son of experimental results gathered at different times must be made with reference to the season, especially the reproductive condition in seasonally reproducing marine invertebrates.

A. L. LAWRENCE*
J. M. LAWRENCE
A. C. GIESE

Hopkins Marine Station,
Pacific Grove, California

References and Notes

1. A. C. Giese, J. S. Tucker, R. A. Boolootian, *Biol. Bull.* **117**, 81 (1959).
2. J. S. Tucker and A. C. Giese, *J. Exptl. Zool.* **150**, 33 (1962).
3. A. Farmanfarmaian, A. C. Giese, R. A. Boolootian, J. Bennett, *ibid.* **138**, 355 (1958).
4. M. A. Nimitz, thesis, Stanford University (1963).
5. H. Hoffmann in *Klassen und Ordnungen des Tier-reichs,* H. G. Bronns, Ed. (Akademische Verlagsgesellschaft, M. G. H., Leipzig, 1930), vol. 3, p. 271.
6. A. C. Giese and G. Araki, *J. Exptl. Zool.* **151**, 259 (1962).
7. A. L. Lawrence, J. M. Lawrence, A. C. Giese, unpublished observations.
8. V. Fretter, *Trans. Roy. Soc. Edinburgh* **59**, 119 (1937).
9. A. C. Giese, *Ann. Rev. Physiol.* **21**, 547 (1959).
10. K. P. Mauzey, *Bull. Ecol. Soc. Am.* **44**, 47 (1963).
11. A. Fugi, *Bull. Fac. Fisheries, Hokkaido Univ.* **11**, 195 (1961).
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* Present address: Department of Biology, University of Houston, Houston, Tex. 77004.

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Changes in the Tail Feathers of the Adolescent Lyrebird

Abstract. *The changes which take place in the tail of the male lyrebird as it develops to maturity over a period of 7 to 8 years are attributable both to moulting and to processes whereby certain plain feathers are transformed into filamentaries and medians by growth of the rachis and loss of barbs and barbules.*

The tail of the mature male lyrebird (*Menura superba*) consists of 16 retrices which are normally carried extended behind the bird's body, but which are reversed over the body during display (Fig. 1). Each of two of these retrices, known as the lyrates, consists of a quill on the inner side of which is a vane which varies uniformly in width from about 5 to 6 mm at the proximal end to about 19 mm at the distal end. The color is light grey below and brownish-black above. To the outer side of the quill is attached a vane approximately 8 cm wide, which is characterized by a number of apparently transparent V-shaped "windows." This effect is due to the absence of barbules in the V-shaped areas; the thickness of the barbs decreases steadily as the distance from the shaft (rachis) increases. Figure 2 shows a portion of a mature lyrate feather, the detail of the window being illustrated in the inset. The margins of the V-shaped windows are colored yellow-to-orange on the underside, the tips of the vane being dark brownish-black. The end of the lyrate feather is dark brownish-black and club-shaped. Two of the retrices are very narrow, being only about 3 mm wide over the greater part of their length, and practically devoid of vane, especially on the inner side. The vane increases in width to a maximum of about 10 mm. Along the inner side are very fine barbules, ranging in length from about 2.5 to 3 mm over the greater part of the quill to up to about 19 mm over the last 15 to 18 cm distal from the base. These two feathers, generally referred to as the medians, are dark-colored above and silvery-gray on the underside.

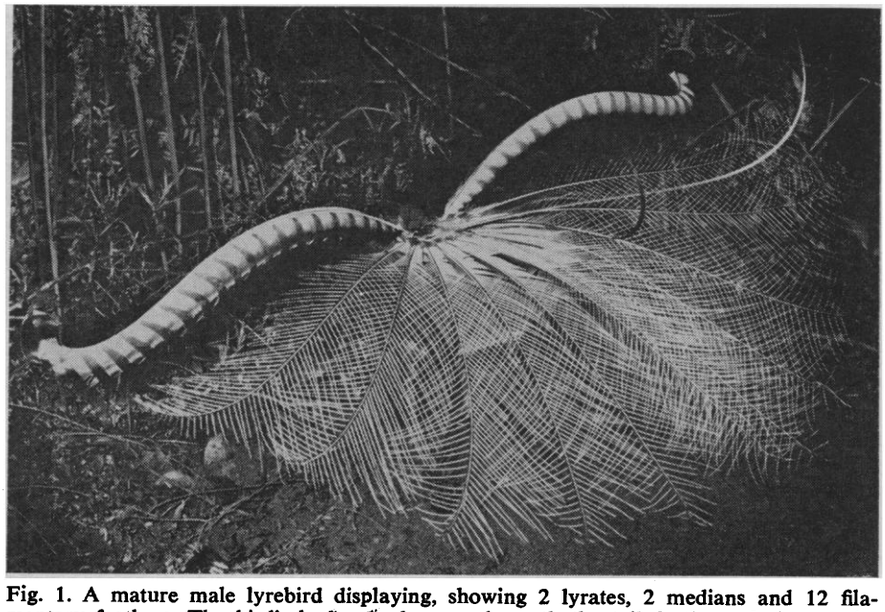
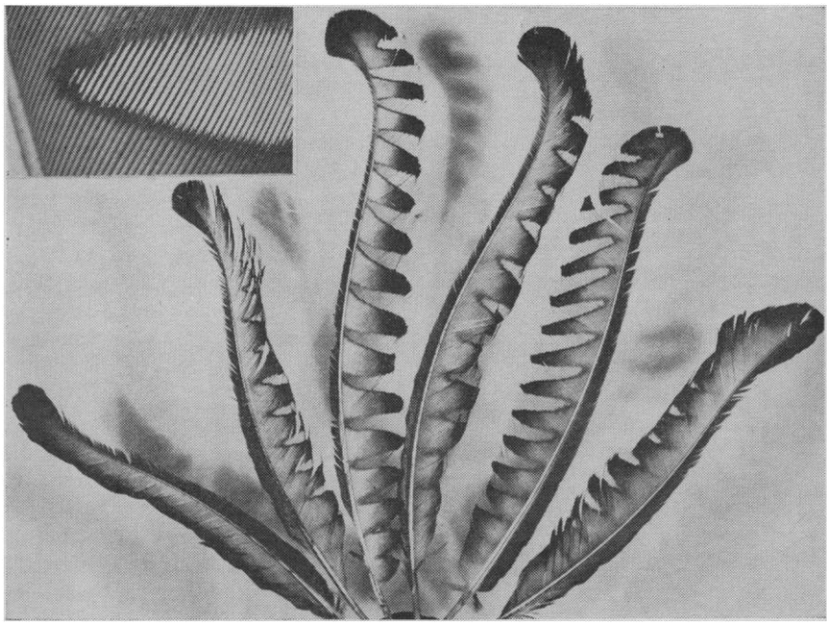


Fig. 1. A mature male lyrebird displaying, showing 2 lyrates, 2 medians and 12 filamentary feathers. The bird's body can be seen beneath the tail feathers, facing to the right.

The remaining 12 retrices (known as the filamentary feathers, or filamentaries) each consist of a quill with a "vane" of peculiar design. The proximal portion consists of barbs and barbules which fasten themselves to present a continuous appearance, the length of this part of the feather being greater in the middle than at the extremities of the group (see Fig. 1). The remainder of the feather consists of a number of fine filaments (barbs), up to 20 cm in length, attached to either side of the shaft. The coloring is similar to that of the medians—that is, dark above and light below. Different birds exhibit considerable variation in the length of these feathers (retrices) which range from 70 to 72 cm in some cases and up to over 80 cm in others.

When the young lyrebird leaves the nest at the age of approximately 6 weeks, its tail consists of 16 feathers and is about 12 cm long. At the age of 6 months the tail is similar in length (about 30 cm) and appearance to that of the female; but the juvenile windows in the lyrate are very poorly defined—that is, the lines of demarcation between the vane-like areas are not sharp.

It is difficult to be positive on this point, but observations made on the lyrebirds in Sherbrooke Forest, in the Dandenong Ranges, 48 km east of Melbourne, Australia, suggest strongly that the lyrates are moulted approximately annually and replaced by new ones. Occasionally, immature birds have



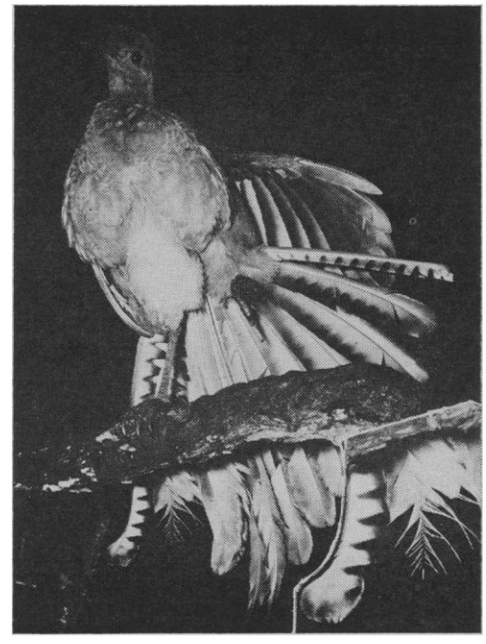


Fig. 2 (left). Lyrate feathers moulted by immature male lyrebirds. The inset shows the detail of a "window." The feather on the left came from a very young bird which had been killed by a predator. Lengths of these feathers from left to right are 27, 32, 41, 39, 35, and 30 cm. Fig. 3 (right). This immature male lyrebird was preening when photographed in July 1956. One plain feather shows the beginning of the filamentation process. The inset photograph shows the effect in greater detail. The end of the lyrate feather is immature in character.

been observed carrying two pairs of lyrates, one more advanced than the other, indicating that moulting of the old lyrates does not always precede the growth of the others. Frequently, too, the two new lyrates are not grown simultaneously, so that they remain "out of phase" and do not match completely even after growing has apparently ceased. As the bird grows older, each generation of lyrates is noticeably more mature in appearance, the windows being clearer and the tip of the feather more club-like in appearance; the area of pigmentation (black) is also greater. Figure 2 illustrates the manner in which the lyrate develops toward the mature form (Fig. 1) with successive moults.

The process whereby the adolescent male lyrebird acquires the filamentary feathers is of outstanding interest. When the bird is approximately 4 years old, one of the "plain" feathers appears to "split" at the distal end, due to the loss of barbules (Fig. 3). This process continues toward the base, while at the same time the barbs increase in length and the rachis also becomes longer. Later, other "plain" feathers undergo a similar transformation, so that a point is reached at which the tail may consist of several plain feathers and one or more filamentaries which have been formed from the other plain feathers. It is important to recognize that this in fact is the mechanism by which the filamentary feathers develop—the appearance of the filamentary feather is not necessarily preceded by a moult. A metamorphosis occurs.

In some cases, the process is not continued until all 12 feathers have been "converted." Certain birds have been observed to moult some of their converted filamentaries, while still retaining several plain feathers, so that one plain feather may be seen to accompany 11 filamentaries of which

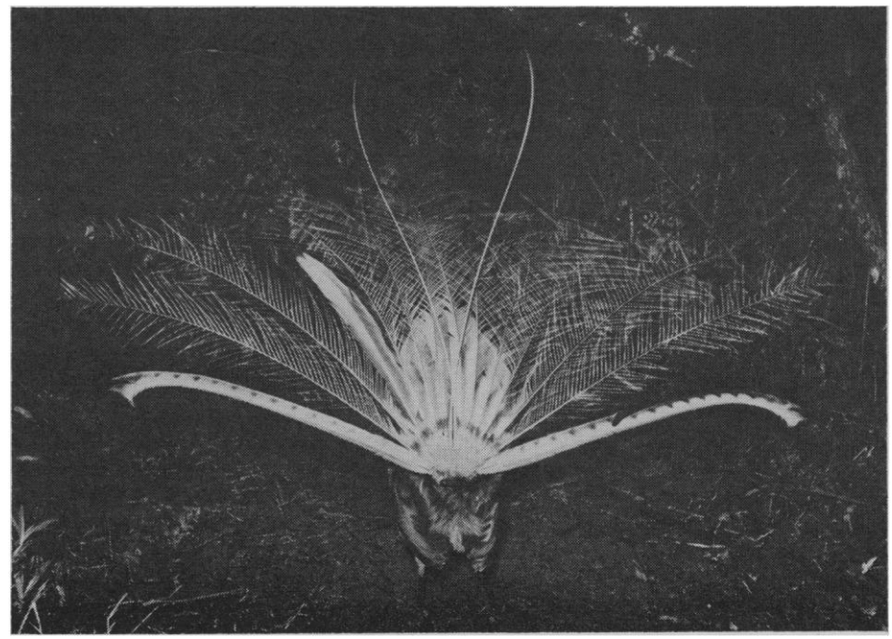


Fig. 4. A "near-mature" male with only one plain feather remaining. The medians are of the mature type.

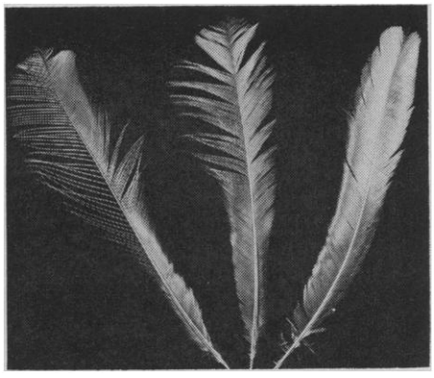


Fig. 5. The transformation of a plain feather (right) to a partly filamented feather (left). The dimensions of these feathers from right to left are: length, 42, 43, 46 cm; width, 7, 12, 16.5 cm, respectively.

some at least are "first generation" filamentaries (Fig. 4). The latter can frequently be identified by virtue of the fact that some of the barbs have still retained a few barbules near the distal end, which cause the barbs to fasten together to some extent (Fig. 5 and cover). The barbs (filaments) in a "second generation" filamentary feather do not bear barbules in such a position. Once the transformation of the tail has begun, it frequently happens that a plain feather is moulted; in this case it is replaced by a filamentary of the "second generation" type.

The development of the medians is not normally apparent in the adolescent bird until the 5th year, possibly the 6th. The central plain feathers are observed to grow longer, frequently extending far beyond the remainder of the feathers, and to taper at the distal end. However, these two feathers are derived from two which have been carried at least for several years and probably from the beginning.

The first pair of new medians consists of two feathers having a central shaft and vane about 25 mm wide. Commencing at a point about 15 cm from the base, and extending to a point 15 cm from the tip, the outer side of the vane is filamented, the inner side having the usual vane-type structure. These medians grow until they extend far beyond the remainder of the tail, which may at this stage consist of several plain (that is, unchanged) feathers, along with a number of filamentaries (most of which have been acquired by the transformation of plain feathers). In due course, the first pair of true medians is moulted and replaced by a new pair which are narrower than the first pair. In these and subsequent medians, the filamentation extends along the outer side of the feather right to the tip. The medians, which are about 25 mm wide, are not always in phase—that is, one begins before the other. Three and probably four sets of medians are grown before the bird is fully mature at the age of 7 or 8 years.

It is thus clear that for several years the feathers other than the lyrates remain in a "passive" condition and then slowly and irregularly undergo a remarkable series of changes. While the young male lyrebird may be seen displaying and singing on the mound at the age of 1 year, both his displays and singing are juvenile; but, through constant practice, by the time he attains the age of 4 years, he is frequently an accomplished artist. During the adolescent period singing and display activities increase markedly.

The work of Marshall (*1*) and others has established a definite relationship between hormonal activity and singing and display activities. It is clearly not possible to sacrifice lyrebirds for comparative studies; but it may safely be assumed that the same holds in the case of this species also. Since the acquisition of adult characteristics is clearly a manifestation of increased hormonal activity, there appears to be a definite relationship between the enhanced singing and display, feather transformation, and hormone activity in the case of the adolescent male lyrebird.

Since each of the vaned retrices behaves independently—that is, undergoes transformation at different times spread over a period of 3 to 4 years—it seems clear that in each case the cytoplasm must receive its coded genetic information from nuclear sources which retain their independence throughout adolescence, but which are gradually coordinated as the bird matures. The mature male sheds all 16 retrices over a period of 3 to 4 weeks (and frequently less), the new feathers growing practically simultaneously over a period of 4 to 4½ months. However, occasionally a mature male lyrebird, after moulting, will grow its lyrates out of phase, possibly because of some retarding influence.

The bird shown in Fig. 6 has an interesting history. It was hatched in August 1959 and, as is usual, left the nest at the age of 6 weeks. By the following March, its tail feathers had grown to the usual length (about 30 cm), but it had acquired a pair of fine medians comparable in structure

Fig. 6. An immature male lyrebird showing one filamentary feather which has been derived from a plain feather. This feather is longer and wider than the remaining plain feathers. The bird also has its first pair of true medians. When photographed, the bird was almost 5 years old.

with that of a mature male, being practically devoid of vane on the inner side and having very little on the outer side. It shed these after approximately 1½ years, and thereafter resembled other young lyrebirds. One of its vaned retrices began to undergo filamentation in 1962 and by December of that year filamentation was well advanced. It retained that feather until September 1964, by which time it had acquired its first pair of new medians. Figure 6 shows its state of development at the end of May 1964. The increase in length and width of the filamentary feather as compared with the plain feathers may clearly be seen. Visual observations and photographic records establish the fact that this feather had grown considerably since December 1962. At the time of writing (November 1964) this bird had shed its lyrates and all but two of the plain feathers, as well as the solitary filamentary and the medians shown in Fig. 6; it is now growing new filamentaries, lyrates, and medians. Among these the two plain feathers are conspicuous.

It is not yet known whether the barbs and barbules which disappear during the transformation of a plain feather are actually lost by the breaking of their attachments to the rachis and barbs, respectively, or whether they are reabsorbed. At any rate, the whole process of the development of the tail feathers must be under the control of some complicated hormone system which has yet to be investigated.

L. H. SMITH

National Parks Authority,
276 Collins Street,
Melbourne, Australia

Reference

1. A. J. Marshall, *Biology and Comparative Physiology of Birds* (Academic Press, New York, 1960).

2 November 1964

Successive Transformations of an Established Cell Line by Polyoma Virus and SV40

Abstract. *Two different oncogenic viruses, polyoma and SV40, are capable of transforming mouse cell line 3T3. The properties of the transformed cells produced by the two viruses are in some ways similar, but in other ways they are specific for the infecting virus. This fact permits testing whether a cell line transformed by the one oncogenic virus is still susceptible to the tranforming action of the second virus. Two different clonally isolated polyoma-transformed lines when infected with SV40 give rise to cells with properties characteristic of SV40-transformed cells. The frequency of transformation, however, is considerably reduced compared to that of the parent cell line, 3T3.*

The established mouse fibroblast line, 3T3, which is strongly contact-inhibited in culture, is susceptible to transformation by two different oncogenic viruses, polyoma and SV40 (*1*). In each case the transformed cells are characterized by their ability to grow readily over one another under conditions where the untransformed cells remain strictly confined to a monolayer. This loss of contact inhibition of cell division is a stable property, passed on to all the progeny cells. The transformed cells produced by the two viruses may be distinguished from each other; SV40-transformed 3T3 cells are able to grow to a considerably higher saturation density (up to 15 × 10^5 cells/cm^2) than polyoma transformed 3T3 cells (2 to 4 × 10^5 cells/cm^2) and therefore produce much denser colonies when inoculated sparsely (*2*). The transformed cells produced by the two viruses may also be distinguished from each other on the basis of detailed colonial morphology (*1*). Because of these characteristic differences it is now possible to demonstrate SV40 transformation of a cell line already transformed by polyoma virus.

Two polyoma-transformed cell lines were used. One, PY-3T3-31, is a polyoma virus transformed line that has been cloned twice and has a saturation density (2 × 10^5 cells/cm^2) which is in the low range for polyoma-transformed cell lines. The line was produced by infection of a stationary phase culture of 3T3 with polyoma virus in the presence of 100 μg of 5-iodo-2′-deoxyuridine (IDUR) per milliliter. The transformation frequency under these conditions is not reduced and may in fact be increased (*3*). The other line was PY-3T3-11, a clonal isolate of a line transformed in the absence of IDUR, and having a saturation density of 4 × 10^5 cells/cm^2. Whereas many polyoma-transformed 3T3 cultures continue for a long time to show a cytopathic effect and release high titers of virus, these two clones no longer did so. Both clones were maintained in culture by transfers of 1:1000 dilutions of subconfluent cultures.

Cultures of PY-3T3-31 in exponential growth were exposed for 3 hours to 0.5 ml of a stock of SV40 strain 776 (*4*) containing $10^{8.2}$ tissue culture infective doses per milliliter (*5*). The next day the cells were plated, as were uninfected cells, at 100 to 40,000 cells per plate. Two weeks after plating, the cells were fixed and stained. By this time the larger inocula of both the control and infected cells had grown to saturation density, and the cultures appeared as fairly homogeneous layers of interlacing cells, a few cells thick. However, the cultures infected with SV40 also contained colonies of tightly packed epithelioid cells that showed a consid-

Table 1. Transformation frequency of polyoma-transformed cell lines after infection with SV40.

Plating efficiency* (%)		Cells per plate	Total cells plated (× 10^{-3})	Transformed colonies		Transformation frequency† (%)
Control	Infected			Control	Infected	
			Cell line 3T3			
44	67	100	4.0	0	73	2.7
44	67	1000	50	0	781	2.3
			Cell line PY-3T3-31			
43	34	100	2.4	0	0	—
43	34	4000	132	0	13	0.03
43	34	40,000	240	0	18	0.02
			Cell line PY-3T3-11			
48	56	100	1.2	0	0	—
48	56	12,000	72	0	22	0.05

* Plates inoculated with 100 cells and fixed 10 days later. Average of six or more plates.
† $\frac{\text{Transformed colonies}}{\text{Cells plated}} \times 100 \times \frac{100}{\text{plating efficiency}}$.