge musculature 271 and gearing (2,46) may tune the transmission ratio and enable modulation of wingstroke param-272 eters without changing wingbeat frequency. Thus, a combination of effects on  $t_o$  and  $T_n$  likely 273 pushes millimeter-scale fliers closer to resonance than bees, making way for extreme kinematic 274 and morphological adaptation to facilitate maneuverability (47). 275

Our results demonstrate that the physiology of asynchronous muscle activation under cyclic 276 strain constrains many insects to operation at or above resonance, suggestive of a widespread 277 advantage to supra-resonant flight. The apparent ubiquity of supra-resonant flight also demon-278 strates that resonance tuning is not necessary for insect flight systems. While smaller insects 279 generally beat their wings faster and have higher resonant frequencies, competing biomechan-280 ical and physiological pressures from muscle, wing, and exoskeleton make perfectly resonant 281 wingbeats a precarious target for selection. The capacity for supra-resonance widens the phe-282 notypic space for successful flight, opening up the possibility of evolutionary tuning of insect 283 resonant properties for control, efficiency, or speed (8, 12, 48, 49). 284

Operation above resonance may enable increased frequency-modulation capacity in asynchronous insects via modulation of resonant properties, such as during different buzzing modes in bees, or during sensory feedback driven maneuvers in flies (*31, 50*). For example, decreas-

ing the transmission ratio by keeping the wings retracted (such as during bumblebee defense 288 buzzing) would drastically decrease the resonant period, and could easily result in a doubling 289 of wingbeat frequency without any modification of muscle properties (Fig. 4b) (51). Such a 290 mode of frequency modulation would not be possible if bumblebees had much faster muscle 291 stretch activation kinetics  $(t_o)$ , because the low- $t_o$  region of the parameter space has a flat rela-292 tionship between emergent and natural frequency (Fig. 3a, 4b). Similar modulation capacity in 293 synchronous insects is possible by transient neurogenic frequency changes during perturbation 294 recovery (8, 12, 48). 295

Temperature-dependent modulation of wingbeat frequency via changing stretch-activation 296 time constants may be important in thermogenesis buzzing, or for insects that fly in cold condi-297 tions such as alpine honeybees (52). One unique challenge faced by insects at the size of a fruit-298 fly and smaller is that they cannot maintain flight muscle temperatures significantly above ambi-299 ent temperature. This temperature constraint may cause faster-flapping insects to have a slower 300  $t_o$  since stretch-activation timescales are known to be highly temperature-sensitive (23, 53). 301 Modulation of wingbeat frequency by calcium-dependent potentiation of asynchronous muscle 302 force may also be more effective above resonance (54). In general, our results suggest that 303 the timescale of asynchronous muscle's stretch activated dynamics  $(t_o)$  and the timescale of 304 the mechanics of resonant spring-wing thorax  $(T_n)$  are independent axes by which the emer-305 gent wingbeat frequency can change over long timescales by selection, or short timescales by 306 phenotypic plasticity (Fig. 4b). 307

Asynchronous flight was a key evolutionary innovation that opened the possibility for su-308 perfast wingbeat frequencies, enabling insects to miniaturize and diversify. Contrary to the 309 longstanding hypothesis of resonance tuning, our materials testing, muscle physiology, dynam-310 ical and robophysical modeling demonstrate that many asynchronous insects flap significantly 311 above their resonant frequency. We highlight a mechanism for asynchronous supra-resonance: 312 a tug-of-war between intrinsic physiological timescale of asynchronous muscle and the resonant 313 mechanics of the thorax and wings. Supra-resonance also generalizes to multiple synchronous 314 orders, despite their wingbeat frequency being determined neurally. Thus, supra-resonance is 315 an underappreciated and widespread property of insect flight, that underscores the importance 316 of balancing efficiency and agility across Earth's smallest aerial locomotors. 317

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