A wrinkle in flight: the role of elastin fibres in the mechanical behaviour of bat wing membranes

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Bats fly using a thin wing membrane composed of compliant, anisotropic skin. Wing membrane skin deforms dramatically as bats fly, and its three-dimensional configurations depend, in large part, on the mechanical behaviour of the tissue. Large, macroscopic elastin fibres are an unusual mechanical element found in the skin of bat wings. We characterize the fibre orientation and demonstrate that elastin fibres are responsible for the distinctive wrinkles in the surrounding membrane matrix. Uniaxial mechanical testing of the wing membrane, both parallel and perpendicular to elastin fibres, is used to distinguish the contribution of elastin and the surrounding matrix to the overall membrane mechanical behaviour. We find that the matrix is isotropic within the plane of the membrane and responsible for bearing load at high stress; elastin fibres are responsible for membrane anisotropy and only contribute substantially to load bearing at very low stress. The architecture of elastin fibres provides the extreme extensibility and self-folding/self-packing of the wing membrane skin. We relate these findings to flight with membrane wings and discuss the aeromechanical significance of elastin fibre pre-stress, membrane excess length, and how these parameters may aid bats in resisting gusts and preventing membrane flutter.

1. Introduction

Skin plays an important role in the locomotion of many vertebrates. The expansion of the skin connecting adjacent skeletal elements of limbs is an important adaptation in all flying and gliding mammals, as well as in many aquatic vertebrates [1]. This skin expansion increases surface area and has allowed the skeletal structure of a limb to be exapted for novel use to generate fluid-dynamic force, as a paddle or wing. Bats, the only mammals capable of powered flapping flight, fly using a wing membrane comprising skin that spans the hands, arms, legs and trunk. The skin of the wing is extremely thin, roughly an order of magnitude thinner than body skin [2]. The thinness and compliance of bat wing skin has important consequences for the aeromechanics of bat wings.

Because bat wing skin is compliant and slender (here, we use the term slender to refer specifically to thickness relative to length), expanses of skin behave mechanically as elastic membranes that lack bending stiffness; therefore, these membrane wings cannot maintain a predetermined three-dimensional form. Membrane wings deflect when they experience aerodynamic loading, passively adopting a cambered shape, and support the aerodynamic load through tension in the membrane (figure 1a) [3]. Thus, membrane wings of both bats and human-engineered devices experience considerable passive shape change under aerodynamic loading [4–7]. Among the consequences of this aeroelastic coupling are that shape change: occurs with no additional energy input from the bat, can provide rapid mechanical response to varying flow conditions, delays stall to higher angles of attack, enhances lift and can alleviate the effects of gusts [3,8].

Relative to the basal condition for mammalian skin, bat wing membrane skin possesses distinctive morphological traits that may relate directly to flying with membrane wings. Although bat wing membrane is exceptionally thin, it contains
an array of muscles [9,10] along with macroscopic (approx. 0.1 mm wide) fibre bundles composed primarily of elastin fibrils (figure 1b) [11–13]. Many of the wing membrane muscles likely modulate membrane skin stiffness and consequently wing shape during flight [14]. Although the effect of the unusually large elastin fibres on membrane mechanics remains unclear, it is possible that elastin fibres aid wing folding/packing and provide membrane reinforcement and tear resistance [12,13,15]. Additionally, the presence of elastin fibres is associated with prominent corrugation or wrinkles in bat wing membranes [11–13].

Elastin is known to be responsible for the compliant toe-region of the J-shaped stress–stretch behaviour in a multitude of biological tissues [16–18]. Elastin performs this specific mechanical role, discrete from the stiffer surrounding constituents, because it is pre-stretched relative to those components, which are essentially buckled [19–22]. In this configuration, as tissue is loaded in progressively greater tension, elastin bears load at low stresses until buckled components elongate to an extent that allows them to begin bearing load. Although the organization of elastin with respect to other skin constituents is quite different in bat wing membranes than in other connective tissues, elastin fibres likely still contribute to compliance in wing membrane skin. Mechanical testing of wing membranes in a number of bat species has established that the membrane is mechanically anisotropic [15]. The membrane is most compliant along the wingspan, and this tissue compliance is correlated with orientation of wing wrinkles or corrugations (figure 3a).

Here, we develop a tractable model of the wing membrane to further understand the contribution of elastin fibres, wrinkles and matrix to wing membrane mechanical behaviour. Because the tissue is a fibre composite, we use the traditional material science term ‘matrix’ to refer to the tissue not consisting of elastin fibres. This is not to be confused with the extracellular matrix. Aided by our model, we can better understand how elastin fibres and matrix contribute to wing shape configuration.

We hypothesize that wing membrane anisotropy arises specifically from highly oriented elastin fibres, not collagenous elements of the membrane. Based on a study of one bat species, stiff collagen is likely to be randomly oriented within the plane of the tissue [13], but we expect elastin fibres will show preferential orientation consistent with a major role in dictating anisotropy. We further hypothesize that the macroscopic elastin fibres in bat wings are pre-stretched relative to the ventral and dorsal layers of the skin, placing the surrounding matrix in compression when the skin is resting or unloaded. Compressive loading of the slender wing membrane results in the matrix buckling, producing the characteristic wrinkles. The strong anisotropic response arises from wrinkles delaying onset of stretch and stress in the surrounding matrix until they have unfurled.

To address these hypotheses, we first characterize the architecture of the elastin fibre network. The few species previously studied demonstrate substantial variability in elastin fibre quantity, branching density and, to an extent, orientation [9,10,12,13]. We then test whether matrix wrinkles result from the presence of elastin fibres, or if wrinkles are intrinsic to matrix structure. Our hypothesis of a close structural relationship between membrane wrinkles and elastin fibres has not been supported by a previous analysis of wrinkle and elastin fibre orientation, which failed to find a strong correlation [15]. Subsequently, we quantify wing membrane stress–stretch behaviour, both parallel and perpendicular to elastin fibres, normalized to (i) a relaxed configuration and (ii) a high-stress configuration. We propose that force contribution by elastin fibres is negligible at the high-stress configuration, which
allows for testing of matrix isotropy in samples with and without contribution from elastin fibres. Finally, because the wrinkled architecture should allow for greater tissue stretch parallel to elastin fibres, we measure wing membrane extensibility parallel and perpendicular to elastin fibres.

2. Material and methods

2.1. Tissue

Tissue used for mechanical testing was excised from the wing membranes of three male Carollia perspicillata, Seba’s short-tailed fruit bat (body mass: 17.1, 18.4, 18.9 g). We selected this species because phyllostomid bats are particularly well studied with respect to wing membrane function during flight [14,15], and their availability allowed mechanical testing to begin immediately after euthanasia, which minimized the effects of tissue desiccation and breakdown. Each individual was euthanized using carbon dioxide, and tissue samples were collected and placed within a humid storage chamber.

Additional tissue used for dissection was taken from naturally deceased individuals of C. perspicillata. Individuals were frozen after death and thawed prior to dissection. Tissue was manually hydrated as needed.

All experiments were conducted in accordance with approved protocols (Brown University IACUC, Division of Biomedical Research and Regulatory Compliance of the Office of the Surgeon General of the US Air Force).

2.2. Elastin fibre orientation and removal

Because elastin fibres in bat wings exhibit strain birefringence, we used cross-polarizers to determine elastin fibre orientation. Wings were illuminated using a light box covered with linear polarizing film (TechSpec, Edmund Optics Inc., Barrington, NJ), and imaged with an SLR camera with a lens-mounted circular polarizing filter (Nikon Inc., Melville, NY). Elastin fibres could be more clearly discriminated from wing membrane muscles under cross-polarized lighting than standard backlighting.

To determine whether elastin fibres are the cause of wing membrane wrinkling, we carried out a micro-dissection experiment. Prior to dissection, we photographed a wing sample to document the degree of wrinkling. We then removed the elastin fibres and visualized the degree of wrinkling following fibre removal. To remove elastin fibres, we first carefully separated the ventral and dorsal layers of the wing membrane skin. An incision was made beginning at the elbow, along the forearm, over the wrist and down digit V, to form a triangular flap of tissue. The two skin layers were then separated by blunt dissection. Some elastin fibres were removed by separation of the two layers, and the rest were dissected away. For imaging, the wing was lit at a shallow angle to highlight the peaks and troughs of tissue wrinkles.

2.3. Sample preparation for mechanical testing

Two to three tissue samples were taken from each wing for uniaxial mechanical testing, totalling 15 samples. Samples were oriented either parallel or perpendicular to elastin fibres, and taken from both arm- and handwing (figure 2a). Each wing was gently extended until the membrane was flat but still compliant, and far from the point of plastically deforming. We determined fibre orientation by visualizing the wing membrane with cross-polarizing filters. Before excising wing tissue samples, a pair of rectangular, die-cut, adhesive-backed paper frames was placed on the ventral and dorsal surfaces (8-mil Carpet Tag, RippedSheets.com, Benton City, WA). These frames served to keep the skin material flat, simplifying tissue handling and preventing crumpling in response to elastin pre-stress. Frames were carefully aligned and oriented either parallel or perpendicular to elastin fibres (figure 2c). Frames enclosing tissue samples were then cut away from the wing and mounted in the mechanical testing apparatus. Once mounted, the sides of the frame were removed. The region of each membrane sample subjected to testing was sample thickness × 5 × 20 mm. We placed an array of white paint markers on each sample for video-based analysis of sample length change, but found the reliability of such measurements inadequate for our purposes, and do not report them here (see §2.4).

Figure 2. Elastin fibres form a parallel array, allowing uniaxial testing parallel and perpendicular to the fibres. (a) Polarized light image of wings in C. perspicillata (ventral view). Elastin fibres run primarily along the wing unfolding axis, approximately spanwise. The more intensely birefringent muscles in the armwing run orthogonal to elastin fibres. Digits III – V are labelled. Rectangles illustrate location of sampling for specimens with elastin fibres parallel (image left, bat’s right wing) and perpendicular (image right, bat’s left wing) to the long axis of the test frame. (b) Diagrammatic representation of the elastin fibre network in the hand- and armwing illustrating generally parallel organization except for the reticulate pattern between metacarpals IV and V and directly adjacent to bones. Striped area of schematic was not studied. (c) Wing membrane samples framed and excised. Samples were tested along the designated uniaxial tension axis, and either parallel or perpendicular to the principal elastin fibre network orientation. Samples are 20 × 5 mm.
that tissue texture and elasticity did not notably change over the
slowly to minimize viscous contributions to stress. We found
constant elongation rate, each with a cycle period of 100 s (elec-
tensile load bearing was negligible relative to the contribution by
state. We could not estimate a single typical maximum
volume, potentially placing the wing membrane in a tension-free
their wings, compressing the large wing surface area into a small
should capture a fully relaxed configuration, because bats fold
from a fully relaxed starting configuration may not be necessary.

2.4. Uniaxial tensile testing
We tested wing membrane skin using a uniaxial mechanical testing
Tensile force was measured using a 1 N load cell (ULC-1N, Inter-
face Inc., Scottsdale, AZ), and recorded from an A/D converter
(USB 6218-BNC, National Instruments Corp., Austin, TX) using a
custom MATLAB script (MathWorks, Inc., Natick, MA). Sample
length was measured from the device crosshead position, because
wing membrane wrinkling and twisting made digitizing from
video-based length measurements unreliable.

Prior to testing tissue samples, we determined the length range
over which cyclic tests would be performed. Our aim was to test
each sample over a length range corresponding to skin length at
an untensioned state and to the length at approximately one
body weight of tension. To determine these critical lengths, all
pre-stretch in the sample was first removed by reducing crosshead
height until the wing membrane displaced out of plane, indicating
a relaxed state. We then slowly stretched the sample to approxi-
amately one body weight of tension, 0.18 N, and measured
sample length at this load. To minimize loading history-dependent
effects on subsequent tests, each sample was then returned to the
relaxed state and allowed to recover for 5 min.

We selected minimum and maximum tension values to cover a
biologically meaningful loading range as well as to ensure that
assumptions of our model were met. In vivo, skin is often under ten-
sion in its natural configuration [23,24]; therefore, studying skin
from a fully relaxed starting configuration may not be necessary.
However, we reasoned that our test of bat wing membrane skin
should capture a fully relaxed configuration, because bats fold
their wings, compressing the large wing surface area into a small
volume, potentially placing the wing membrane in a tension-free
state. We could not estimate a single typical maximum in vivo ten-
sion value with confidence. Instead, we selected a value that was
likely well above the normal biological range. We chose a suffi-
ciently high value to ensure that the contribution of elastin to
tensile load bearing was negligible relative to the contribution by
matrix, which allowed us to test for matrix isotropy.

Sample testing occurred over seven stretch–relaxation cycles at
constant elongation rate, each with a cycle period of 100 s (elec-
tronic supplementary material, figure S1a). Tissue was elongated
slowly to minimize viscous contributions to stress. We found
that tissue texture and elasticity did not notably change over the
test duration, and rate-dependent effects at this speed were
small enough not to affect our model assumptions. We did not
characterize the viscous contribution to mechanical behaviour,
because strain rates in flight are still poorly understood. Further,
this phenomenological characterization would be best perfor-
med under biaxial loading conditions, which are beyond the
scope of this study [25]. We analysed the elongation portion of
the seventh cycle only and consider the previous six cycles to
serve as preconditioning of the tissue. The stress–stretch response
changed little over cycles 2–6 (electronic supplementary material,
figure S1).

2.5. Signal processing
Wing membrane is very compliant, and relatively little tissue
area can be tested; therefore, the measured forces and signal-to-
noise ratio are low. Load and displacement measurements were
digitally filtered to better resolve tissue mechanical response
compared with electrical noise. We smoothed the recorded
signal using a moving window that applied a first-order poly-
nomial over a window size of 1 s of data collection. We tested
alternative approaches to smoothing and filtering and these did
not change the conclusions of the study.

2.6. Estimation of wing membrane thickness
Uniaxial tensile force was converted to stress by normalizing to
wing membrane sample cross-sectional area. We estimated wing
membrane thickness by sectioning four wing membrane samples
from the arm- and handwing. After flash freezing, each sample
was sectioned using a cryostat and membrane thickness was
measured using [26]. Because wing thickness bulges locally
wherever there are muscles or elastin fibres, thickness measure-
ments were taken from regions between these structures. All arm-
and handwing tension measurements were normalized to
their respective average thickness values, 0.03 and 0.02 mm,
respectively. These measurements are consistent with other
published values for bat wing membrane thickness [2,27].

2.7. Mechanical behaviour equations
We describe mechanical behaviour of the wing membrane using
stress–stretch properties and anisotropy of the tangent modulus.
Stretch is the sum of one plus engineering strain, and is one of the
conventional means of describing large deformations. The more
familiar engineering strain is generally reserved for small de-
f ormations, and poorly approximates strain at large deformations
[28]. We calculate stretch as

\[ \lambda = \frac{L}{L_{ref}} \]

where \( L \) is the deformed length and \( L_{ref} \) is sample length at the
reference configuration. Then using engineering stress, \( \sigma \), tissue
stiffness is

\[ E = \frac{\sigma}{\lambda} = \frac{E_0}{\lambda^k} \]

where \( E \) is the tangent modulus. From the tangent modulus, we
quantify tissue anisotropy as an index of the stiffness parallel to elas-
tin fibres (\( E_\perp \)) relative to that perpendicular to elastin fibres (\( E_\parallel \))

\[ \frac{E_\perp}{E_\parallel} \]

If elastin fibres are pre-stressed, then their resting length is
shorter than that of the surrounding matrix. Therefore, to under-
stand the mechanical behaviour of both constituents, we must
consider two different reference configurations. In the case of
bat wing membranes, the high-frequency, large-amplitude

Figure 3. Elastin fibres are necessary for wing wrinkling. Ventral view of
distal armwing. Elbow is at the left, digit V to the right. (a) Wing membrane
wrinkles run parallel to elastin fibres. Shallow raking light reveals illuminated
and shadowed faces of the high spatial-frequency wrinkles. (b) Removal of
elastin fibres by dissection eliminates wing wrinkling. Ventral epidermis is
also removed. Wing wrinkling still occurs where residual elastin fibres
remain (region around asterisk).
wrinkles in the resting configuration demonstrate that matrix length likely far exceeds the length of elastin fibres. Accounting for matrix length in excess of the fibre length is necessary for accurately assessing its mechanical behaviour. The two reference configurations \( (L_{ref}) \) we employ are a relaxed configuration, the most common normalization approach for mechanical studies (which we designate \( L_{0 \text{MPa}} \)), and a high-stress configuration (discussed in §2.9, which we designate \( L_{1 \text{MPa}} \), the configuration at 1 MPa stress). The high-stress configuration is designed to account for excess matrix length parallel to elastin fibres by placing the stiff matrix at an equal state of stretch, regardless of sample orientation. The two reference configurations are further discussed below.

### 2.8. Low-stress-referenced mechanical behaviour

To describe mechanical behaviour of the wing membrane of our study species at the tissue level, we carried out tests in which sample length was normalized to the length at 5 kPa, the approximate resolution of our load cell. After normalization, we compared stress–stretch behaviour of all wing membrane samples.

### 2.9. High-stress-referenced mechanical behaviour

To test for isotropy in the wing membrane matrix itself, we had to account for elastin’s contribution to stress and the wrinkling of the matrix. Matrix wrinkling is important because it potentially indicates excess matrix length, and therefore, that the measured stretch in the elastin–matrix composite (parallel to elastin fibres) is greater than matrix stretch. To account for these contributions to the stress–stretch behaviour, we normalize sample length to the length at a high-stress configuration, i.e. at stress = 1 MPa. This length was chosen because we expected that at this stress level the contribution by elastin to bearing load would be negligible. Consistent with this, at 1 MPa stress the tangent modulus falls within the range typically observed in mammalian skin [29]. If load bearing by elastin is negligible, then when samples of differing orientations are at equal stress, the matrix component would be at equal stretch if it is isotropic. After normalization, we compared stress–stretch behaviour of wing membrane samples and measured the anisotropy within the plane of the membrane with respect to stress (see §2.10).

### 2.10. Surface anisotropy

We calculate anisotropy within the plane of the wing membrane, also referred to as surface anisotropy [30], as an index describing the ratio of the tangent moduli measured parallel to elastin fibres relative to that perpendicular to elastin fibres. The anisotropic index was calculated for sample pairs taken from a single individual and from the same wing region, i.e. armwing, handwing between digits IV and V, and handwing between digits III and IV (figure 2a). Seven pairs of samples were used to calculate mean ± standard error for the anisotropic index over the stretch domain occupied by all samples. We calculated the anisotropic index for samples at the same state of stretch, as well as for samples at the same state of stress.

### 2.11. Effect of elastin fibre orientation on membrane elongation

To address whether elastin fibres increase the extensibility of wing membrane as a composite material, we compared the extensibility of wing membrane with contribution from elastin fibres (tested parallel to elastin fibres) and without contribution from elastin fibres (tested perpendicular to elastin fibres). We measured the stretch required to deform samples from a relaxed state to a high-stress state, 1 MPa. The ratio of average extensibility parallel relative to perpendicular to elastin fibres described the effect of elastin and tissue wrinkling on wing extension.

### 3. Results

#### 3.1. Elastin fibre orientation and influence on wing membrane wrinkling

Using polarized light, we determined that elastin fibres are arranged in a generally parallel array, along the axis of wing unfolding, approximately spanwise, in the wing membrane of C. perspicillata (figure 2b). Exceptions included small regions of the wing membrane directly adjacent to wing bones, and areas where elastin fibres branched frequently, such as between metacarpals IV and V. Samples for mechanical testing were not taken from regions with branching fibre architecture, and thus contained only fibre arrays that were close to completely parallel (figure 2c). The elastin fibre network was highly similar in both left and right wings and among all three individuals tested (electronic supplementary material, figure S2).

We found that elastin fibres caused the wrinkled state of the relaxed wing membrane. After removing the ventral epidermis, the remaining dermis and dorsal epidermis of the wing membrane remained wrinkled parallel to the elastin fibres. However, after elastin fibre removal, we no longer observed high spatial-frequency wrinkling of the dorsal epidermis; instead, the wing membrane showed disorganized low spatial-frequency buckling modes (figure 3). With the wing membrane relaxed, elastin fibre removal decreased wrinkle spatial frequency by an order of magnitude. We found similar results when we removed the dorsal epidermis instead of the ventral epidermis.

#### 3.2. Stress–stretch mechanical behaviour: low-stress configurations

Stress–stretch behaviour of the wing membrane was nonlinear. In addition, comparison of stress–stretch curves among samples demonstrates both that the membrane is anisotropic and that variation in curves among samples was relatively high when data are presented in reference to a relaxed configuration (figure 4; electronic supplementary material, figure S3). For all samples, the wing membrane was compliant at low stretch and stiffened with increasing stretch in a nonlinear fashion. Samples tested parallel to elastin fibres remained compliant over a greater range of stretch than samples tested perpendicular to elastin fibres. The stretch required to reach one body weight of tension was highly variable.

#### 3.3. Stress–stretch mechanical behaviour: high-stress configuration

Stress–stretch behaviour of the wing membrane was similar in tests conducted parallel and perpendicular to elastin fibres when results are referenced to sample length at 1 MPa stress (figure 5). For stress exceeding 0.1 MPa, stretch in all samples was similar (standard deviation = 1.1% stretch). At lower stresses, a given stretch, stress was greater in samples tested parallel to elastin fibres than those tested in the perpendicular configuration (figure 5a, inset). Wing membrane anisotropy was not discernible above 0.34 MPa (figure 5b).

#### 3.4. Elastin fibre effect on extensibility

Extension of the wing membrane was greater in samples tested parallel to elastin fibres compared with samples perpendicular to elastin fibres; on average, samples tested parallel to elastin fibres elongated 289% more over the studied range (i.e. to \( \lambda^* \)).
The mechanical behaviour of bat wing membrane skin depends upon a number of structural features, including wing membrane wrinkles, elastin fibres, and the surrounding matrix. We found that wrinkles result from pre-stretched elastin fibres, which compress and buckle the slender wing membrane (figure 3). Parallel and perpendicular to the elastin fibres and wrinkles, bat wing membrane skin was anisotropic, exhibiting greater extensibility and compliance parallel to the elastin fibres (figure 4). We found that anisotropy was not a result of the matrix properties, as stress–stretch behaviour of the matrix showed isotropy once parallel- and perpendicular-fibre samples were normalized to approximately the same state of matrix stress/stretch (figure 5). Therefore, elastin fibres are responsible for wing membrane anisotropy and provide the wing membrane skin with its dramatic spanwise extensibility and initial compliance.

Our results provide a simple and tractable model for wing membrane skin, which can be considered a two-element composite of parallel, pre-stretched, compliant elastic fibres that are embedded in a stiffer but buckled isotropic matrix when the wing is not under load. With this architecture, elastin fibres provide an initial compliant phase when wing membrane is loaded in tension, until the wrinkles in the isotropic matrix are unfolded. When stretch reaches a level at which the wrinkles have dissipated, the stiffer matrix dominates wing membrane mechanical behaviour, and the higher stiffness portion of the stretch–stress curve is reached. Below, we address the biphasic behaviour of bat wing membrane parallel to elastin fibres, and discuss the likelihood that this provisional interpretation of wing membrane mechanics is applicable to other bat species. Finally, we discuss how this wing membrane architecture may relate to the dynamics of the wing membrane during bat flight.

### 4.1. Elastin fibres: orientation and effect on wing wrinkling

Elastin fibres are necessary for the characteristic wrinkling of wing membranes: following their removal, the wrinkles dissipate (figure 3), demonstrating that membrane wrinkles are not an intrinsic part of the matrix structure. Instead, wrinkles result from compressive buckling imposed on the thin matrix by elastin fibres. Although the pattern of skin wrinkling observed in bat wings is unique, our interpretation of elastin architecture, that it is pre-stretched relative to its surrounding matrix, is consistent with its architecture in the skin of other mammals [18,31]. It is because elastin is pre-stretched relative to the matrix that it can be responsible for the stress–stretch behaviour at low stress in bat wing membrane skin [16]. Further, because the fibres and wrinkles have a distinct orientation, the matrix wrinkles create an abundance of excess length along the length of the fibres that must be accounted for when measuring the degree of anisotropy.

The pattern of spanwise-oriented elastin fibres may be broadly, and possibly universally, distributed among bats. All bat wings undergo large spanwise deformations during wing retraction in flight, which would be aided by the extensibility provided by elastin. Spanwise elastin fibres have been reported in bat species from diverse families, including Vespertilionidae, Rhinolophidae, Pteropodidae and Molossidae [9,10,12,32,33]. However, many of these species have not been described in detail, so there may be exceptions to this pattern at the species level. In particular, variation may occur within localized anatomical regions of the wing, such as the reticulated elastin fibre network between metacarpals IV and V in C. perspicillata (figure 2b), or parts of the armwing in Tadarida brasiliensis [12]. Nonetheless, the wing membranes of all bats studied to date are more extensible in the spanwise than the chordwise direction, and this is consistent with elastin fibres and their associated wrinkles running in a similar orientation, and suggests an evolutionarily conserved pattern [15].

### 4.2. Stress–stretch behaviour: anisotropic effect of elastin at low-stress configurations

The stress–stretch behaviour of wing membrane skin exhibited substantial anisotropy within the plane of the membrane when measurements were referenced to a relaxed configuration (figure 4). In particular, samples tested parallel to elastin fibres display compliance over a larger range of stretch values than samples of perpendicular fibre orientation. The observed stress–stretch behaviour of samples tested parallel to elastin fibres was close to biphasic, and, after an extended compliant phase, behaved much like that of samples tested perpendicular to elastin fibres. This biphasic behaviour is consistent with a typical role of elastin in diverse biological tissues: contracting the resting state of the material and providing a compliant...
phase over the contracted length \([16–18]\). Hence, comparison of mechanical behaviour in parallel versus perpendicular orientations of relaxed skin is functionally a comparison of mechanical behaviour of elastin fibres to matrix. Therefore, sample length parallel to elastin fibres approximately equals elastin fibre length and thus provides an underprediction of the matrix length until higher loads are applied, and the matrix is unwrinkled.

4.3. Matrix: isotropic and stiff

Stress–stretch data for bat wing membrane skin referenced to a high-stress configuration demonstrate that the matrix is approximately isotropic. Normalizing sample length to that at moderately high stress (1 MPa) accounts for the compliant phase of elastin fibres as well as for matrix unwrinkling. Employing this approach, we found that the nonlinear stress–stretch behaviour of samples tested perpendicular to elastin fibres were strikingly similar (figure 5). Differences in mechanical behaviour between the two orientations remained only at low stress or stretch, where the contribution of elastin to load bearing was not negligible (figure 5 inset). Matrix isotropy is consistent with its mechanical constituents, such as collagen, being randomly oriented, which has been observed using histological techniques in the matrix of another bat species [13]. A lack of preferential orientation in load-bearing matrix elements would produce the uniform response that we observed.

Elastin typically dominates mechanical behaviour at low stress in a variety of tissues [16–18]. This observation guided our analytical approach to identifying matrix isotropy, although elastin is not commonly found in large homogeneous bundles within skin outside of bat wing membranes (the only exception that we are aware of is the ventral groove blubber in rorqual whales [34]). We hypothesized that the contribution by elastin fibres to load bearing was negligible at moderately high stress, and thus we employed sample length-normalization instead of accounting for elastin fibre contribution explicitly. Had we found anisotropy at the high-stress configuration, we could not have distinguished whether it arose from anisotropy in the membrane matrix and/or from elastin fibres. However, our measurements relative to the high-stress configuration demonstrate that load bearing by elastin fibres can be considered negligible at higher stresses and that the matrix is mechanically isotropic (figure 5).

It is not surprising that the mechanical role of elastin at higher stresses is small in bat wing membrane skin. Elastin is one to two orders of magnitude more compliant than mammalian skin as a whole [29], and its fibre volume fraction is not large (as apparent in [2,13]). However, although the macroscopically visible fibres in bat wing membranes are composed predominantly of elastin, they possess a small fraction of collagen inside and around the periphery of the fibre bundle [2,12]. The function of this collagen is unknown. The collagen fibrils could subtly modulate the stress–stretch behaviour at a finer scale than our present methodology can resolve, or serve other functions, such as increasing fibre yield strength. Further analyses, including more detailed study of tear propagation and failure in wing membranes, could address this latter hypothesis.

4.4. Elastin contribution to stretch

Elastin fibres delay the onset of stretch in the matrix, suggesting that a key role of elastin fibres is to enhance membrane elongation. By imposing high spatial-frequency wrinkles on...
the membrane (figure 3), elastin fibres allow the wing to collapse its large surface area into a relatively small volume (electronic supplementary material, figure S4). This, in turn, allows the unfurling wing membrane to stretch much more parallel with than perpendicular to elastin fibres. Human-engineered composites often use a similar design to allow a wrinkled/buckled element to undergo displacements without deforming and failing, such as in shirred fabrics and flexible electronics [35]. As a consequence of the elastin fibre architecture in the bat wing, wing membrane skin at the whole tissue level may reach stretch values that exceed the yield stretch of the matrix.

4.5. The role of elastin in wing membrane aeromechanics

The wrinkles of the bat wing membrane result from the length of the matrix exceeding the length of the elastin fibres. This architecture is essentially analogous to matrix slack, but we describe it as excess length following Rojalsirikul et al. [6]. The effect of excess length in membrane aeromechanics is to increase membrane deflection, which can manifest as various wing reconfigurations depending upon how the wing membrane is anchored along the edges [6,8]. If the leading and trailing edges are anchored, increased camber results [3,5]. However, if the trailing edge is free to deflect, as in the bat armwing, the wing will twist, which can result in decreased lifting efficiency but also have the beneficial effect of reducing sensitivity to gusts [36]. Thus, interactions between elastin fibres and matrix may be critical determinants of three-dimensional wing form and aerodynamic performance of bats during flight.

Elastin fibres may also have important functional roles other than interaction with the wing membrane skin matrix. The fibre–matrix interaction dictates that elastin fibres within the wing membrane are under greater stretch than the surrounding matrix, and can experience net tension even when the surrounding matrix is buckled in compression (figure 3). As a result of elastin pre-tension and its overall compliance, the wing membrane can stretch notably more parallel to elastin fibres than perpendicular to them; this difference can be almost threefold at 1 MPa stress. Finally, as a consequence of these mechanics, when the wing matrix buckles as the wing is retracted during upstroke, elastin fibres maintain a low level of tension in the wing membrane over much, if not all, of this part of the wingbeat cycle. Increased spanwise tension in membrane wings with free trailing edges results in an overall decrease in membrane deflection, but perhaps more importantly, a decrease in flutter [7]. This effect may be critically important for bats because fluttering can dramatically decrease aerodynamic performance of a membrane wing [8].

4.6. Wing membrane curvature: an interplay between elastin fibres and wing membrane muscles?

Membrane wings interact with aerodynamic pressure to adopt a curved profile, which, depending on aerodynamic conditions, can be beneficial or detrimental [6]. Bats fly within a broad spectrum of flow conditions, and we hypothesize that their membranes have evolved two morphological features to control wing curvature: wing membrane muscles and elastin fibres. The muscles, fully embedded within the wing membrane, activate during downstroke and are hypothesized to

![Figure 6. Schematic summary of findings: bat wing membrane is an anisotropic composite of spanwise pre-stressed compliant elastic fibres embedded in an isotropic stiff matrix with excess length parallel to the fibres. Samples tested perpendicular to elastin fibres display stiff nonlinear stress–stretch behaviour dominated by matrix (left grey line). Samples tested parallel to elastin fibres, undergo a similar stiff phase, but first must proceed through a compliant phase dominated by elastin (black line), which then transitions into matrix-dominated mechanical behaviour (black line transitions into right grey line). Once excess matrix length has unfurled, mechanical behaviour is approximately isotropic.](http://rsif.royalsocietypublishing.org/content/12/104/1286.full.pdf)
aeromechanical role of each of these units can be better addressed through future studies.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

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