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# Sexually Dimorphic Muscles in the Forelimb of the Japanese Toad, *Bufo japonicus*

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*ABSTRACT* During the breeding season, male anurans display clasping behavior by holding females with their forelimbs. This behavior is peculiar to males, and may require specializations in forelimb musculature. The present study revealed that five kinds of forelimb muscles were heavier in the male Japanese toad than in the female: the flexor carpi radialis (FCR), the flexor antibrachii medialis caput superius (FAMsup), the abductor indicis longus (AIL), the extensor carpi radialis caput superius (ECRsup), and the flexor antibrachii lateralis superficialis caput superius (FALSsup). In addition, one breast muscle, the coracoradialis (CR), was also heavier in males than in females. A quantitative analysis of muscle fibers processed for myosin ATPase activity showed that, in such "sexually dimorphic muscles" of the female, both fast (twitch) and slow (tonic) muscle fibers were of smaller diameter than in other forelimb muscles of both sexes (all male muscles plus "nondimorphic muscles" of the female). Moreover, both types of fibers were less numerous than in the corresponding muscles of the male. These results suggest that the "sexually dimorphic muscles" are used especially for clasping by the male and are degenerative or subnormal in the female. Slow muscle fibers were neither peculiar to, nor abundant in, these clasping muscles, although they may well be necessary for tonic and prolonged contractions of the forelimb muscles during clasping. The mechanism of sexual dimorphism may be a direct action of androgens on clasping muscles or an indirect action on clasping muscles via the innervating motoneurons.

Clasping is one of the most conspicuous aspects of sexual behavior in male anurans. During the breeding season, a sexually mature male toad firmly clasps a female; the forelimbs of the male are strongly flexed toward the female, and the second digits, with well-developed nuptial pads, are hooked into her axillary region. This clasping posture usually lasts for many hours. Therefore, tonic and long-lasting contractions of the forelimb muscles are necessary during clasping. This suggests specializations in the male's forelimbs. In fact, male toads usually have much stouter forelimbs than females.

In his detailed textbook on the anatomy of the frog *Rana esculenta*, Gaupp (1896–1904) stated briefly that the flexor carpi radialis (FCR), the extensor carpi radialis caput superius (ECRsup), and the abductor indicis longus (AIL) are larger in males than in females, and suggested that these muscles play an important role in clasping. However, he did not go into further detail, and little attention has since been paid to his interesting observation (Muller et al., '69; Melichna et al., '72).

In this study, we first made a comparison of wet weights between the male and female forelimb muscles of the Japanese toad and confirmed in this species that the FCR, ECRsup, and AIL were also heavier in males than in females. In addition, we found that two other forelimb muscles, the flexor antibrachii medialis caput superius (FAMsup) and the flexor antibrachii lateralis superficialis caput superius (FALSup), and one breast muscle, the coracoradialis (CR), were also

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heavier in males than in females. To examine the qualitative and quantitative sexual differences in the constituent muscle fibers, we made histochemical preparations of the muscle fibers processed for myosin ATPase and for succinic dehydrogenase (SDH) activity. According to the staining properties, we distinguished two general types of muscle fibers: fast (twitch) and slow (tonic) (Smith and Ovalle '73; Putnam and Bennett, '83). Then the number and size of muscle fibers were recorded for each type of fiber in both "sexually dimorphic" and "nondimorphic" muscles in both sexes.

## MATERIALS AND METHODS

### *Animals*

Adult Japanese toads (*Bufo japonicus*, nomenclature after Kawamura et al., '80) of both sexes were used in this experiment. They were captured at breeding sites in the suburbs of Tokyo from February to August, maintained at room temperature, and fed crickets. Experiments were carried out in spring (March, reproductive season) and summer (June to August, nonreproductive season). Because the histochemical and quantitative properties of muscle fibers did not differ significantly by season (tested by the two-tailed Student's t test), the results were consolidated. Eighteen males (snout-vent length 13.6±0.8 cm; body weight 205±42 gm, mean±SD, range 120–275 gm) and 12 females (snout-vent length 13.6±0.9 cm; body weight 195±35 gm, mean±SD, range 143–260 gm) were used.

### *Preparation of frozen sections*

The toads were anesthetized either by injection of a tricaine methane sulfonate (MS222) solution (20 mg/100 gm body weight) or by immersion in crushed ice. Forelimb muscles were removed in toto at room temperature within 15–20 min after anesthesia and cleaned of extraneous tissues. Each muscle was then briefly rinsed in a 0.65% NaCl solution, lightly blotted, and immediately weighed. The wet weights of all the forelimb muscles and the one breast muscle, CR, were measured and proportionally corrected for 200 gm of body weight. Next, the muscles were immersed in the embedding matrix (Lipshaw) and quenched in liquid nitrogen. The samples were stored at −80°C until sectioned. Transverse frozen sections were cut at 12–14 μm on a cryostat at −20°C, and the sections were thaw-mounted on slides.

### *Histochemical procedures*

Five "sexually dimorphic muscles," one "semidimorphic muscle," and the "nondimorphic muscles" were histochemically processed for myosin ATPase and/or SDH activity.

#### Procedure for myosin ATPase (modified from Guth and Samaha, '70)

An incubation solution of 40 ml of a 0.1 M tris-maleate buffer containing 320 mg calcium chloride, 60 mg citric acid, 100 mg ATP (disodium salt, Sigma), and 800 mg gelatin was freshly prepared and adjusted to pH 7.4 with a 1 N NaOH solution. The mounted sections were incubated for 30 min at 37°C, washed in three 2-min exchanges of distilled water, and transferred into a freshly prepared 2% cobalt chloride solution for 3 min. They were then rinsed in three 2-min exchanges of distilled water and immersed in a 1% ammonium sulfide solution for 1 min. The slides were then washed in running tapwater for 3 min, dehydrated in a graded series of ethanol solutions, cleared in xylene, and mounted in Canada balsam.

#### Procedure for SDH (modified from Barka and Anderson, '63)

The mounted sections were incubated for 50 min at 37°C in a mixture of 10 ml 0.2 M phosphate buffer (pH 7.4), 5 ml 0.65% NaCl solution, 25 ml 0.2% nitro blue tetrazolium solution (Wako Pure Chem. Ind., Ltd), and 10 ml 0.06 M sodium succinate (dissolved in 0.2 M phosphate buffer, pH 7.4). After incubation, the sections were rinsed in cold distilled water for 2 min, transferred into 10% formalin for 15 min, rinsed in distilled water, and mounted in glycerine jelly.

### *Data analysis*

The sections processed for ATPase activity were used for the quantitative analysis of the muscle fibers. The sections were projected onto a Minicopy Reader (Fuji Film), and the number of muscle fibers were counted on a section that had been cut at the greatest girth of the muscle belly and at a right angle to the long axis of the muscle. For a measurement of muscle fiber diameters, the same sections were projected onto a color video monitor. Several areas were chosen at random from the entire cross sections of the muscles, and the large and small diameters (approximating the muscle fibers as oval) of

all the muscle fibers within a region on the screen were measured with the aid of a Video Image Processor (model VIP-21ch, Olympus). The significance of the difference in the number and the diameter of the muscle fibers was tested by the two-tailed Student t test. When the variances were not equal, the Cochran-Cox method was used.

## RESULTS

### *Macroscopic observations*

Figure 1 shows lateral, medial, and dorsal views of the forelimb muscles of a male and a female Japanese toad. The anatomical location of each forelimb muscle was essentially the same as that of *Rana esculenta* as described by Gaupp (1896–1904). Therefore, we followed his nomenclature. A comparison of the forelimbs of the two sexes revealed that, in every case, five of the 14 muscles were larger in males than in females (Fig. 1, underlined muscles) (for statistics, see next section): FCR and FAMsup (FAMsup is not illustrated in Fig. 1) on the medial side (innervated by n. brachialis longus inferior), and AIL, ECRsup, and FALSsup on the lateral side (innervated by n. brachialis longus superior).

The origin and the insertion of each muscle were examined in detail and the function of each was inferred. The contraction of the FCR, FAMsup, ECRsup, and FALSsup muscles in concert causes a strong flexion of the forearm, bringing about the clasping posture. The contraction of the AIL abducts the second digit so that it is pressed into the axillary region of the female. Therefore, all five forelimb muscles (FCR, FAMsup, AIL, ECRsup, and FALSsup) seem to be involved in clasping.

In addition, one of the breast muscles, CR, was also larger in males than in females (not illustrated in Fig. 1). This muscle, with an extremely long and stout insertion tendon attached to the radius, also acts as a strong flexor of the forearm and thus may also be involved in clasping.

### *Wet muscle weights*

The sexually dimorphic nature of these muscles became more evident upon examination of their wet weights (Fig. 2).

The sexual differences in the wet weights of the five forelimb muscles (FCR, FAMsup, AIL, ECRsup, and FALSsup) were highly significant ($P < 0.001$); the five female muscles, on the average, weighed less than 20% of the corresponding male muscles. In the breast muscle CR the sexual difference in wet weights was also significant ($P < 0.001$), although in this case the female muscle weighed about 60% of the male muscle. No significant sexual difference was found in other forelimb muscles: flexor carpi ulnaris (FCU), palmaris longus (PL), FAM caput inferius (FAMinf), ulnocarpalis (UC), epitrochleocubitalis (ETC), ECR caput inferius (ECRinf), extensor digitorum communis longus (EDCL), FALS caput inferius (FALSinf), flexor antibrachii lateralis profundus (FALP), extensor carpi ulnaris (ECU), epicondylocubitalis (ECC), and anconaeus (AC; quadriceps). On the basis of these results, we refer to the five forelimb muscles (FCR, FAMsup, AIL, ECRsup, and FALSsup) as "sexually dimorphic muscles," the breast muscle (CR) as a "semidimorphic muscle," and the other forelimb muscles as "nondimorphic muscles."

### *Histochemical properties of muscle fibers*

Fast (twitch) and slow (tonic) muscle fibers could be distinguished on the basis of the staining properties in both histochemical methods; fast muscle fibers reacted strongly

*Abbreviations*

| | | | |
|---|---|---|---|
| AC, | m. anconaeus (quadriceps) | FALSinf, | m. flexor antibrachii lateralis superficialis caput inferius |
| ACscap, | m. anconaeus caput scapulare | FALSsup, | m. flexor antibrachii lateralis superficialis caput superius |
| AIL | m. abductor indicis longus | FAMinf, | m. flexor antibrachii medialis caput inferius |
| CR, | m. coracoradialis | FAMsup, | m. flexor antibrachii medialis caput superius |
| ECC, | m. epicondylocubitalis | FCR, | m. flexor carpi radialis |
| ECRinf, | m. extensor carpi radialis | FCU, | m. flexor carpi ulnaris |
| ECRsup, | m. extensor carpi radialis caput superius | PL, | m. palmaris longus |
| ECU, | m. extensor carpi ulnaris | UC, | m. ulnocarpalis |
| EDCL, | m. extensor digitorum communis longus | | |
| ETC, | m. epitrochleocubitalis | | |
| FALP, | m. flexor antibrachii lateralis profundus | | |

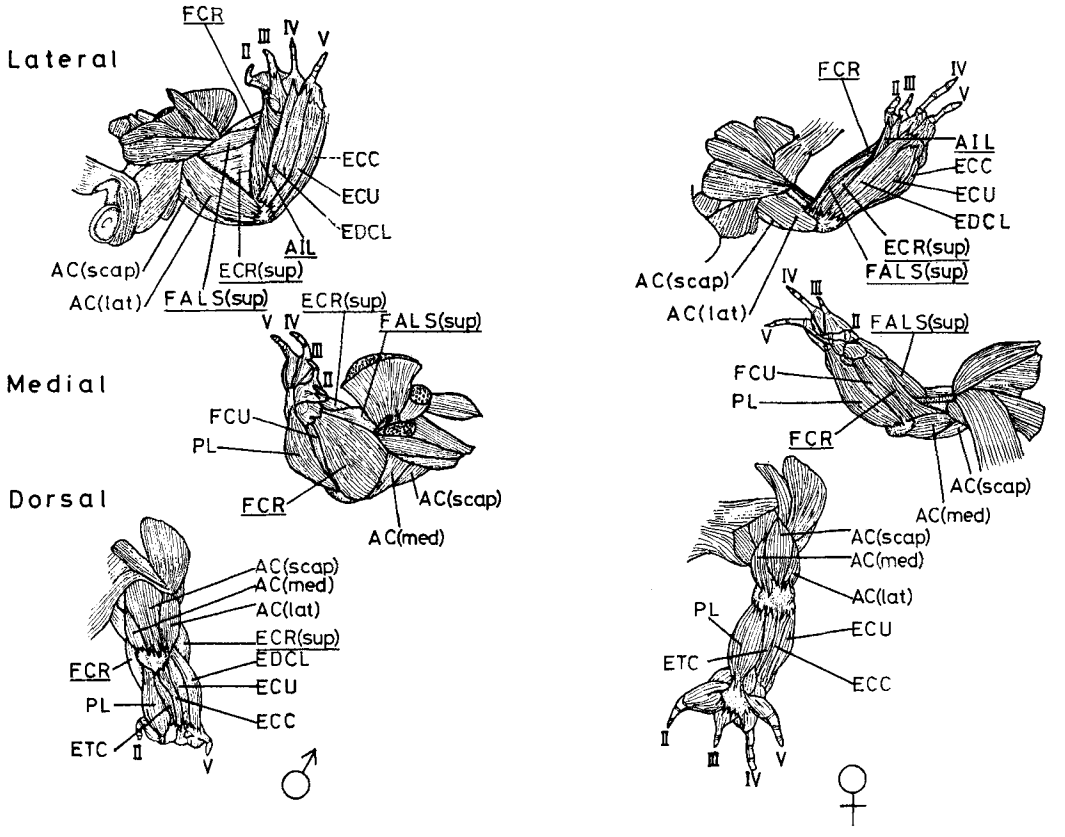


Fig. 1. Lateral, medial, and dorsal views of the forelimb muscles of the male (left-hand frame) and the female (right-hand frame). Underlining indicates four of the five sexually dimorphic muscles (FCR, ECRsup, FALSsup, and AIL). The deep sexually dimorphic muscle (FAMsup) is not shown here.

for both enzymes, whereas slow muscle fibers reacted only slightly for both. In SDH preparations, slow muscle fibers had a fine-grained appearance in contrast to the coarse-grained appearance of fast muscle fibers (Fig. 3). To simplify the following quantitative analysis, we did not further subdivide the fiber types.

In all of the muscles examined, slow muscle fibers were rarely grouped together, but were intermingled with fast muscle fibers. There seemed to be no sexual difference in the histochemical properties of either type of muscle fiber (Fig. 3).

*Quantitative analysis of muscle fibers*

The total numbers of muscle fibers in sexually dimorphic muscles, the semidimorphic muscle, and one of the nondimorphic muscles, AC caput scapulare (ACscap)*, were counted from cross sections processed for myosin ATPase activity (Table 1). The sexual difference in the total number of muscle fibers was highly significant, males having more than females in FCR ($P < 0.005$), ECRsup ($P < 0.005$), and FALSsup ($P < 0.002$); the difference was also significant in FAMsup ($P < 0.05$) and AIL ($P < 0.05$).

*Because our preliminary observations revealed no quantitative differences among the nondimorphic muscle fibers of both sexes, ACscap was arbitrary chosen as a representative nondimorphic muscle.

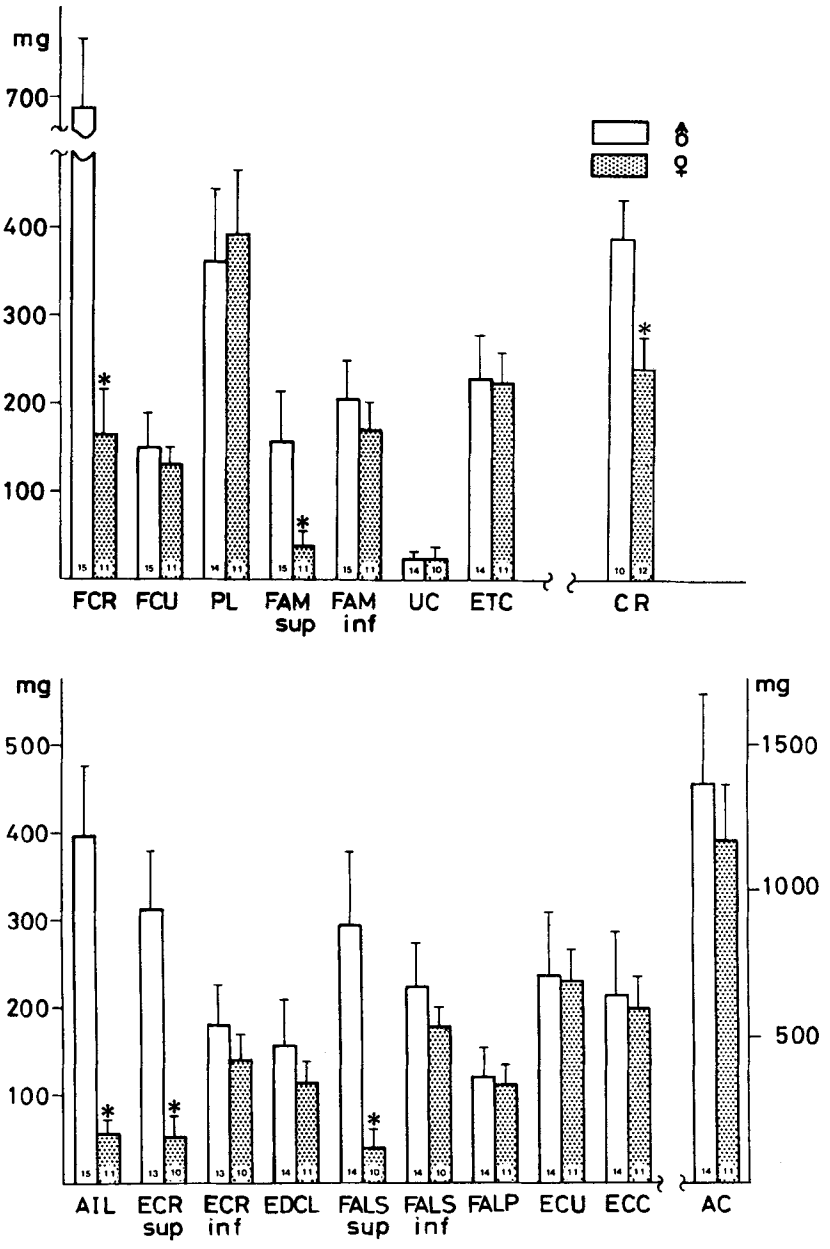


Fig. 2. Wet weights (proportionally corrected for 200 gm of body weight) of forelimb muscles. The vertical lines on the bars indicate SD values, and the numbers in the columns show the number of muscles. *: $P < 0.001$.

There was no significant sexual difference in the number of muscle fibers in ACscap ($P > 0.1$) and CR ($P > 0.2$). These results show that the sexual difference in the wet weights of five dimorphic muscles is attributable to a difference in the numbers of muscle fibers.

The fiber-type compositions of each muscle, represented in Table 1 as the percentage of the number of fast and slow muscle fibers against the total, were not significantly different between sexes. Likewise, there was no clear tendency for the sexually dimorphic

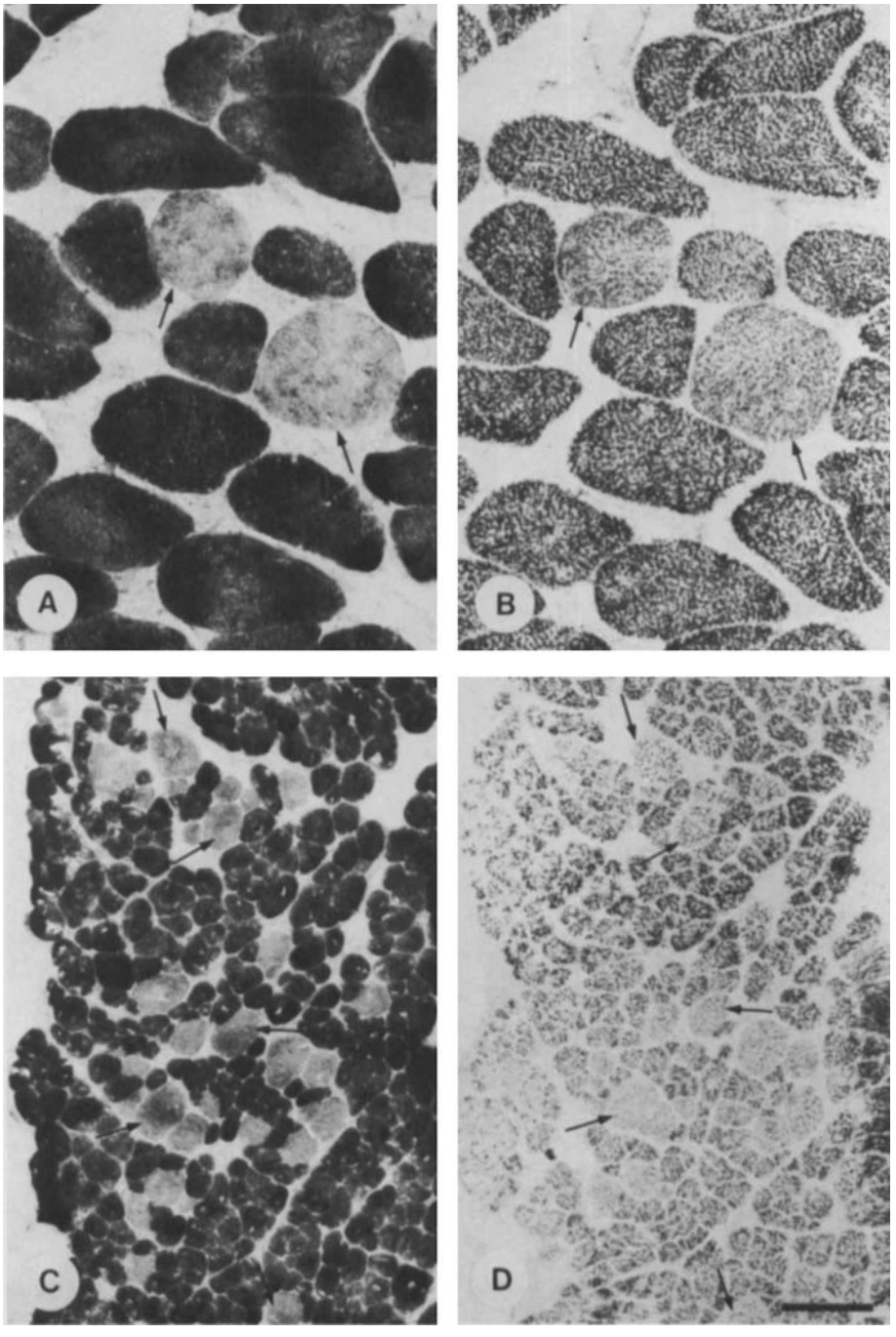


Fig. 3. Cross sections of the FALSsup of the male (A and B) and the female (C and D), processed for myosin ATPase (A and C) and for SDH (B and D) activity. Some of the slow muscle fibers are indicated by arrows. Scale bar = 100 $\mu$m. ×125.

*TABLE 1. Number of muscle fibers*

| Muscle | Male[1] No. of fast muscle fibers | Male[1] No. of slow muscle fibers | Male[1] Total | Female[1] No. of fast muscle fibers | Female[1] No. of slow muscle fibers | Female[1] Total | Male[2] total vs female total |
|---|---|---|---|---|---|---|---|
| FCR | 4,880 ± 1,067 (5) 95.5% | 229 ± 82 (5) 4.5% | 5,109 ± 1,125 (5) | 2,414 ± 535 (3) 93.3% | 172 ± 51 (3) 6.7% | 2,989 ± 596 (6) | $P < 0.005$ |
| FAMsup | 1,829 ± 509 (4) 90.8% | 186 ± 136 (4) 9.2% | 2,014 ± 612 (4) | 777 ± 131 (5) 92.6% | 62 ± 34 (5) 7.4% | 850 ± 134 (6) | $P < 0.05$ |
| AIL | 3,044 ± 401 (3) 95.3% | 150 ± 98 (3) 4.7% | 3,193 ± 374 (3) | 2,393 ± 541 (3) 96.9% | 77 ± 35 (3) 3.1% | 2,444 ± 400 (6) | $P < 0.05$ |
| ECRsup | 2,297 ± 427 (5) 94.5% | 133 ± 67 (5) 5.5% | 2,430 ± 461 (5) | 1,639 ± 194 (4) 96.2% | 65 ± 25 (4) 3.8% | 1,596 ± 245 (6) | $P < 0.005$ |
| FALSsup | 2,086 ± 344 (6) 92.4% | 171 ± 81 (6) 7.6% | 2,257 ± 407 (6) | 1,213 ± 192 (4) 91.6% | 111 ± 42 (4) 8.4% | 1,394 ± 259 (6) | $P < 0.002$ |
| ACscap | 1,560 ± 299 (5) 93.1% | 115 ± 41 (5) 6.9% | 1,675 ± 323 (5) | 1,947 ± 320 (4) 92.8% | 152 ± 74 (4) 7.2% | 2,045 ± 502 (6) | $P > 0.1$ |
| CR | 2,963 ± 202 (3) 92.8% | 231 ± 177 (3) 7.2% | 3,194 ± 27 (3) | 2,600 ± 351 (2) 92.4% | 215 ± 141 (2) 7.6% | 2,856 ± 355 (3) | $P > 0.2$ |

[1]Values are mean ± SD, number of samples observed (in parentheses), and percentage of the muscle fibers against the total.
[2]Student's t test.

muscles and semidimorphic muscle to have more slow fibers than the nondimorphic muscles.

The large and the small diameters of the muscle fibers were measured in the seven muscles mentioned above (Figs. 4–6). The sexual difference in the diameters of both fast and slow muscle fibers was highly significant ($P < 0.001$) in the five sexually dimorphic muscles (Fig. 5; see also Figs. 3 and 4). Diameters of the female muscle fibers were about 30% of those of the male fibers (about 9% of the male cross-sectional areas). In the semidimorphic muscle and the nondimorphic muscle the female muscle fibers were slightly smaller than the male fibers. However, the sexual difference in muscle fiber diameters was not statistically significant except for the slow muscle fibers of the CR (Fig. 6; see also Fig. 4).

An unusual feature of the muscle fibers of the female FCR was that they were clearly divided into two parts. One part was composed of only fast muscle fibers comparable in size to the male muscle fibers (A in the inset of Fig. 5); the other was composed of fast and slow muscle fibers far smaller than those in the male (B in the inset of Fig. 5).

When the muscle fiber diameters are compared not between the two sexes but between the sexually dimorphic muscles and the nondimorphic muscles, it is clear that the dimorphic muscle fibers of the male are not thicker than the nondimorphic muscle fibers of either sex, but are relatively similar (compare Figs. 5 and 6; see also Fig. 4). On the other hand, the muscle fibers of the sexually dimorphic muscles of the female are smaller in diameter than those of other forelimb muscles in both sexes (all male muscles plus nondimorphic muscles of the female).

In summary, the quantitative analysis of the muscle fibers shows that the sexual difference in the wet weights of five dimorphic muscles can be attributed not only to the difference in the number of muscle fibers, but

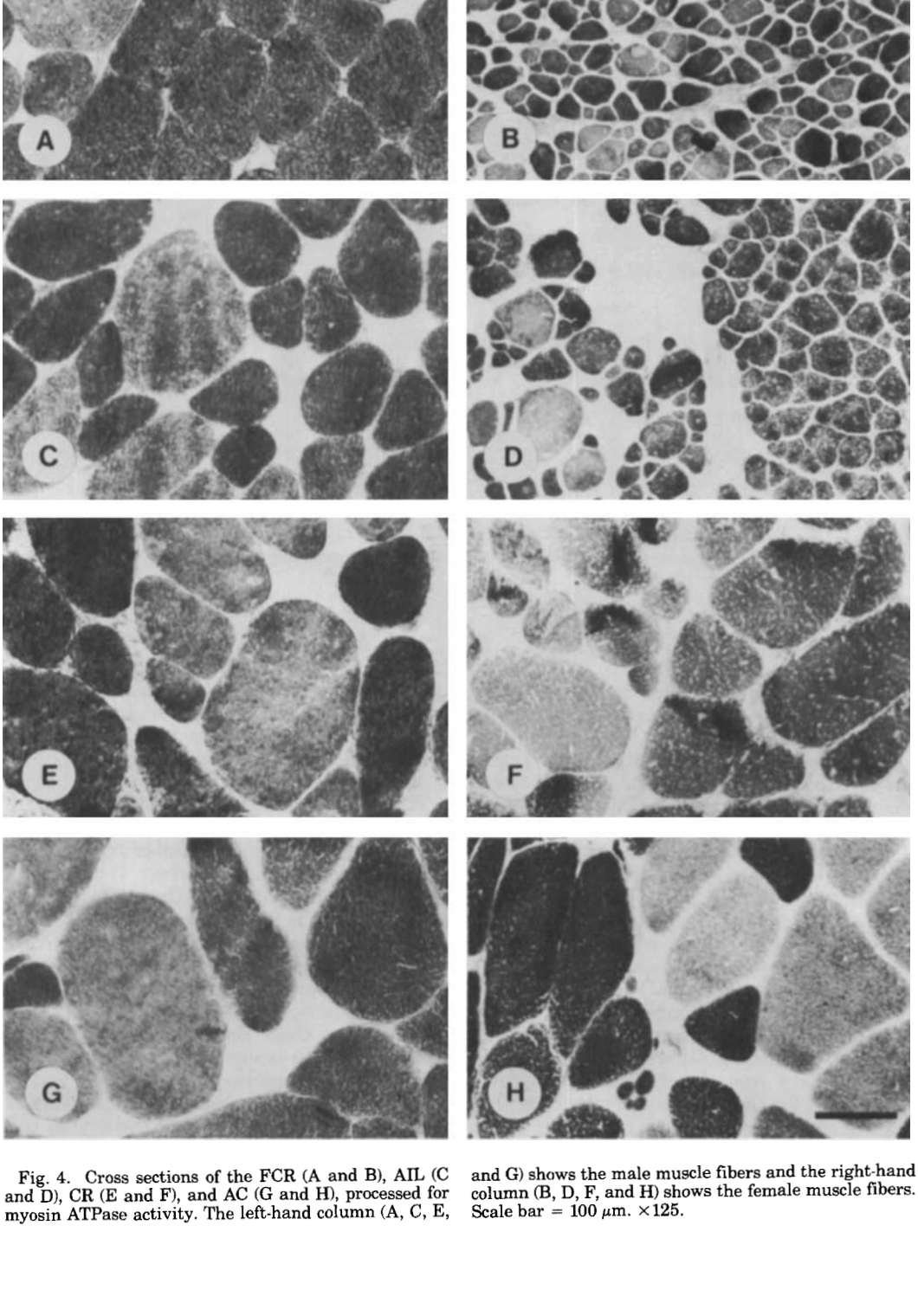


Fig. 4. Cross sections of the FCR (A and B), AIL (C and D), CR (E and F), and AC (G and H), processed for myosin ATPase activity. The left-hand column (A, C, E, and G) shows the male muscle fibers and the right-hand column (B, D, F, and H) shows the female muscle fibers. Scale bar = 100 $\mu$m. ×125.

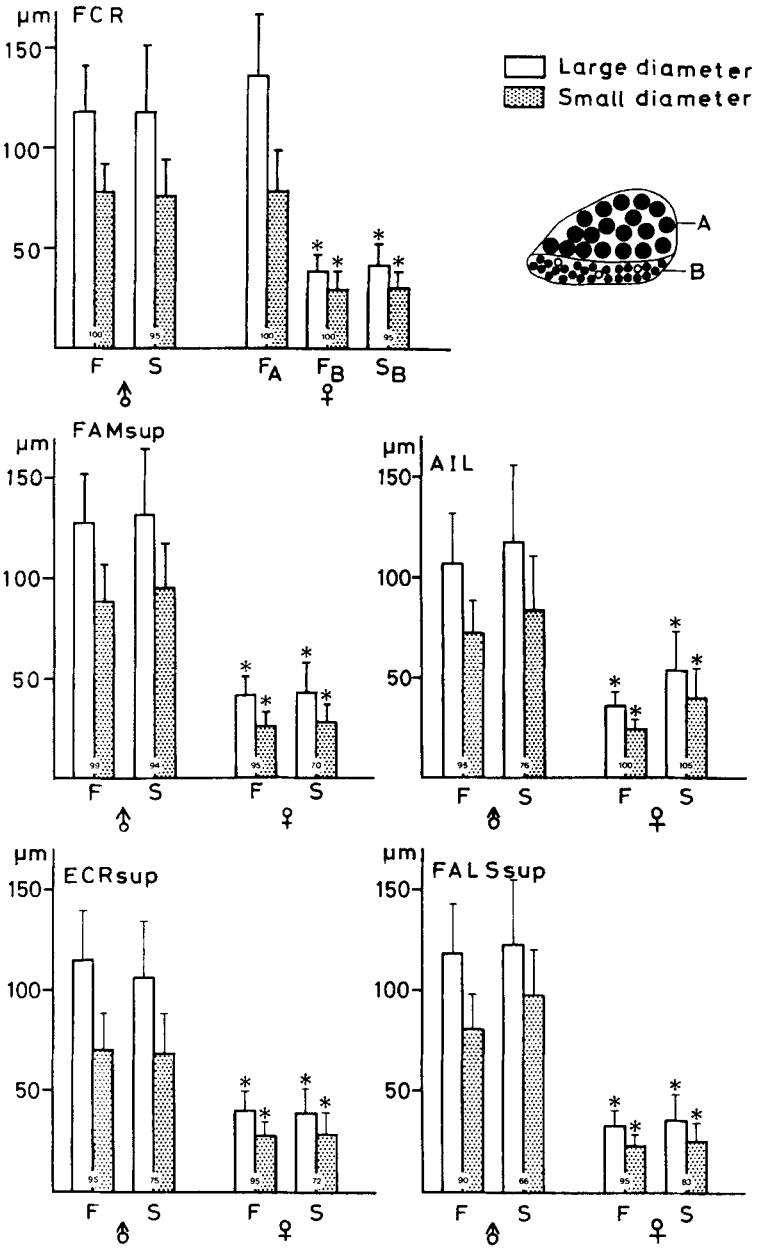


Fig. 5. Muscle fiber cross-sectional diameters of five sexually dimorphic muscles processed for myosin ATPase activity. Results are presented for both fast muscle fibers (F) and slow muscle fibers (S). The inset drawing of FCR shows schematically the cross section of the female FCR. The female FCR can be divided into two parts. The larger part (A) consists of fast muscle fibers (•); the smaller part (B) consists of both fast (•) and slow (◦) muscle fibers of smaller sizes. The vertical lines on the bars indicate SD values, and the numbers in the columns show the number of muscle fibers. All sexual differences in the large and the small diameters of muscle fibers are highly significant (*: $P < 0.001$) except for the FA fibers of the female FCR, which are similar in size to those of the male.

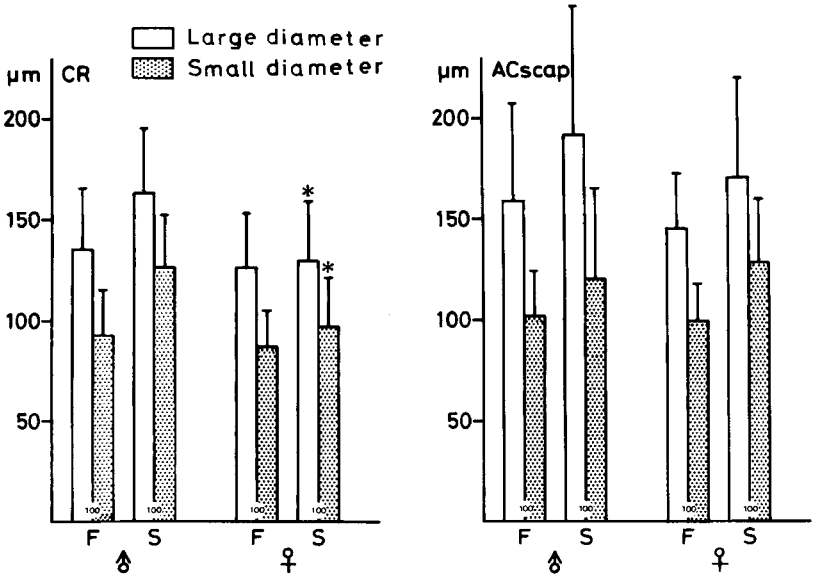


Fig. 6. Muscle fiber diameters of the semidimorphic muscle (CR) and the nondimorphic muscle (ACscap). The vertical lines on the bars indicate SD values, and the numbers in the columns show the number of muscle fibers. There are no significant sexual differences except for the slow muscle fibers of the CR (*: $P < 0.001$).

also to differences in the sizes of muscle fibers. The moderate sexual difference in the wet weight of the semidimorphic muscle (CR) can be attributed to the difference in the size of slow muscle fibers.

## DISCUSSION

### *Histochemical typing of muscle fibers*

We distinguished fast (twitch) and slow (tonic) muscle fibers on the basis of the staining properties of muscles processed for myosin ATPase and SDH activity. Examination of the SDH preparations suggested more than two subdivisions of muscle fibers and additional histochemical analysis might reveal further subdivisions such as the types 1–5 of Smith and Ovalle ('73). However, to simplify the quantitative analysis, we did not further subdivide the fiber types.

Judged by staining properties of muscles processed for myosin ATPase and SDH activity, our fast muscle fibers correspond to the fast-twitch, oxidative, glycolytic (FOG) muscle fibers of Putnam and Bennett ('83) or type 2 and 3 muscle fibers of Smith and Ovalle ('73); our slow muscle fibers correspond to the tonic muscle fibers of Putnam and Bennett ('83) or type 4 and 5 muscle fibers of Smith and Ovalle ('73). Neither the fast-twitch, glycolytic (FG) muscle fibers of Putnam and Bennett ('83) nor the type 1 muscle fibers of Smith and Ovalle ('73) were observed in the forelimb muscles of the Japanese toad. This is in agreement with the earlier histochemical observation in the toad FCR (Putnam and Bennett, '83).

### *Sexual dimorphism in the forelimb muscles of anurans*

Only a little attention has been paid to the Gaupp's suggestion (1896–1904) of the sexual dimorphism in some forelimb muscles of the amphibian (Muller et al., '69; Melichna et al., '72). The present study is the first that has quantitatively compared the muscle fibers between the two sexes as well as between the sexually dimorphic and nondimorphic muscles.

One of the salient (but, in a sense, confusing) findings in the present study is that the sexually dimorphic muscles of the male are not hypertrophied. On the contrary, it was found that, in the sexually dimorphic muscles of the female, both fast and small muscle fibers are of smaller diameter than those in other forelimb muscles of both sexes (all male muscles plus nondimorphic muscles of the female) and are less numerous than in the corresponding muscles of the male. From

these results, it is speculated that the sexually dimorphic muscles of the female are degenerative or subnormal, in terms of size and number of muscle fibers. The earlier studies may have overlooked this because of the lack of comparisons between the sexually dimorphic and nondimorphic muscles.

Melichna et al. ('72) studied sexual dimorphism in fiber pattern of the FCR of *Rana temporaria*. They reported that, in the female muscle, all muscle fibers processed for ATPase activity stained darkly, whereas in the male muscle, some muscle fibers stained lightly. The difference between our results and theirs might have resulted from the different species used. However, their histochemical observations may have been inadequate in that they did not survey the whole muscle. In the present study, female FCR was clearly divided into two parts. Melichna et al. ('72) may have observed only the part A of the FCR and have overlooked part B (see the inset of Fig. 5).

### *Functional significance of the sexually dimorphic muscles in behavior*

Because the sexual dimorphism was specifically observed in those muscles that could act as flexors of the forearm and abductors of the second digit, and not in the other forelimb muscles, this dimorphism may be directly correlated to clasping. Melichna et al. ('72) studied contraction properties of the FCR of *Rana temporaria* and reported that the male FCR contracts considerably more slowly than that of the female and that this "tonic" function of the male FCR may be suited for clasping behavior.

The slow (tonic) muscle fibers of the frog have been studied for a long time, by physiological (Kuffler and Gerald, '47; Kuffler et al., '47; Kuffler and Vaughan-Williams, '53a,b), electron-microscope (Peachey and Huxley, '62), and histochemical (Lännergren and Smith, '66; Engel and Irwin, '67; Smith and Lännergren, '68; Smith and Ovalle, '73; Lännergren, '75, '78, '79; Luff and Proske, '79) techniques. Although the slow muscle fibers have some properties advantageous for tonic and prolonged contractions of the forelimb muscles during clasping, Kuffler and Vaughan-Williams ('53b) concluded that the slow muscle fibers play an important role in the general postural activity of the frog, instead of playing a particular role in clasping behavior. Our results support and extend this view; the slow muscle fibers are neither clasping-specific nor abundant in the clasping-related muscles (see Table 1).

Thibert and Nicolet ('75) further studied the mechanical activities of the isolated FCR of the male *Rana temporaria* and compared them to those of the sartorius muscle. Although they found that the behavior of the FCR as a whole muscle was close to that of slow muscle fibers, their results did not support the presence of a great number of slow muscle fibers in the FCR, and the "tonic" nature of the male FCR has not been reasonably explained thus far.

The physiological properties of the nondimorphic muscles of the forelimb found, in the present study, to be similar in size and histochemical properties to the dimorphic muscles of the male (but not necessary for clasping), have not been studied thus far. Therefore, to examine whether there is a physiological specialization of the male sexually dimorphic muscle (suited for the tonic and prolonged contractions of the forelimb during clasping), it may be necessary to compare the contractile properties or metabolic activities of the whole muscle and single muscle fibers among the female dimorphic muscles, the male dimorphic muscles, and the nondimorphic muscles of both sexes, instead of comparison between the male dimorphic muscle and the sartorius (Thibert and Nicolet, '75).

### *Mechanisms of sexual dimorphism*

The direct actions of steroid hormones on muscles have been well studied in the syringeal muscle of songbirds and in the levator ani muscle of rats (reviewed by Arnold, '81). In amphibians, Muller et al. ('69) studied the effects of castration and testosterone treatment of the FCR of *Rana temporaria*, and they reported that muscle fibers of the FCR of the mature male frog showed seasonal fluctuations and that the fiber size was reduced by castration and restored by testosterone treatment. They concluded that the fiber size of the FCR was affected by the androgen levels. Although their results cannot be directly compared to ours, it may be possible that the androgens also act directly on the forelimb muscle fibers to produce sexual dimorphism. Since the present study was aimed at the sexual dimorphism of the forelimb muscles, our sampling was not enough to detect seasonal fluctuations in fiber size.

The indirect action of steroid hormones on muscles via the innervating motoneurons

may be also possible (see Erulkar et al., '81). Recent autoradiographic studies of the localization of hormone-concentrating cells in the brain of *Xenopus* have revealed androgen-concentrating neurons in the cranial nerve nucleus IX-X, which contains motoneurons innervating laryngeal muscles (Kelley et al., '75; Kelley, '80, '81), and in the brachial spinal cord, which contains motoneurons innervating probable clasping-related muscles (Erulkar et al., '81). Sexual dimorphism in the laryngeal muscles of the frog, which are involved in mate calling by the male, has also been reported (Schneider, '70; Eichelberg and Schneider, '73, '74). Therefore, to test the hypothesis of the indirect action of steroid hormones on muscles via the innervating motoneurons, it may be necessary to examine whether there is sexual dimorphism also in the motoneurons innervating the sexually dimorphic muscles (c.f. Breedlove and Arnold, '80).

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