

# Anatomy and Histochemistry of Spread-Wing Posture in Birds. 3. Immunohistochemistry of Flight Muscles and the “Shoulder Lock” in Albatrosses

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**ABSTRACT** As a postural behavior, gliding and soaring flight in birds requires less energy than flapping flight. Slow tonic and slow twitch muscle fibers are specialized for sustained contraction with high fatigue resistance and are typically found in muscles associated with posture. Albatrosses are the elite of avian gliders; as such, we wanted to learn how their musculoskeletal system enables them to maintain spread-wing posture for prolonged gliding bouts. We used dissection and immunohistochemistry to evaluate muscle function for gliding flight in Laysan and Black-footed Albatrosses. Albatrosses possess a locking mechanism at the shoulder composed of a tendinous sheet that extends from origin to insertion throughout the length of the deep layer of the pectoralis muscle. This fascial “strut” passively maintains horizontal wing orientation during gliding and soaring flight. A number of muscles, which likely facilitate gliding posture, are composed exclusively of slow fibers. These include *Mm. coracobrachialis cranialis*, *extensor metacarpi radialis dorsalis*, and *deep pectoralis*. In addition, a number of other muscles, including *triceps scapularis*, *triceps humeralis*, *supracoracoideus*, and *extensor metacarpi radialis ventralis*, were found to have populations of slow fibers. We believe that this extensive suite of uniformly slow muscles is associated with sustained gliding and is unique to birds that glide and soar for extended periods. These findings suggest that albatrosses utilize a combination of slow muscle fibers and a rigid limiting tendon for maintaining a prolonged, gliding posture. *J. Morphol.* 263:12–29, 2005.

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**KEY WORDS:** flight; albatross; functional morphology; immunohistochemistry

Compared to flapping flight, gliding and soaring flight has been shown to be more efficient in a number of ways. First, measurements of oxygen consumption have shown that gliding is only about twice as costly as resting (Baudinette and Schmidt-Nielsen, 1974), whereas flapping flight incurs a cost of about seven times that of resting (Tucker, 1972). Second, the heart rate of gliding pelicans was found to be about 150 beats per minute (BPM), compared with 190 BPM for birds flapping at 50 m (Weimerskirch et al., 2001). Third, Goldspink et al. (1978) and Meyers (1993) showed that gliding flight in Herring Gulls (*Larus argentatus*) and American Kestrels (*Falco sparverius*), respectively, requires less muscle activity than flapping flight as de-

termined by electromyography. Gliding is a more energy-efficient form of locomotion than flapping flight because fewer muscle fibers are required to be active.

Gliding and soaring flight are static forms of locomotion in which the wings are held stationary in a horizontal position, while movement of the atmosphere provides much of the necessary energy (Norberg, 1985; Pennycuick, 1989). The gliding or soaring bird must be able to maintain its outstretched wings and resist the force of air from below and in front, as well as support its body mass. The use of static, isometric contractions are better suited for such postural roles, due to the longer actin–myosin interaction and the reduced number of ATP molecules needed per second for repriming the cross-bridges. Thus, slow muscle fibers are more efficient for such postural activities (Goldspink, 1980, 1981).

A correlation between avian muscle fiber histochemistry and muscle function has been described previously (e.g., Simpson, 1979; Maier, 1983; Rosser and George, 1985; Welsford et al., 1991; Meyers, 1992a, 1993; Sokoloff et al., 1998), in that muscles thought to be used for activities such as locomotion have greater proportions of fast-twitch fibers, and muscles thought to have a postural role have higher proportions of slow fibers. Extensive studies of various birds (Rosser and George, 1986a,b; Rosser et al., 1994), indicated that a number of taxa possess slow-contracting muscle

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fibers presumed to function in posture, gliding flight, or underwater swimming.

Avian slow muscle fibers can be classified by morphological, physiological, or biochemical criteria as slow tonic (ST) or slow-twitch oxidative (SO; see Goldspink, 1981; Hikida, 1987; Rosser et al., 1987; Williams and Dhoot, 1992). With respect to other physiological and morphological characteristics, ST fibers fail to respond to single nerve impulses, slowly shorten when stimulated repetitively (Morgan and Proske, 1984), and have multiple “en grappe” nerve terminals (Hess, 1967), whereas slow-twitch (and fast-twitch) muscle fibers generate an action potential and exhibit a rapid rise in tension after nerve stimulation (Morgan and Proske, 1984; Torrella et al., 1993). Histochemically, avian SO fibers react like mammalian SO fibers, but avian SO fibers, like slow tonic fibers, are multiply innervated (Baier and Gatesy, 2000). Recent work on the biochemistry of avian myosin (Bandman and Rosser, 2000) has shown that birds possess nine myosin heavy chains (MyHC): five fast and four slow/cardiac. Two slow MyHCs, MyHC1 and MyHC2, have been found in avian slow muscles (typically studied are the chicken *M. latissimus dorsi pars anterior* and the “red-strip”<sup>1</sup> of the pectoralis), with most mature fibers expressing MyHC2 only (Bandman and Rosser, 2000). The deep layer of the pelican’s pectoralis exhibits a slow myosin isoform (SM) similar to that of the chicken *latissimus dorsi* (Rosser et al., 1994; Bandman and Rosser, 2000). These slow myosins do not appear to be homologous with those in mammals (Bandman and Rosser, 2000).

Albatrosses are elite avian gliders. These birds, some with wing spans of up to 12 feet, spend a great amount of time gliding on the winds of the oceans, presumably for months (Goldspink et al., 1978). They are considered to be among the most economical energy users among flying birds (Costa and Prince, 1987), possessing a metabolic cost during soaring (plus take-off and landing) of only about three times their basal metabolic rate (BMR). In comparison, Sooty Terns and Ring-billed Gulls show increases of 4.8 and 7.5 times their BMR when flying (Costa and Prince, 1987). Clearly, this relates to the amount of time albatrosses spend soaring in comparison to the flapping flight of these other species. Further, Adams et al. (1986) found that the energy used by Wandering Albatrosses was 1.83 times BMR, the lowest value measured among breeding birds. This economy is related to the soaring lifestyle of albatrosses and to the anatomical specializations they possess.

A wide variety of avian taxa (including albatrosses, petrels, pelicans, frigatebirds, cormorants, storks, cranes, cathartid vultures, and various others) possess a deep layer of the pectoralis muscle (the deep pectoralis; see Meyers and Mathias, 1997, and references therein). It has been suggested that the deep layer of the pectoralis should be a slow tonic muscle to aid in gliding (Pennycuick, 1972; Meyers, 1993), and Pennycuick (1972) calculated that the energy used by the deep pectoralis of vultures is 5.86 kcal/h. The deep pectoralis muscle of vultures and pelicans was studied histochemically by Rosser and George (1986a) and Rosser et al. (1994), respectively, and was found to be composed of slow muscle fibers. Rosser and co-workers indicated that the deep pectoralis is specialized for gliding and soaring flight and the superficial pectoralis is specialized for flapping flight. However, very little is known about the fiber types in the pectoralis muscles of most soaring birds (Meyers, 1993), and nothing is known about the fiber types of their other flight muscles.

Pennycuick (1982) discovered a shoulder locking mechanism in albatrosses and giant petrels and proposed that the deep pectoralis in these birds need not be composed of slow fibers. This was contrary to his previous prediction (1972; see above) regarding the fiber type of the deep pectoralis. He described the lock as being made up of a tendinous sheet within the superficial pectoralis and suggested that because of the lock, the deep pectoralis in albatrosses and giant petrels should be a “sprint” muscle (FG/fast-fatigable/white) and presumed it to function during high-frequency wing movements observed during landing. According to Pennycuick, the lock restricted movement above the horizontal when the wing is moved into a fully protracted position, but releases when retracted a few degrees. In addition, an elbow lock was reported by Hector (1894). This was rejected and replaced by an elbow “fixing” mechanism (Joudine, 1955; Yudin, 1957).<sup>2</sup>

We had two principal goals in this study. First, we wanted to quantify the presence of slow muscle fibers within muscles that we believe are associated with gliding and soaring behavior in albatrosses using immunohistochemical techniques. Meyers and Mathias (1997) predicted that larger gliding birds, such as albatrosses, pelicans, and vultures, would have more muscle fibers devoted to posture and distinct muscles relegated to a postural role. Second, we sought to describe the locking mechanism of the shoulder by anatomical investigation. Preliminary results of this work were presented in Stakebake and Meyers (2000).

<sup>1</sup>The “red strip” is a well-studied zone of slow tonic muscle fibers in the deep part of the pectoralis of chickens. It represents a remnant of slow myosins that are more widespread during early pectoralis development (see Matsuda et al., 1983).

<sup>2</sup>The articles cited as Joudine (1955) and Yudin (1957), written in French and Russian, respectively, are in fact by the same person. Both articles share identical figures and were written at the Zoological Institute in Leningrad.



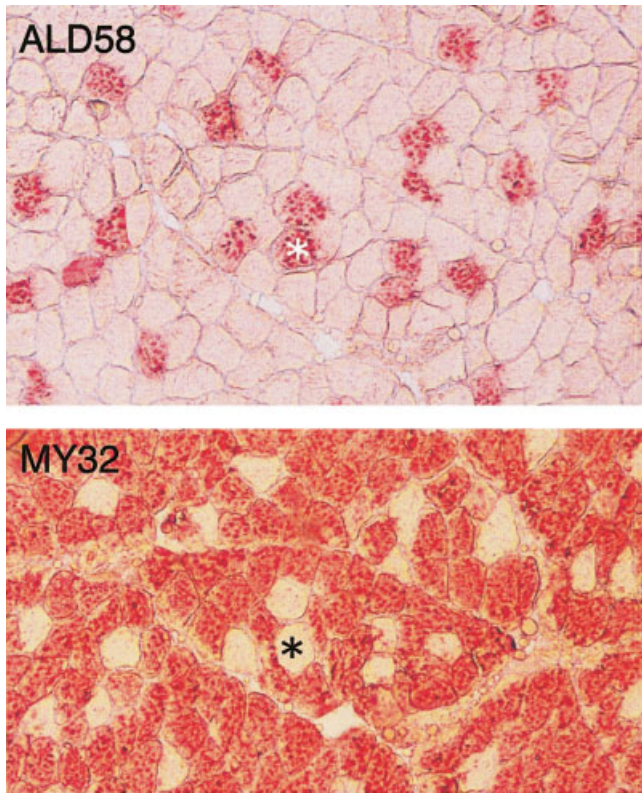


Fig. 1. *Diomedea immutabilis*. Serial sections of *M. extensor metacarpi radialis pars ventralis* to illustrate ALD 58 and MY32 antibody reactions in the Laysan Albatross. A positive reaction for the anti-slow ALD58 antibody (top) results in red-stained fibers (asterisk), whereas a negative reaction for a homologous fiber results in no color with the anti-fast MY32 antibody (bottom). Note that there is no overlap of reaction with these two antibodies.

## MATERIALS AND METHODS

Two Laysan (*Diomedea immutabilis*) and two Black-footed (*D. nigripes*) Albatrosses were dissected to study muscle attachments, muscle fiber types, and biomechanical functions. Fresh specimens salvaged by the United States Fish and Wildlife Service (USFWS), were received and stored in freezers prior to immunohistochemical and anatomical examination. Muscle fiber types were assayed using the histochemical protocols of Hermanson and Cobb (1992). After thawing, muscles on one side of the birds were removed, cut perpendicularly to fascicle orientation at mid-belly, and mounted on cork blocks with 5% gum tragacanth. The muscles were then frozen in 2-methylbutane, cooled with liquid nitrogen to approximately  $-150^{\circ}\text{C}$ , and stored at  $-70^{\circ}\text{C}$  prior to sectioning at  $12\ \mu\text{m}$  in a cryostat (Tissue-Tek II, Microtome/Cryostat, models 4551 and 4553, respectively) set at  $-20^{\circ}\text{C}$ . Slow fibers were analyzed by reaction with ALD 58 (University of Iowa, Hybridoma Bank), an antibody that labels both slow-twitch and slow-tonic fibers in mammals (Han et al., 1999) as well as birds (Meyers, 1997). Because ALD 58 reacts with both slow-twitch and slow-tonic bird muscle fibers, no discrimination between the two was possible. Fast fibers were identified with MY 32 (Sigma Chemical, St. Louis, MO), an antibody that labels fast-twitch fibers in mammals and birds. Figure 1 illustrates the typical reactivity observed with both antibodies for fast and slow fibers. Samples were reacted against the antibodies in a humidified chamber at  $4^{\circ}\text{C}$  for 16–18 h (ALD 58) or at  $25^{\circ}\text{C}$  for 2 h (MY 32).

After rinsing in phosphate-buffered saline, samples were incubated in goat antimouse antibody and stained with streptavidin peroxidase system (SPS kit; Zymed Labs, San Francisco, CA) (Meyers and Mathias, 1997). Overlapping black and white photographs (Olympus PM 10AD microphotography system or Nikon Coolpix 995 Digital camera with ocular adapter on an Olympus BH-2 compound microscope at  $40\times$  or  $100\times$ ) of prepared muscle cross-sections were taped together to create a montage of whole muscles or whole sample sections of muscles, and fiber types within each muscle or muscle sample were counted. The percentage of fiber types (fast vs. slow) of whole muscle sections (six muscles) or samples of larger muscles (four muscles), and the standard deviations were calculated (see Table 1). Muscle cross-sectional areas were estimated by tracing the muscle shapes from glass slides and transferring the outline to graph paper. Muscle outlines were cut out and weighed and compared to the mass of a square cm of the graph paper. Simpson's Rule (Thomas and Finney, 1996) was used to verify the calculations. Muscle areas (see Table 2) are provided to illustrate the range of muscle size and to account for the resulting variability of muscle fiber numbers. Since Laysan and Black-footed Albatrosses are closely related (Kuroda et al., 1990; Robertson and Nunn, 1998), we pooled fiber numbers from both species when calculating means.

Muscles were studied and examined with regard to their anatomy and actions and divided into the following functional categories.

### Body Support

This refers to postural musculature that prevents undesirable wing elevation and also functions to keep the body from "falling through the wings" (Meyers, 1993). Here, *M. pectoralis*, superficial layer (SP), and deep layer (DP), as well as the locking mechanism associated with the pectoralis, were examined from four individuals. Because of the size of DP and SP, samples were taken from throughout the bellies of these muscles. The locking mechanism of the shoulder was studied by wing manipulation of fresh-frozen-thawed specimens and by dissection. The object was to identify the components of the lock, the sites of muscle and tendon attachment, and joint mechanics associated with the shoulder.

### Wing Elevation

Wing elevation is critical to flight. Like the wings of an aircraft, bird wings also need to be held horizontally during gliding flight. In birds, this position is primarily maintained by atmospheric movement and muscles that elevate and hold the wings at the horizontal. Meyers (1993) reported activity of *M. supracoracoideus* (SC) during gliding flight in kestrels. The present study examined *Mm. supracoracoideus* (SC) and *deltoideus major* (DM) as the principle wing elevators from three birds. Whole cross-sections were taken of both heads of DM, in addition to samples from all three heads of SC.

### Wing Protraction

These are muscles responsible for pulling the wing cranially and resisting forces caused by the air pushing against the leading edge of the wing. We studied *Mm. coracobrachialis cranialis* (CBC) and the cranial edge fascicles of the superficial layer of *M. pectoralis* (PCr). Meyers (1993) reported activity of the cranial edge of the pectoralis during gliding flight in kestrels. Due to its mechanically advantageous position, it is a protractor of the wing (Fisher, 1946; Stegmann, 1964; Meyers, 1993). Whole cross-sections of CBC from four individuals were used; tissue blocks averaging  $48\ \text{mm}^2$  were removed from the cranial border of the superficial layer of the pectoralis from three birds (see Table 2).

### Wing Extension

These muscles extend or straighten the wing at the elbow and wrist joints, and also participate in the automatic extension-

TABLE 1. Total fiber numbers, mean number of slow fibers, and relative slow fiber percentages, for nine muscles from Laysan and Black-footed Albatrosses, *Diomedea immutabilis* and *D. nigripes*

Muscle	Bird*	# of Fibers		Mean # slow fibers $\pm$ SD%	% Slow fibers fibers $\pm$ SD%	Mean % Slow fibers $\pm$ SD%
		slow	fast			
M. pectoralis, deep layer	1	N/A	0	—	100	100
	2	N/A	0	—	100	
	3	N/A	0	—	100	
	4	N/A	0	—	100	
M. supracoracoideus, coracoid head	2	3329	22123	2985 $\pm$ 691	13	14 $\pm$ 1
	3	2020	11152		15	
	4	3605	22887		14	
M. deltoideus major, pars cranialis:	2	636	13253	4511 $\pm$ 4332	5	13 $\pm$ 11
	3	2339	24570		9	
	4	10559	30742		26	
M. pectoralis, cranial fascicles	2	5688	10735	13806 $\pm$ 7189	35	38 $\pm$ 10
	3	23165	23926		49	
	4	12564	30107		29	
M. triceps humeralis	4	5966	N/A			
M. triceps scapularis	1	4538	N/A			
	4	21552	N/A			
M. extensor metacarpi radialis, pars ventralis:	1	7939	11624	7442 $\pm$ 3479	41	40 $\pm$ 13
	2	6111	23585		21	
	3	3061	2938		51	
	4	12657	14906		46	
M. extensor metacarpi radialis, pars dorsalis:	1	3614	0	2937 $\pm$ 971	100	99 $\pm$ 2.0
	2	3135	97		98	
	3	3702	0		100	
	4	1298	0		100	

\*Birds 1 and 2 are *D. immutabilis*, 3 and 4 are *D. nigripes*.

flexion mechanism of the avian wing (Fisher, 1957; Vazquez, 1994). We examined whole cross-sections of the two heads of M. triceps brachii, the triceps humeralis (TH) and triceps scapularis (TS) from three birds. Meyers (1993) reported activity of these elbow extensor muscles during gliding flight in kestrels.

In addition, whole cross-sections of the two wrist extensors, Mm. extensor metacarpi radialis pars dorsalis (EMRd) and extensor metacarpi radialis pars ventralis (EMRv), were examined from four individuals.

## RESULTS

### Body Support

**Anatomy.** The superficial layer of the pectoralis (SP) originates from the keel and body of the sternum, the furcula, and the sterno-coraco-clavicular membrane. A narrow Fascia pectoralis (Meyers, 1992b) extends along the length of the origin, adjacent to the keel, and provides additional surface

for fiber attachment (Fig. 2). The middle one-third of the muscle has a combination of tendinous and muscular fibers on its surface. The varied fiber orientation is well known (see Dial et al., 1988), with craniodorsal fascicles oriented transversely (to protract the wing), cranioventral fascicles oriented dorsoventrally (to depress the wing), and caudoventral fascicles oriented from caudoventrally to craniodorsally (to retract the wing) (Boggs and Dial, 1993).

The SP inserts by a thick, dense tendon onto the base of the deltopectoral crest (Fig. 2), just cranial to the insertion of the deep layer (Fig. 3). It also attaches onto the biceps brachii tendon where the latter attaches onto the bicipital crest of the humerus (Figs. 3, 4). Such an attachment of SP to the

TABLE 2. Muscle areas, for eight muscles studied from Laysan and Black-footed Albatrosses, *Diomedea immutabilis* and *D. nigripes*

Muscle	Bird*				Mean (mm <sup>2</sup> ) $\pm$ SD
	1	2	3	4	
Supracoracoideus, coracoid head	41	28	30	33	33.0 $\pm$ 5.7
Deltoideus major, pars cranialis	N/A	14	41	59	38.0 $\pm$ 22.6
Pect thoracicus, cranial fascicles	N/A	28	60	55	47.7 $\pm$ 17.2
Coracobrachialis cranialis	7	9	10	13	9.8 $\pm$ 2.5
Triceps humeralis	N/A	56	70	58	61.3 $\pm$ 7.6
Triceps scapularis	116	66	109	68	89.8 $\pm$ 26.4
Extensor metacarpi radialis, pars ventralis	21	26	10	25	20.5 $\pm$ 7.3
Extensor metacarpi radialis, pars dorsalis	9	10	10	10	9.8 $\pm$ 0.5

\*Birds 1 and 2 are *D. immutabilis*, 3 and 4 are *D. nigripes*.

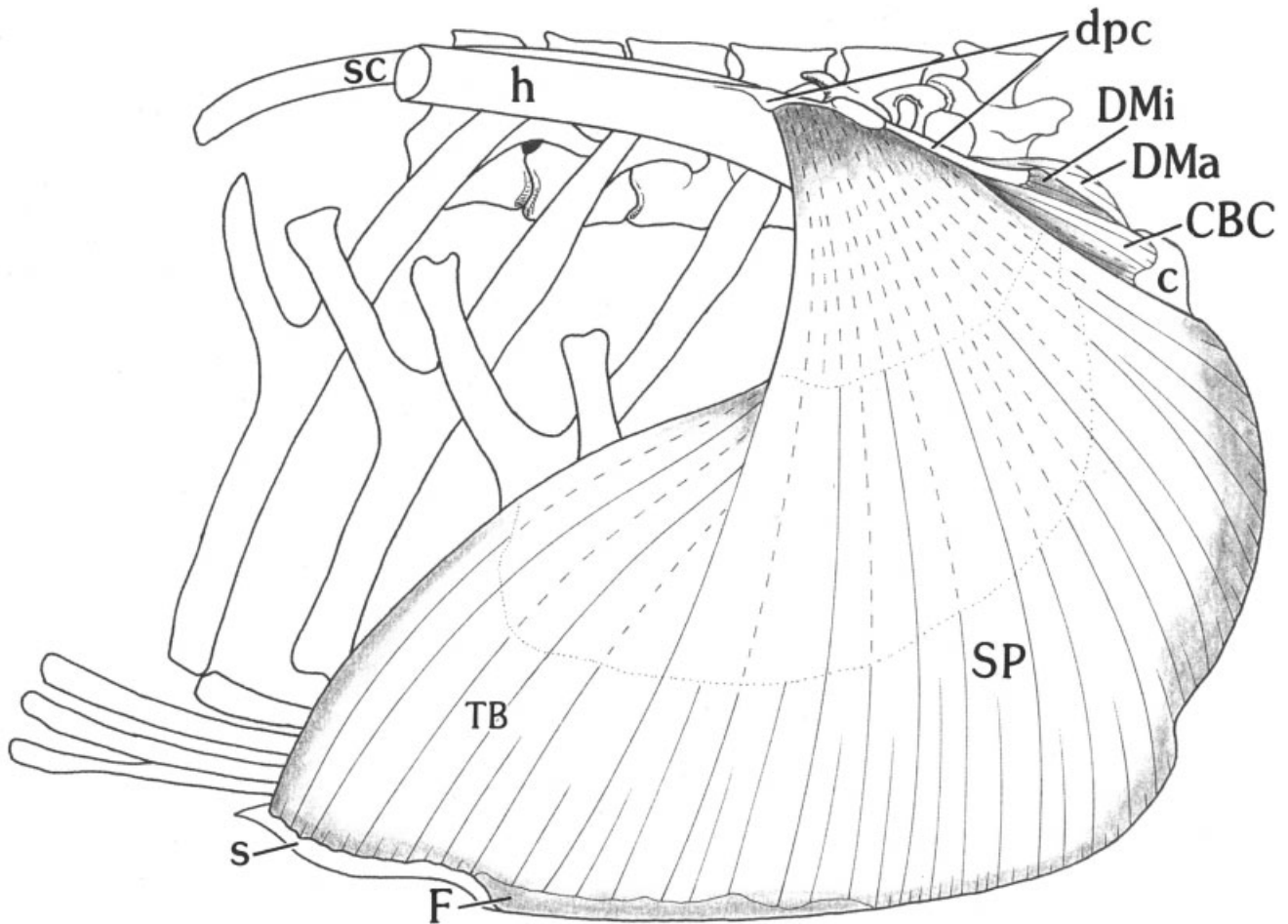


Fig. 2. *Diomedea immutabilis*. Lateral view of the right thoracic wall and shoulder girdle of the Laysan Albatross, showing the superficial pectoralis and coracobrachialis cranialis muscles. Propatagialis muscles have been removed. The fine dotted line on the pectoralis about one-third from the origin represents a transition from muscle fibers to a mixture of muscle and tendon. The line about two-thirds from the origin indicates a transition to a fully tendinous region. Similar patterns are also visible on other figures. c, coracoid; CBC, M. coracobrachialis cranialis; DMa, M. deltoideus major; DMi, M. deltoideus minor; dpc, deltopectoral crest; F, Fascia pectoralis; h, humerus; s, sternum; sc, scapula; SP, M. pectoralis thoracicus, superficial layer, sternobrachialis portion; TB, thoracobrachialis portion of superficial pectoralis.

biceps tendon is not uncommon in birds (see George and Berger, 1966; Meyers, 1992c, 1993).

The deep layer of the pectoralis (DP) lies between SP and the supracoracoideus and is a complex muscle, divided into two unequal parts by the blood vessels passing from the subclavian artery to the superficial layer through a foramen in the muscle (Fig. 4). The cranial part makes up most of the deep layer and originates from the furcula, sternal keel, and sterno-coraco-clavicular membrane. Most of the muscle fascicles of this part converge and form a flat tendon that passes deeply to the SP and inserts onto the base of the deltopectoral crest, just caudal to the insertion of SP (Figs. 3, 4).

The caudal part is oval in shape and arises from the caudal aspect of the sternum (Fig. 4). It has a tendinous surface and inserts with the biceps tendon onto the bicipital crest of the humerus. At this

location it is joined by fascicles of the SP that also insert onto the biceps tendon. The tendinous insertions of the two parts of DP are continuous with each other (Fig. 4).

The superficial fascia of DP is relatively thick and extends from origin to insertion across the entire surface of the muscle. Relatively short muscle fascicles insert at an angle on an internal tendon that parallels the superficial fascia throughout the length of the muscle from origin to insertion (Fig. 3). This internal tendon and thickened fascia have a ligamentous function and make up the "shoulder lock," which passively limits wing elevation above the horizontal.

**Immunohistochemistry.** All samples of DP from four birds reacted against ALD 58 revealed a uniform pattern of slow muscle fibers (Fig. 5). In contrast, all samples of SP revealed uniform fast fibers after reaction against both antibodies (Fig. 5),



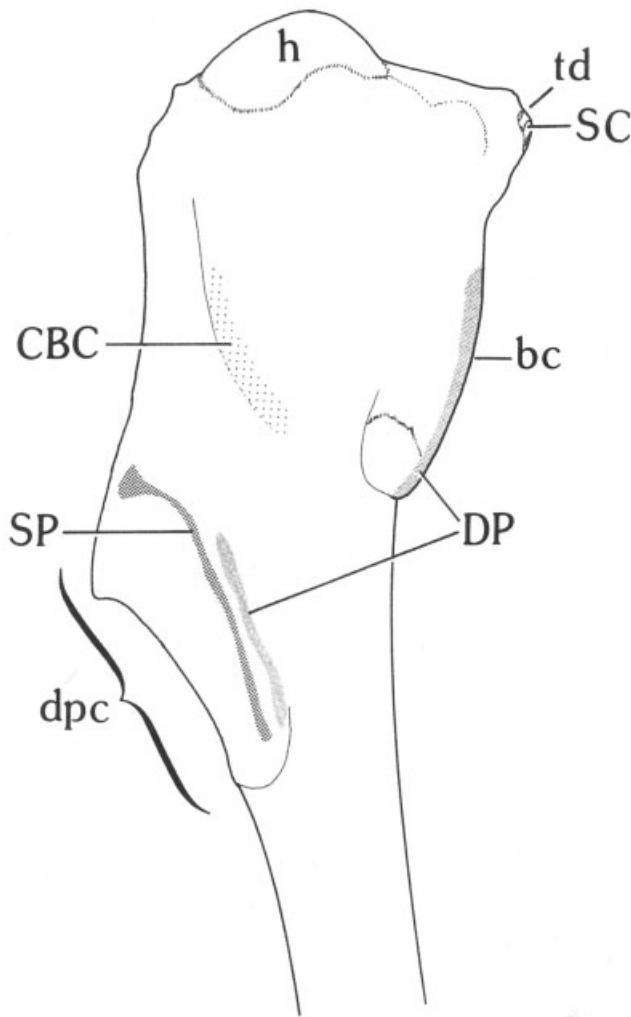


Fig. 3. *Diomedea immutabilis*. Ventral surface of right humerus of the Laysan Albatross, showing muscle attachments onto the proximal end. In the avian anatomical position, cranial is to the left and lateral to the bottom of the page. bc, bicipital crest; CBC, insertion of *M. coracobrachialis cranialis*; dpc, deltopectoral crest; h, humeral head; SC, insertion of *M. supracoracoideus*; DP, insertion of *M. pectoralis*, deep layer; SP, insertion of *M. pectoralis thoracicus*, superficial layer; td, tuberculum dorsale.

with the exception of the cranial border of SP, discussed below.

### Wing Elevation

**Anatomy.** The *M. supracoracoideus* (SC) has three distinct heads originating from the sternum, coracoid, and furcula (Fig. 6). All three heads converge to form a thick tendon that inserts onto the tuberculum dorsalis along the deltopectoral crest of the humerus (Fig. 3). A fourth “deep” part of SC was described and illustrated in *Diomedea nigripes* by Kuroda (1960), who also cited its existence from Forbes (1882). Subsequent articles (Kuroda, 1961; George and Berger, 1966; McKittrick, 1991) refer to a

deep layer or four-part SC in albatrosses, citing each other or Kuroda (1960). Vanden Berge and Zweers (1993) identified this deep layer, which arises from the sterno-coraco-clavicular membrane, as being the ventral head of *M. deltoideus minor* (see Fig. 6). This was supported by Kovacs and Meyers (2000), who noted that this deep muscle in the puffin *Fratercula arctica* is innervated by the axillary nerve and is more properly considered a part of *M. deltoideus minor*.

The *M. deltoideus major* (DM) extends from the cranial scapula to the dorsal surface of the pectoral crest and adjacent proximal shaft of the humerus (Fig. 7). A larger cranial head makes up most of the muscle; the caudal head lies along the caudal border of the latter. A typical scapular anchor attaches to the DM (Meyers, 1992c; Fig. 7). Both SC and DM act to elevate the wing.

**Immunohistochemistry.** All three heads of SC as well as both of DM possessed numerous, evenly distributed populations of slow fibers throughout these muscles (Fig. 8). The coracoid head was arbitrarily selected for fiber quantification and was found to possess  $14 \pm 1\%$  slow fibers (Table 1). The cranial head of DM was chosen likewise and possessed  $13 \pm 11\%$  slow fibers. This is the first known, documented description of slow fibers in SC or DM in any bird.

### Wing Protraction

**Anatomy.** The cranial edge of the superficial layer of the pectoralis (PCr) has fibers that cross the cranial aspect of the shoulder joint and are positioned to protract the wing (Fig. 2). Species with highly bowed furculae (e.g., alcids) typically utilize these cranial fibers for wing protraction during flapping flight (see Stegmann, 1964).

The *M. coracobrachialis cranialis* (CBC) lies at the deep aspect of the cranial shoulder joint and extends from the coracoid to the cranial surface of the humerus. It is a thin, flat muscle, arising from the processus acrocoracoideus of the coracoid, and crosses over the cranial surface of the shoulder articulation (Figs. 4, 6). CBC inserts onto the Impressio coracobrachialis of the humerus, deep to *M. pectoralis* (Figs. 2, 3). The CBC is overlain by a dense sheet of collagenous tissue (Lig. acrocoracohumeral) that extends from the muscle's origin to the distal part of the deltopectoral crest (beneath the pectoralis) and also to the bicipital crest (Fig. 6).

**Immunohistochemistry.** The PCr had  $38 \pm 10\%$  slow fibers. In a muscle sample of  $47.7 \pm 17 \text{ mm}^2$  there were an average of 13,806 slow fibers (Table 1). These slow fibers were distributed more densely along the cranial edge of the superficial pectoralis and became more diffuse and less numerous caudoventrally. All samples from birds studied ( $n = 3$ ) revealed a similar pattern of slow fibers (Fig. 8). No

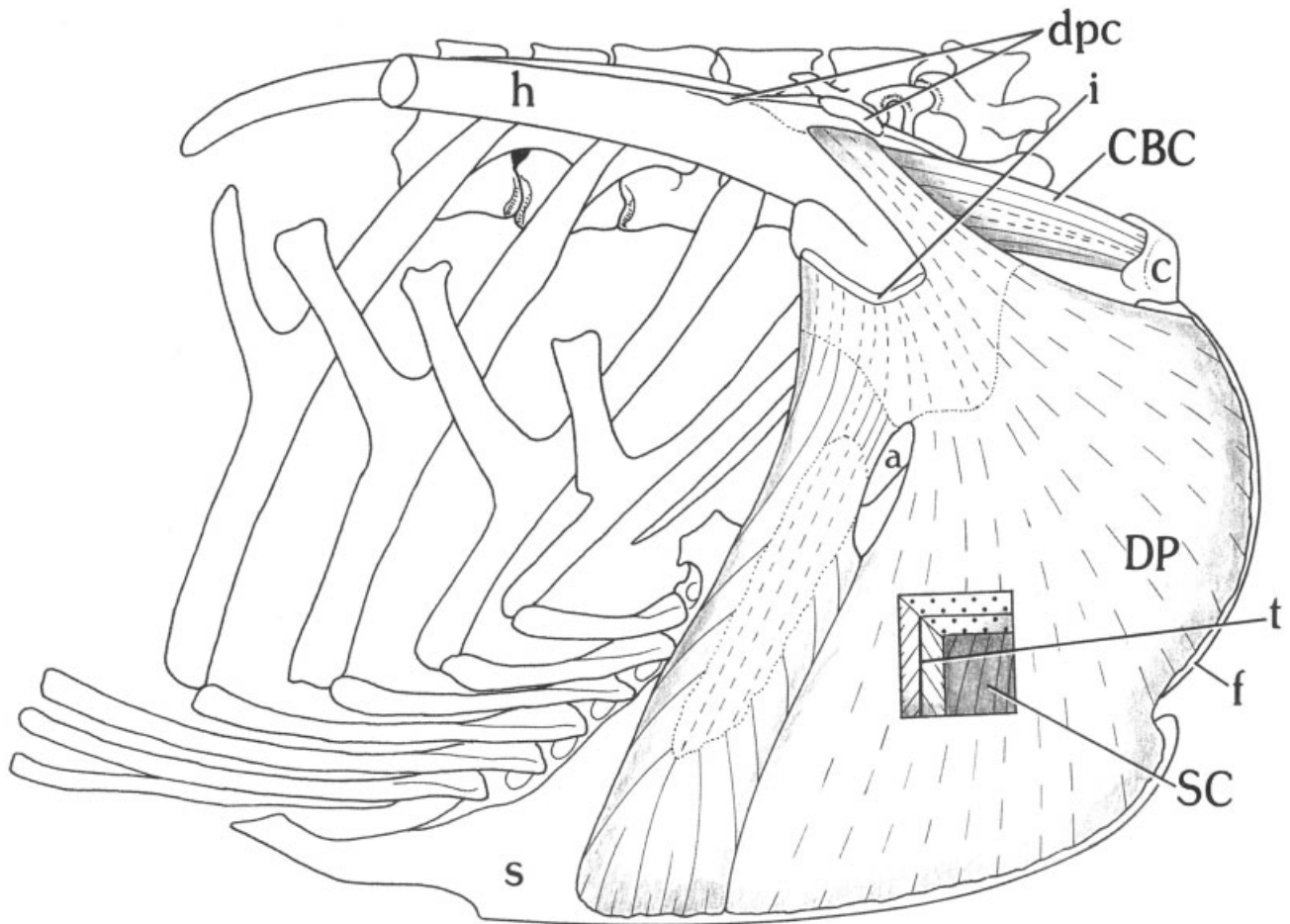


Fig. 4. *Diomedea immutabilis*. Lateral view of the right thoracic wall and shoulder girdle of the Laysan Albatross, showing the deep pectoralis and coracobrachialis cranialis muscles. Superficial pectoralis and deltoid muscles have been removed. A window cut into the deep pectoralis shows its fascicle orientation and the presence of an internal tendinous sheet, which forms the shoulder lock. The SC can be seen deep to the deep pectoralis through the window. a, opening through which blood vessels pass from supplying the superficial pectoralis—it separates the deep pectoralis into cranial and caudal parts; c, coracoid; CBC, M. coracobrachialis cranialis; DP, M. pectoralis, deep layer; dpc, deltopectoral crest; f, furcula; h, humerus; i, area of insertion of the deep pectoralis onto the bicipital crest—the superficial pectoralis inserts here as well; s, sternum; SC, M. supracoracoideus, t, internal tendon of deep pectoralis, which forms the wing lock.

other area of the superficial pectoralis possessed slow fibers.

The CBC was found to be uniformly slow ( $\bar{x} = 6,576 \pm 2,920$  fibers,  $n = 4$ ) (Fig. 8; Table 1).

### Elbow Extension

**Anatomy.** The two parts of *M. triceps brachii* extend the wing at the elbow in birds. The humeral head (triceps humeralis; TH) originates on the humerus near the Fossa pneumotricipitalis. It can be roughly divided into two parts, with different fascicle orientations. The lateral part has fascicles that extend obliquely from the humeral shaft to the tendon, whereas the medial part extends more in parallel with the tendon. The triceps humeralis inserts distally on the olecranon of the ulna (Fig. 9).

The scapular head (triceps scapularis; TS) arises by a narrow, round area on the lateral surface of the

scapular shaft. An additional attachment, via a “scapular anchor” (Retinaculum m. scapulotricipitis) anchors the cranial edge of the muscle to the proximal humeral shaft. At its insertion, TS is joined to the antebrachial fascia, and inserts onto the Processus cotylaris dorsalis of the ulna, adjacent to the olecranon (Fig. 9).

The tendons of TH and TS are bound together by loose connective tissue for most of their lengths. The insertions, although distinct, are connected lengthwise.

**Immunohistochemistry.** Both heads of *M. triceps* were composed of both fast and slow fibers (Fig. 10). In TH the slow fibers were evenly distributed throughout the muscle. In TS, the concentration of slow fibers was greatest along the caudal end and the ventral edge, then diminished to almost no slow fibers at the cranialmost end. Due to the large size of

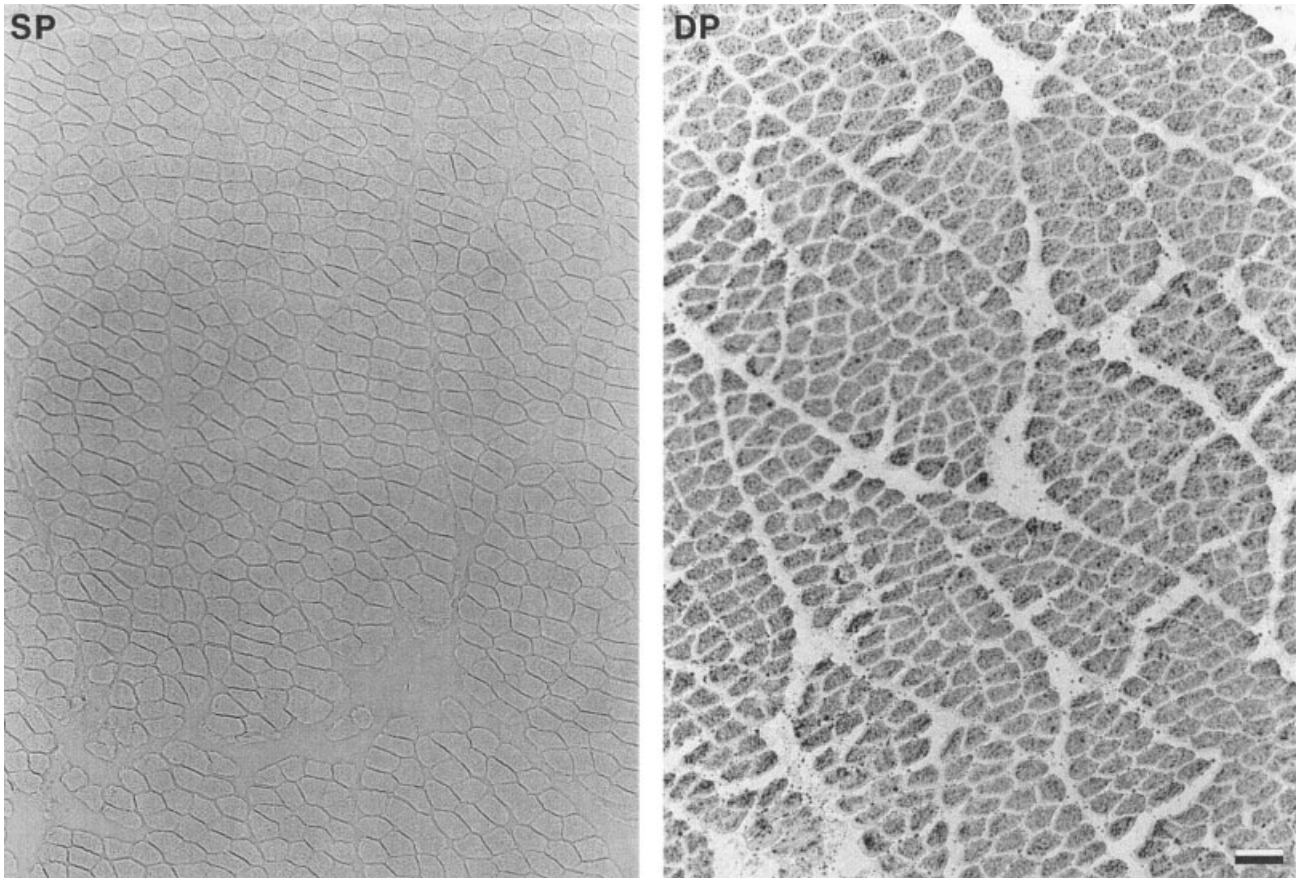


Fig. 5. *Diomedea immutabilis*. ALD 58 antibody reactions in superficial pectoralis (SP) and deep pectoralis (DP) muscles of the Laysan Albatross. Note that the superficial pectoralis has a negative reaction to the slow antibody and is entirely fast, whereas the deep pectoralis has a uniformly positive reaction and is entirely slow. Scale bar = 100  $\mu$ m.

the muscle cross-sections (TH:  $61 \pm 7 \text{ mm}^2$ ; TS:  $90 \pm 26 \text{ mm}^2$ ; see Table 2) slow fibers only were quantified from three muscles. In one bird (*D. nigripes*), there were 5,966 slow fibers in TS, and 21,552 slow fibers in TH. In another (*D. immutabilis*), TS had 4,538 slow fibers.

### Wrist Extension

**Anatomy.** Wrist extension is facilitated by the automatic extension-flexion mechanism of the avian wing (Fisher, 1957; Vazquez, 1994) and also through action of Mm. extensor metacarpi radialis dorsalis (EMRd) and extensor metacarpi radialis ventralis (EMRv). Vazquez (1994) suggested that as a two-joint muscle, EMR can extend both wrist and elbow. EMRd lies dorsal to the pars ventralis, with a fleshy origin from the distal surface of the patagial ossicle (Fig. 9). It tapers quickly to a long tendon, which becomes fused with the tendon of EMRv by about half-way down the length of the ulna.

The EMRv lies deep to the pars dorsalis and originates by a robust, round tendon from the ventral surface of distal humerus, ventral to the Processus

supracondylaris dorsalis (Fig. 9). Both EMR muscles insert by a common tendon onto the Processus extensorius of the proximal carpometacarpus.

**Immunohistochemistry.** The EMRv had a mean of  $40 \pm 13\%$  slow fibers for four birds (Fig. 10; Table 1).

The EMRd was composed exclusively of slow fibers in three birds (Fig. 10), whereas one *D. immutabilis* had 97% slow fibers (see Table 1).

## DISCUSSION

### Slow Muscle Fibers and Posture

Slow muscles have been well studied with respect to their involvement in postural activities across many taxa and musculoskeletal regions (e.g., see Armstrong, 1980; Putnam et al., 1980; Mendiola et al., 1991; Hermanson and Cobb, 1992; Meyers, 1992a; Hermanson et al., 1993; Rosser et al., 1994; Meyers and Mathias, 1997; Gellman et al., 2002). Goldspink (1980, 1981) has indicated that isometric contractions are best suited for postural roles and that slow muscle fibers are known to be more efficient in such roles.



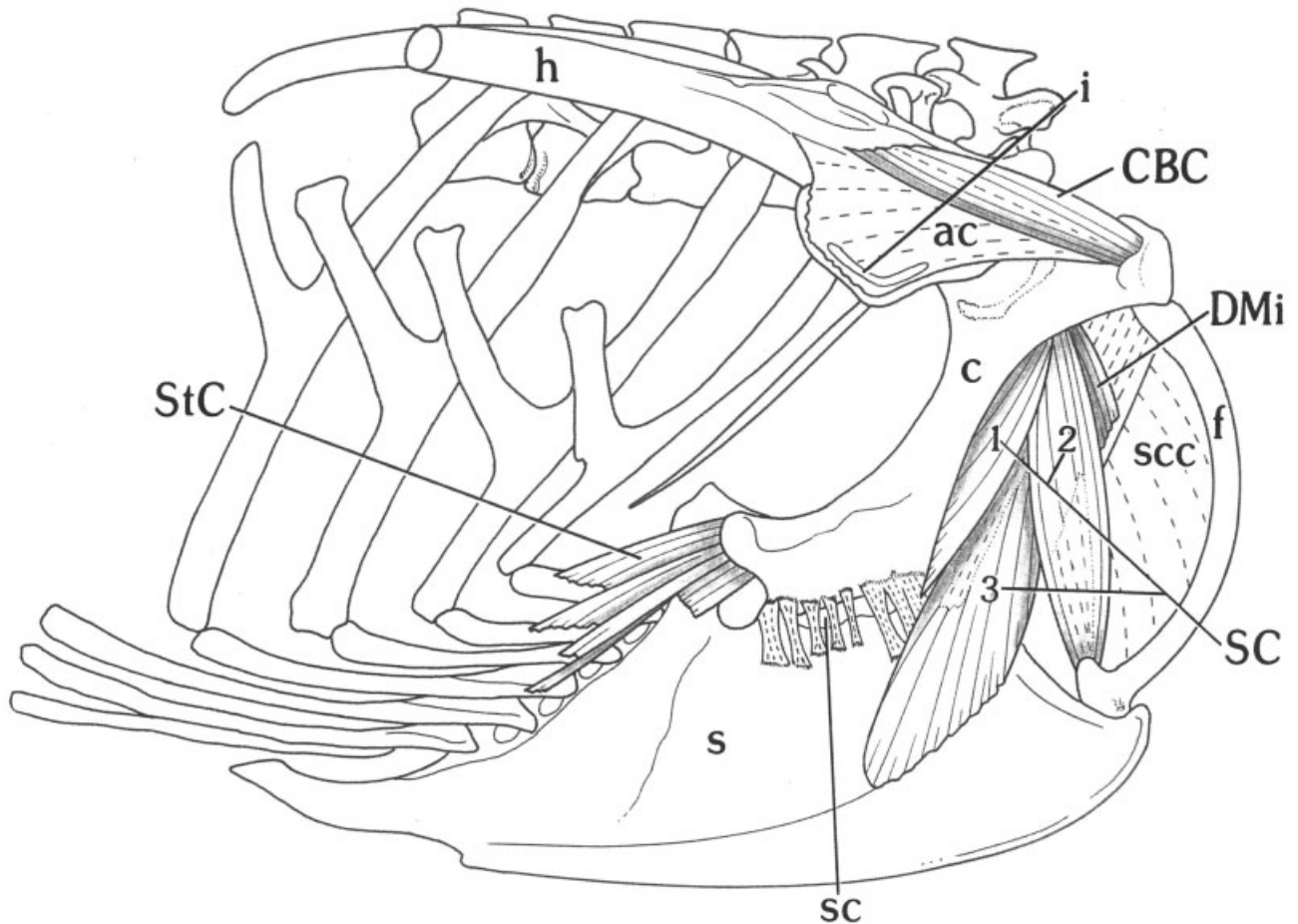


Fig. 6. *Diomedea immutabilis*. Lateral view of the right pectoral region and shoulder girdle of the Laysan Albatross, showing Mm. supracoracoideus, coracobrachialis cranialis, and sternocoracoideus. Deep pectoralis muscle has been removed. ac, acroracoracohumeral ligament; i, area of insertion of the deep pectoralis onto the bicipital crest—the superficial pectoralis inserts here as well; c, coracoid; CBC, M. coracobrachialis cranialis; DMi, M. deltoideus minor, deep layer; f, furcula; h, humerus; SC, M. supracoracoideus (1 = coracoid head; 2 = furcular head; 3 = sternal head); sc, sternocoracoid joint and associated ligaments; scc, sterno-coraco-clavicular membrane; s, sternum; StC, M. sternocoracoideus.

### Body Support

The pectoralis muscle is active during gliding flight as shown by electromyography in gulls (Goldspink et al., 1978) and kestrels (Meyers, 1993). The predominant fast-twitch fibers of the superficial pectoralis muscle would not be suitable for enduring, gliding behavior (but see Meyers and Mathias, 1997).

A deep layer of the pectoralis is common to gliding and soaring birds. Although it can depress the wing, the deep pectoralis is believed to act during gliding to support the body between the wings via isometric muscular contraction.

The diversity of avian species with superficial and deep layers of the pectoralis has been described by Meyers and Mathias (1997; see references therein). Knowledge of this division of the pectoralis goes back at least to Forbes (1882), who said it was characteristic of albatrosses, storks, vultures, *Phaethon*,

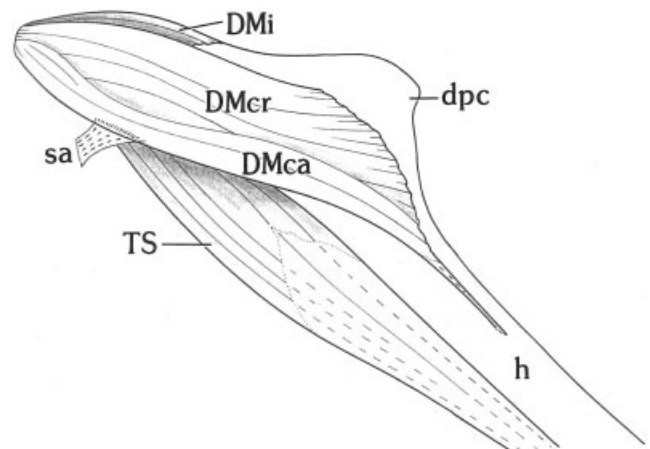


Fig. 7. *Diomedea immutabilis*. Dorsal view of the right shoulder of the Laysan Albatross, showing Mm. deltoideus and triceps brachii. Propatagial musculature has been removed. DMCa, M. deltoideus major, caudal head; DMCr, M. deltoideus major, cranial head; DMi, M. deltoideus minor; dpc, deltopectoral crest; h, humerus; sa, scapular anchor; TS, M. triceps scapularis.

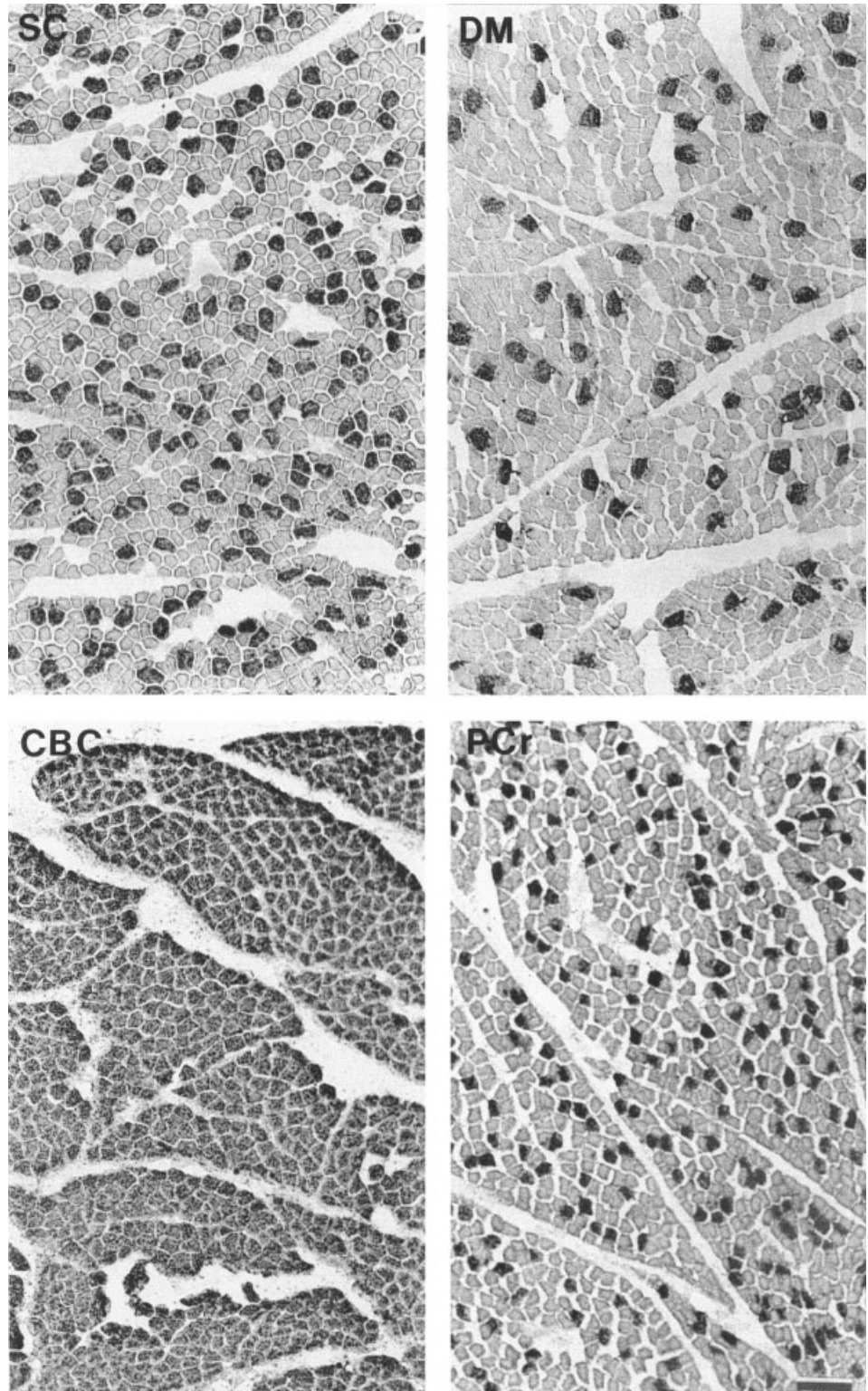


Fig. 8. *Diomedea immutabilis*. ALD 58 antibody reactions in wing elevator and protractor muscles of the Laysan Albatross. SC, supracoracoideus; DM, deltoideus major, pars cranialis; CBC, coracobrachialis cranialis; PCr, Cranial region of the superficial pectoralis. Positive reaction for slow muscle fibers results in a dark staining profile. Note that CBC is uniformly slow and that the other three muscles have various populations of slow muscle fibers. Scale bar = 160  $\mu$ m.

*Fregata*, *Plotus* [= *Anhinga*], *Sula*, and *Pelecanus*. Fisher (1946) indicated that its main function (in vultures) was to hold the wing down against the air moving upwards. Kuroda (1960, 1961) suggested that the deep layer was an adaptation to soaring

flight (and was thus absent from birds that did not soar), that it acted to hold the wing motionless despite wind action from below, and that its fibers should be "white" for this purpose. In 1972 Penny-cuick indicated that the deep layer makes up 8–10%



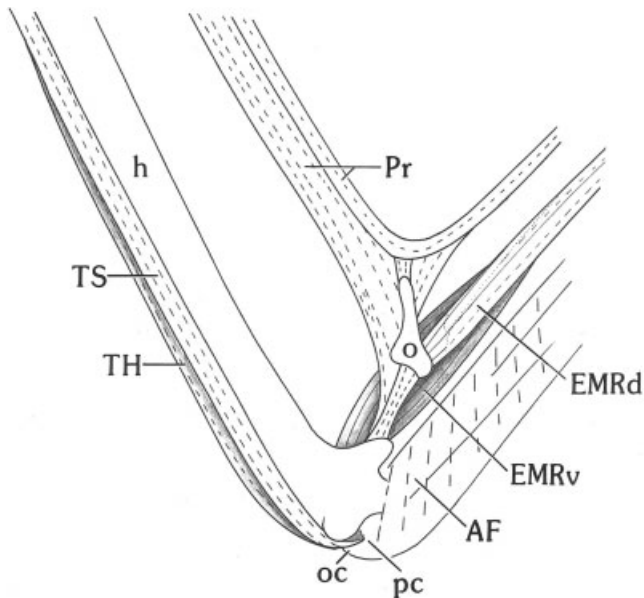


Fig. 9. *Diomedea immutabilis*. Dorsal view of the right elbow region of the Laysan Albatross, showing the insertions of Mm. triceps and the origins of Mm. extensor metacarpi radialis. AF, antebrachial fascia; EMRd, M. extensor metacarpi radialis pars dorsalis; EMRv, M. extensor metacarpi radialis pars ventralis; h, humerus; o, patagial ossicle; oc, olecranon area of ulna; pc, Proc. Cotylaris dorsalis of ulna; Pr, Lig. propatagiale; TH, M. triceps humeralis tendon; TS, M. triceps scapularis tendon.

of the entire pectoralis mass in vultures and storks, that it was most probably a tonic muscle, and also calculated its power consumption. In 1982, Pennycuick described the shoulder lock in albatrosses, and suggested that the deep layer should be a twitch, rather than tonic, muscle (see below).

Rosser and George's (1986a) thorough study on the pectoralis of 43 species of birds revealed only a few species with slow fibers in this muscle. Subsequent examination of Turkey Vultures (Rosser and George, 1986a) and White Pelicans (Rosser et al., 1994) revealed that their deep pectoralis muscles were uniformly slow. The pelican was found to express the SM2 myosin, also found in the tonic anterior latissimus dorsi (Rosser et al., 1994; Bandman and Rosser, 2000), suggesting the deep pectoralis is tonic as well. A small cluster of slow-twitch fibers (~20) was found in the deep region of the *undivided* pectoralis of only one California Gull (Meyers and Mathias, 1997). No slow fibers were found in various regions of the pectoralis of two Herring Gulls or one Ring-billed Gull studied by Rosser and George (1986a). Likewise, only fast fibers were found in the pectoralis of skuas and Herring Gulls (Caldow and Furness, 1993). Meyers and Mathias (1997) suggested that fast fibers may function during gliding and soaring in California Gulls, which lack the deep layer to the pectoralis and any functional quantity of slow fibers. The enduring properties of gull pectoralis muscle during isometric contractions need to be

tested experimentally. Other gliding and soaring taxa that lack the deep layer (e.g., eagles, hawks) need to be investigated as well.

### Shoulder Lock

Pennycuick (1982) discovered the locking mechanism in the albatross shoulder (although Rayner first mentions Pennycuick's finding in his 1981 article), and described it as being made up of "a sheet of tendon forming part of the superficial pectoralis muscle." In three species of albatrosses examined (Wandering, Black-browed, and Light-mantled: *D. exulans*, *D. melanophris*, and *Phoebastria palpebrata*, respectively) he found the lock at a "superficial level" in the pectoralis, with a small amount of muscle lying over it, whereas in the Southern Giant Petrel (*Macronectes giganteus*) he described the lock as being on the deep surface of the *superficial* pectoralis. Due to the presence of the lock, and the pale color of the deep pectoralis muscle, Pennycuick (1982) suggested that the deep layer should be a fast muscle (not slow tonic), specialized to function during landing when albatrosses rotate their wings about the long axis at frequencies twice that of flapping (see Scholey, 1982). Bannasch and Lundberg (1984) examined *Macronectes* and provided more detail on the anatomy of the lock. They reported (as we do) that the locking tendon is found within the deep pectoralis, and also suggested that the muscle should be tonic—even though they agreed with Pennycuick about the unusual wing rotation in albatross landing. Bannasch and Lundberg suggested that the superficial pectoralis can contribute to the rotation described by Scholey and Pennycuick. Our own observations of *Macronectes* support the suggestions of Bannasch and Lundberg. The lock in the giant petrel as well as two additional albatross species we examined (*D. exulans* and *melanophris*) was qualitatively identical to that in *D. immutabilis* and *D. nigripes*, and was clearly located within the deep pectoralis muscle. Furthermore, the pale color Pennycuick observed is typical for the deep pectoralis and avian slow tonic muscles in general (Meyers, pers. obs.).

Pennycuick (1982) described the albatross locking mechanism as a functional consequence of full wing protraction, and reported that the lock could be "released" by retracting the wing a few degrees. Contrary to Pennycuick's findings, we could not get the lock to release using the manipulations he described. The wing lifted easily *only* when the internal tendon of the deep pectoralis was cut (in one specimen excessive manipulation *tore* the deep pectoralis off of the sternum). We suggest that since the lock is made up of collagenous tissue extending from origin to insertion, elevation would require some degree of stretching or a changed skeletal configuration to provide slack in the tendon.



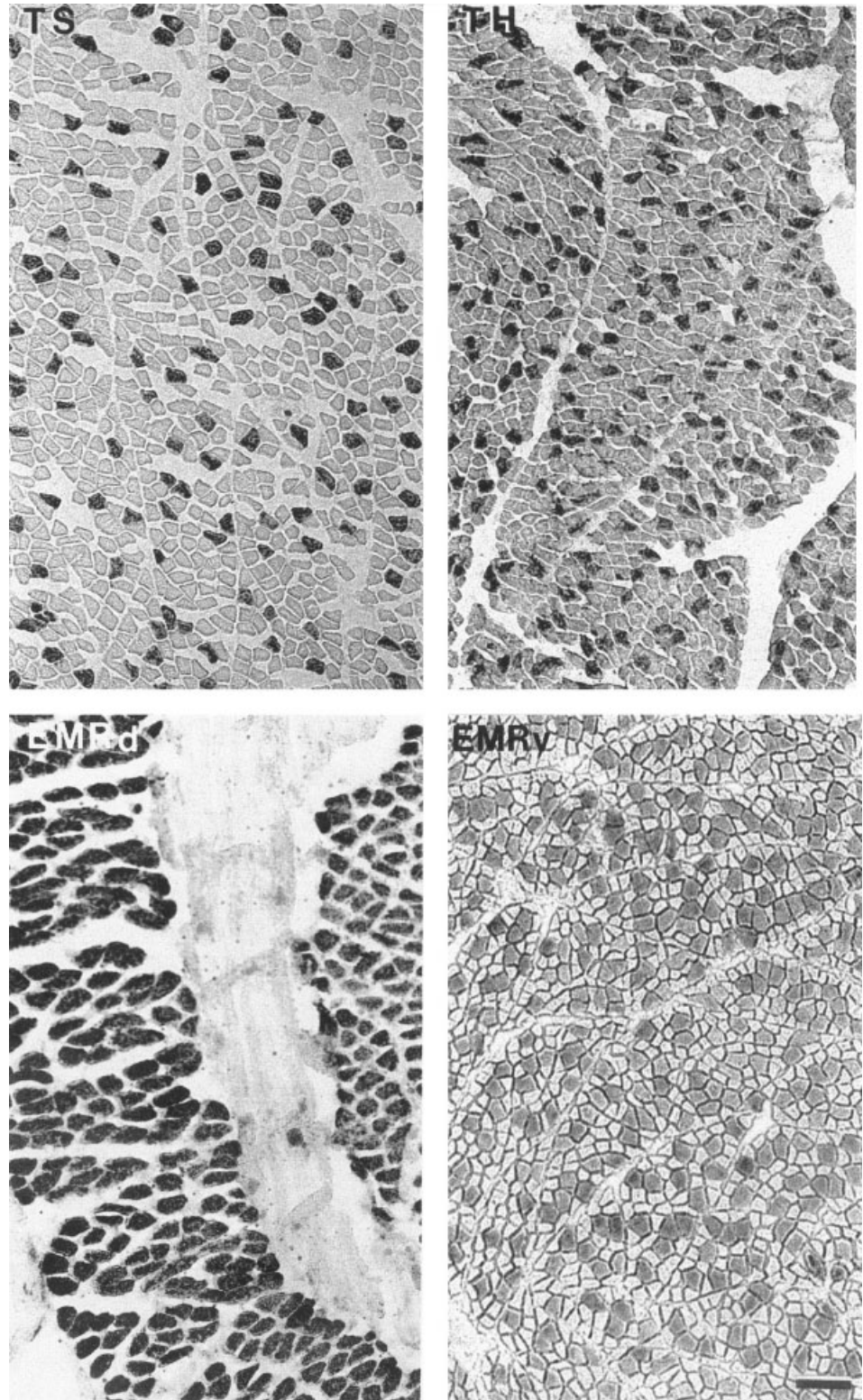


Fig. 10. *Diomedea immutabilis*. ALD 58 antibody reactions in elbow and wrist extensor muscles of the Laysan Albatross. TS, triceps scapularis; TH, triceps humeralis; EMRd, extensor metacarpi radialis pars dorsalis; EMRv, extensor metacarpi radialis pars ventralis. Positive reaction for slow muscle fibers results in a dark staining profile. Note that EMRd is uniformly slow and that the other three muscles have populations of slow muscle fibers. Scale bar = 160  $\mu\text{m}$ .

As is well known, the coracoid forms a saddle-like synovial joint within the coracoid sulcus of the sternum (Baumel and Raikow, 1993; see Fig. 6), and is able to slide or glide within this joint through the action of the *M. sternocoracoideus*. The functional

significance of this sliding movement is not known. It is not believed to function in respiration (Vanden Berge and Zweers, 1993) as previously suggested (Raikow, 1985); Dial et al. (1991) found that it is active at the upstroke–downstroke transition, just

prior to the beginning of downstroke. They indicated that this activity pulls the coracoid caudolaterally, deflecting the acroracoid process laterally and spreading the shoulders. This movement was also reported by Atkins (1977) who showed the effect on coracoid translation on linkage ligaments in the pigeon pectoral girdle. He calculated a coracoid sliding angle of about 30°, corresponding to a decrease in the distance from the glenoid to the sulcus of about 0.6 mm in the pigeon. We suggest that this lateral movement of the coracoid within the sternal sulcus (which we also observed as possible in albatrosses), may also reduce the distance from the pectoralis insertion to the origin. The movement of the coracoid caudolaterally would bring the glenoid (and therefore the pectoralis insertion onto the humerus) towards the sternum, thus decreasing that distance. This reduced distance could make elevation of the humerus possible without stretching the tendinous shoulder lock. Whether any stretching or elasticity of this tendon is possible requires additional *in vivo* physiological measurements, and a further evaluation of the function of *M. sternocoracoideus* in albatrosses.

Other locking mechanisms have been described for bird and bat feet (Quinn and Baumel, 1990; Schutt, 1993) and horse knees (Sack, 1989) and shoulders (Hermanson and Hurley, 1990). The arrangement of an internal tendon within the deep pectoralis, extending from origin to insertion as the limiting strut, is analogous to that found in horse biceps brachii, and that muscle's role in quiet standing (Hermanson and Hurley, 1990). The slow, short-fibered fascicles within the lateral head of the horse biceps brachii were suggested to be important for small amounts of force production required over long periods of time. This provided sufficient tension on the internal tendon for maintaining posture through slight regional adjustments (Hermanson and Hurley, 1990). A similar situation exists in the horse knee joint, which requires a low level of vastus medialis activation to stabilize the knee despite the presence of a "locking mechanism" (Schuurman et al., 2003). The uniform, slow fiber composition of the deep pectoralis provides a postural, enduring stability to the lock that limits wing elevation to, or below, the horizontal during gliding and soaring. This appears to provide fine control of wing elevation when confronted with the buffeting of rigid ocean winds during maneuvers such as banking and turning.

### Wing Elevation

Wing elevation musculature is important to gliding flight as an antagonist to the pectoralis in maintaining static shoulder stabilization in the transverse plane (Meyers, 1993). Based on the uniform slow fiber composition of albatross deep pectoralis (DP), one might predict slow fiber populations for muscles of wing elevation as well. Indeed, all three

heads of supracoracoideus (SC), as well as deltoid major (DM), all possessed about 13% slow fibers. The SC and DM have not been well studied with respect to their fiber types. Neither muscle had any slow fibers in kestrels (Meyers, 1992a), nor did the SC in skuas or Herring Gulls (Caldow and Furness, 1993) or starlings (Goslow et al., 2000). Boesiger (1987, 1992) reported two fiber types in the SC of five passerine and three galliform species, but these were classified as lipid-rich and lipid-poor. No slow fibers were mentioned. That these muscles have both fast and slow fibers in albatrosses seems to agree with their dual role in flight—as powerful wing elevators during take off and landing, as well as stabilizing muscles for prolonged soaring.

### Wing Protraction

We found the *M. coracobrachialis cranialis* (CBC) to be exclusively comprised of slow fibers in albatrosses. This suggests the importance of postural control in wing protraction for gliding flight. Fisher (1946) found larger CBC muscles in condors than vultures and suggested that it was an adaptation for improved soaring ability. Stegmann (1964) indicated that doves use this muscle for wing protraction, since they lack a bowed furcula and could not use the cranial part of the pectoralis for this action. Meyers and Mathias (1997) found the CBC of the California Gull to have about 8% slow fibers, whereas Double-crested Cormorants contain 31% slow-twitch fibers, assumed to function in their wing-drying posture (Meyers, 1997). Meyers (1992a) surveyed CBC along with other kestrel shoulder and brachial muscles and found it to be uniformly fast-twitch. Simpson (1979) described various percentages of tonic fibers in several pigeon muscles, including CBC, but did not quantify fiber types.

The cranial edge of the superficial pectoralis (PCr) has a large population of slow fibers. These slow fibers exist only within that portion of SP that is apparently best suited for wing protraction. Meyers (1993) found this region active during gliding flight in kestrels, yet it was exclusively fast in that species. A similar, although much smaller population of slow fibers (about 100 fibers in only one of three birds examined) was found in this region of the pectoralis of cormorants (Meyers, 1997). These too were assumed to function in the wing-drying posture. No slow fibers were found in this region of the California Gull pectoralis (Meyers and Mathias, 1997).

### Wing Extension

The histochemistry of the triceps muscle is perhaps one of the best studied of the non-pectoralis flight muscles in birds. Kovacs and Meyers (2000) found 1% of the puffin TS to be slow tonic (four out of 480 fibers). No slow fibers were found in the TS of mallards, coots, or Yellow-legged Gulls (Torrella et



al., 1996, 1998a,b, 1999), although Meyers and Mathias (1997) found fewer than 40 slow (twitch) fibers in two out of three California Gulls studied. This discrepancy may be due to the fact that Meyers and Mathias examined the entire muscle in cross-section, whereas Torrella and colleagues relied on three small sample areas of about 500 fibers each (Torrella et al., 1999). Hector's (1894) suggestion that the sesamoids of the patagial fan (subsequently investigated by Mathews, 1936, and Brooks, 1937) act as an elbow lock in albatrosses has been rejected by Yudin (1957) and cannot be substantiated by our anatomical observations. The arrangement of the sesamoid is peculiar, and has been suggested to function as a strut in the long patagial tendons (Mathews, 1936; Brooks, 1937). In his analysis of Hector's proposed mechanism, Yudin described an elbow "fixing" mechanism. When the wing is extended, the proximal radius butts against the distal humerus, thus limiting the degree of wing extension (and therefore the wrist as well). Joudine [Yudin] (1955) suggested that albatrosses and other soaring species (e.g., *Puffinus griseus*, *Fulmarus glacialis*, *Oceanodroma furcata*) possessed this elbow "stop" but those without this specialized flight mode did not.

Like the triceps, EMR histochemistry has been relatively well studied in birds. Cormorants have 29 and 6% slow fibers in their EMRd and EMRv, respectively (Meyers, 1997); almost identical percentages were found in California Gulls (28 and 6%; Meyers and Mathias, 1997). Torrella et al. (1998a) reported 9.4 and 3.6% of SO fibers in their sampled regions of EMRd and EMRv of coots, and 6.1 and 8.1 in Yellow-legged Gulls (Torrella et al., 1998b). Mallards (Torrella et al., 1998a) and House Finches (Meyers, unpubl. obs.) have uniformly fast EMR muscles. These numbers contrast sharply with the slow muscle content in the albatross EMR.

### Development and Evolution of the Deep Pectoralis

Rosser et al. (1994) discussed the evolution of the deep pectoralis in Gruiformes and Ciconiiformes. They suggested that the deep layer developed from a separate neuromuscular compartment of the deep "red strip" of the undivided pectoralis. They also discussed the relationship of the mammalian soleus within the triceps surae as being analogous to the superficial/deep pectoralis in soaring birds.

Peters (1989) indicated that during evolution, neuromuscular compartments can split off from their parent muscle and may allow special functions. Studies of primitive marsupials suggest that the soleus arose as a neuromuscular compartment from the lateral gastrocnemius (Lewis, 1962). In lizards, the homolog to the triceps surae is a single muscle, with slow fibers (a neuromuscular compartment) sit-

uated in the same position and function as the mammalian soleus (Putnam et al., 1980; Peters, 1989).

The pectoralis of chickens possesses muscle fibers that react with antibodies to both fast and slow myosins (Bandman et al., 1982). In 11-day chick embryos, slow myosin light chains were detected throughout the entire pectoralis (Matsuda et al., 1982). By day 16 the slow myosins begin to disappear, and become restricted to the most anterior region of the muscle by day 2 posthatch (Matsuda et al., 1983). Pigeons show some reactivity to the slow myosin antibody (NA8) throughout the pectoralis at 13 days in ovo, but have a uniformly fast pectoralis as adults (Rosser et al., 1998). A similar ontogenetic pattern of widespread slow fibers that are replaced by fast fibers can be found in the pectoralis of the bat, *Myotis lucifugus*. *Myotis* has a deep area of the pectoralis with slow fibers in late fetal life through 5 days postnatally, giving way to a muscle that is 100% fast in adults (Schutt et al., 1994). These studies may provide an explanation for the development of slow fiber regions within the pectoralis muscle of gliding and soaring species. Since slow myosin is typically expressed first, those species with a deep layer may retain this early developmental feature. This can also explain the existence of slow fibers in the cranial part of the pectoralis, where they are not part of a distinct, isolated muscle, but are presumed to be a functional neuromuscular compartment. Ontogenetic studies may elucidate the development of these pectoralis fiber types.

Meyers (1993) described the evolution of gliding morphology and identified birds with a "partly divided" pectoralis. These birds (including kestrels, ravens, dippers, Sandhill Cranes) have a region of muscle fascicles, largely arising from the furcula and adjacent connective tissues, which insert onto the biceps tendon overlying the bicapital crest of the humerus (Meyers, 1993) (Fig. 11). This morphology is similar to that of the deep pectoralis, and Meyers (1993) indicated that this could represent an intermediate step in the evolution of gliding and soaring birds. In kestrels, this region was active during gliding flight in a windtunnel, but had no slow muscle fibers (Meyers, 1993). The muscle fiber types within the region of other species with the "partly divided" morphology are presently unknown.

Chick limb muscles typically cleave from adjacent muscles from origin to insertion (or vice versa) but not in the middle (Schroeter and Tosney, 1991; Kardon, 1998). Schroeter and Tosney (1991) reported that most of the thigh muscles cleave from insertion to origin and stated that a similar pattern also exists within the forelimb (see also Sullivan, 1962). This cleavage pattern does not appear to be related to the pattern of blood vessels, activity, or innervation. Therefore, the "partly divided" morphology described by Meyers (1993) might represent a partial or incomplete cleavage beginning at the insertion and stopping before the muscle was cleaved in two.



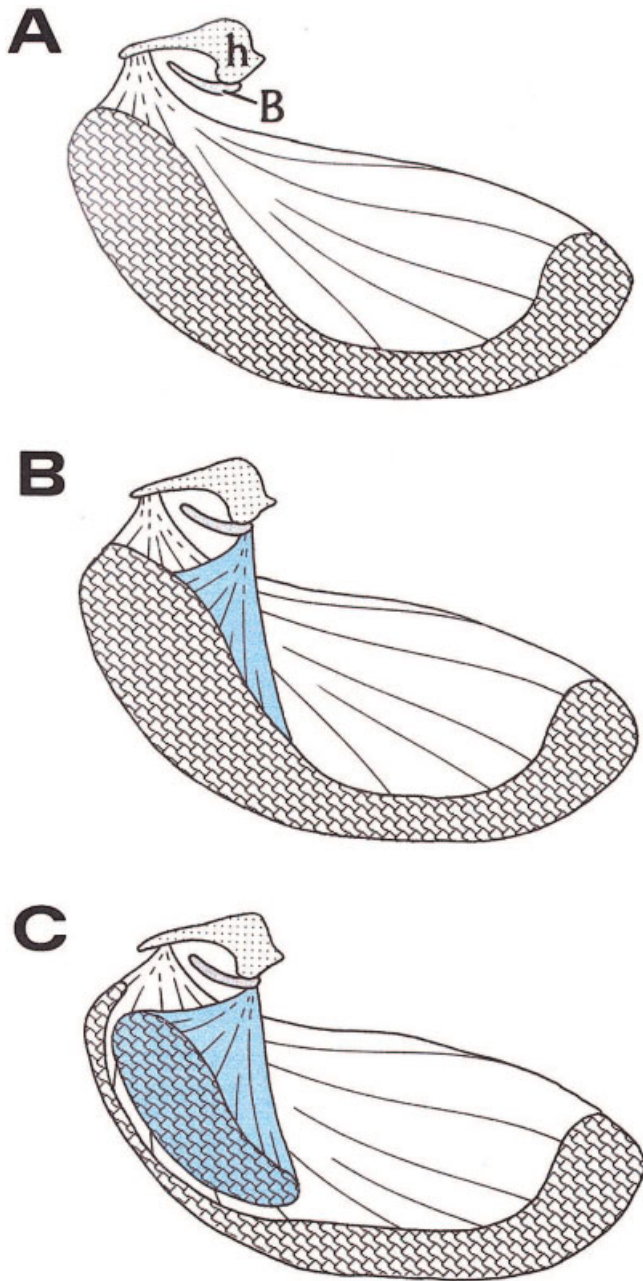


Fig. 11. Diagram of the hypothetical evolution of the deep layer of pectoralis muscle, seen from medial view. The humerus and *M. biceps brachii* are shown in cross-sections. **A:** Typical undivided pectoralis inserting onto the deltopectoral crest. **B:** "Partly divided" pectoralis, with fascicles arising from the furcula and sterno-coraco-clavicular membrane and attaching onto the biceps tendon. **C:** Divided pectoralis of albatrosses and others, with a deep, slow layer inserting separately onto the biceps tendon. B, *M. biceps brachii*; h, humerus.

In species with a completely separate, slow, deep layer, cleavage would complete and produce two muscles (Fig. 10), and the slow muscle fiber component of the deep layer would be retained.

Sullivan (1962) reported that the separation of pectoralis and supracoracoideus is visible by stage

25–26 (~4.5 days; Hamburger and Hamilton, 1951). Presumably, a deep/superficial pectoralis separation would be visible by this stage as well. Muscle development and embryo studies of a readily available species with a slow deep layer (e.g., pelicans) may reveal more on the development and evolution of this structure.

### Biomechanics of the Deltopectoral Crest

In albatrosses, the bulk of the deltopectoral crest is devoid of muscle attachment; the large pectoralis muscle inserts at the base of the crest, and the deltoideus muscle does not reach the edge of the crest (Fig. 3). Thus, the crest protrudes farther than "necessary" for muscle attachment. This is in contrast to birds such as pigeons and starlings, where the entire surface area of pectoralis insertion is onto the crest (Dial and Biewener, 1993). It is well known that the pectoralis muscle produces pronation of the wing in addition to depression (Bannasch and Lundberg, 1984; Dial et al., 1988). An attachment closer to the edge of the crest would increase the muscle in-lever, and hence, pronation ability, since it would move the insertion further from the axis of rotation. Why then is the crest larger than needed? We suggest a few possibilities: 1) The patagialis muscles arise from the furcula and pass over the deltopectoral crest. The more the crest protrudes, the greater the leverage of these muscles, although they do not attach to the crest. 2) The large, "unused" part of the crest may be the result of differential growth of the humerus and not be strictly "adaptive." Further investigations can evaluate these hypotheses and others.

### Use of Frozen-Refrozen Tissue

Jouffroy et al. (1999) reported that they could get reliable immunohistochemical results with fixed tissue. While we were unable to duplicate their success with fixed albatross tissue, we were able to use tissue from previously frozen specimens. In some cases, birds had been stored in the freezer for over 1.5 years. Our specimens were frozen on site in Hawaii, shipped to us, then thawed and refrozen in liquid nitrogen. Comparisons with control tissues showed no affect of refreezing, nor were there any serious freezer artifacts. Previous research of this type has typically made use of fresh, unfrozen specimens, requiring histochemical or immunohistochemical analysis to be completed soon after specimen death (although Rosser et al., 1994, also used previously frozen tissue). We were impressed with the quality of the sections as well as antibody reactivity. This unconventional technique may facilitate future research in muscle histochemistry and immunohistochemistry, by allowing specimen freezing, thawing, reaction, and subsequent photography or analysis without qualitative muscle tissue damage.

## CONCLUSIONS

This study revealed immunohistochemical, anatomical, and biomechanical data heretofore unknown or poorly described in albatrosses. Immunohistochemical reactions were used to identify muscles within the albatross gliding flight apparatus that possessed slow (postural) fibers, including some that were uniformly slow (CBC, EMRd, and DP). This appears consistent with the extreme nature of gliding and soaring behavior in these birds and the low energetic cost of locomotion in these birds (Costa and Prince, 1986), and sharply contrasted with findings of most other birds studied. This study also reinvestigated the anatomy and biomechanics of the shoulder lock discovered by Pennycuik (1982). The tendinous locking mechanism, which functions to limit wing elevation above the horizontal, was discovered to be associated with the *deep* layer of the pectoralis in this investigation, rather than the superficial layer of the pectoralis as described by Pennycuik (1982). Pennycuik's placement of the shoulder lock, its ability to release, and the prediction of fast fiber types in DP are not consistent with the findings of the present study.

## ACKNOWLEDGMENTS

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