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Tooth replacement in the Slow worm (*Anguis fragilis*)

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(With 4 figures in the text)

The dentition of the Slow worm (*Anguis fragilis*) has been carefully observed in a range of individuals from birth to advanced age. Although a considerable volume of carefully prepared dead material has been used, emphasis in the investigation is placed upon three living specimens kept in near-natural conditions and examined by taking impressions of the teeth every week throughout a whole season.

A general description of the gross anatomy of the dentition is given, and the changes that occur with increasing age. The tooth attachment and the process of replacement in the individual tooth is considered and found to conform to the Anguinomorphan type of replacement described by McDowell & Bogert (1954).

The evidence obtained from both dead and living material as to the pattern and order of tooth replacement is presented and found to be in agreement with the theory of Edmund (1960).

Further details of the replacement pattern, discernible only in the records from the living specimens, and the effects of season and age upon tooth replacement are described.

Anguis is found to provide an excellent illustration of the fundamental principles in reptilian tooth replacement, being relatively uncomplicated in the features of its replacement pattern. The species also appears to be a promising subject for the further investigation of tooth replacement phenomena.

Contents

Introduction

Edmund (1960) established that in all lower vertebrate dentitions the teeth are formed along the edge of the dental lamina in a sequence beginning anteriorly, and progressing towards the rear, and that once formed, they migrate in the occlusal direction. In consequence, the teeth come to be arranged on the lamina in a number of oblique rows or

Zahnreihen, and Edmund showed that this principle is traceable through the fossil record for some 350,000,000 years.

All this was learned from dead material, together with embryological considerations, but there are strict limits to the amount of information obtainable from these sources. It has already been shown that there is a great deal of additional knowledge to be derived from the study of tooth replacement in living specimens, and the difficulties of keeping the animals alive in captivity and regularly examining their dentitions, have now been largely overcome (Edmund, 1962 and in current work, Cooper, 1965).

The dentition composed of a number of overlapping oblique rows of teeth is an arrangement which gives rise secondarily to other features, a noteworthy one being sequences of replacement in the alternately-numbered teeth. When living specimens are being observed these sequences of eruption are particularly noticeable and for this reason, they are marked on the records in Fig. 4.

Actual sequences of replacement are not invariably in the two series (odd and even) and may be in every third tooth in some cases, but this is only an occasional occurrence.

Age, temperature, season and disease all have effects on tooth replacement and such effects can only be observed in the living subject. In the present investigation, emphasis is therefore placed upon the evidence from living material.

Materials and methods

During the course of this investigation a large number of live Slow worms was examined and from these a series of 24 individuals was selected, ranging from the newly-born (smallest 62 mm from snout to vent) to ones of advanced age (largest 201 mm from snout to vent).

Twenty were prepared as dry skulls in a manner to avoid as far as possible, loss of the earlier replacement stages that may be held in position only by delicate soft tissue. The heads of the specimens were boiled in plain water for 15 min and the soft tissues removed by hand. Around the replacement teeth small amounts of soft tissue were deliberately left, which when dried, served to hold the teeth in position.

In order to see the very small replacement teeth and ascertain their correct positions, one specimen was cleared by Dawson's technique (Bolles Lee, 1950). After the highly pigmented skin had been removed, all the calcified teeth could easily be seen even without staining.

Three adult female Slow worms were maintained in an outdoor vivarium so that records of tooth replacement in life could be collected. Owing to the remarkable ability of the Slow worm to climb and burrow, it was found necessary to use a vivarium with vertical glass walls 1 ft high and extending beneath the ground to a depth of 6 in. The specimens so kept spent much of the time out of sight and apparently found most of their own food, though this was supplemented whenever the Slow worms were visible and active.

It is difficult to assess the age of Slow worms, especially after they have attained mature size, and the choice of specimens for the experiment centred around one individual of known age. It had been caught as an adult female and had lived for 7 years in the vivarium and so could be assumed to be at least 10 years old. It was decided to select one obviously younger than this and another showing greater signs of ageing. A female estimated to be 3 to 4 years old was chosen by its size, colouration and markings (Smith, 1965), whilst signs such as heavy and hard osteoderms on the head, scarring about the body and unusually large size were used to distinguish the oldest one. Although chosen by these essentially inexact methods, there is no doubt in the author's mind that the 3 specimens differed widely in their ages.

In order to investigate the dentition in life and especially the replacement pattern, it is necessary to inspect and record the state of the tooth rows at suitable time intervals on a large number of occasions. Hence a quick and simple method must be used which neither damages the tissues, nor endangers the life of the specimen. Examination by radiographs, as described by Edmund (1960; 1962) is hardly possible in specimens whose entire jaw measures less than a centimetre in length; and repeated exposure to the X-rays is an unknown and unwelcome hazard. A satisfactory method was found in taking impressions of the teeth with sheets of paraffin wax 0·5 mm thick (dental casting wax). The wax sheet was inserted between the jaws so that the full extent of the tooth rows was covered, and the jaws carefully pressed together so that the teeth were driven into the wax. The wax sheet was then withdrawn and examined under a binocular microscope by transmitted light (impressions of the upper teeth being visible on the one side and of the lower teeth on the reverse side of the wax). This method proved very adequate in showing the circumstances at each tooth position, namely presence or absence of a tooth and whether it was newly erupted or at its final occlusal level.

It was also found possible to examine the teeth of the living specimens directly. The soft tissues of the mouth in *Anguis* are so translucent that under suitable magnification and lighting even the crest of the jaw bone and the unerupted teeth are visible, as also is the vascular network in the gingival tissues.

Once ascertained, the circumstances at each tooth position were recorded by simple symbols— an open circle for a functional, ankylosed tooth, a black dot for a newly erupted tooth and vacant spaces left blank. Series of such records assembled consecutively are shown in Fig. 4. Only one quadrant of the dentition is represented in these records, since replacement is effected with remarkable symmetry not only on the two sides, but also in upper and lower jaws. The upper tooth row is chosen because it is borne on two separate bones, and this adds complexity to the replacement pattern.

The dentition

The dentition of *Anguis fragilis* consists only of marginal teeth borne on the maxilla, premaxilla and dentary (Fig. 1); there are no teeth on the ptergyoid bones or at any other site.

At rest the jaws are held closed, the lower arch of teeth fitting within the upper arch, so that there is contact between the buccal surfaces of the lower posterior teeth and the palatal surfaces of the uppers. The lower anteriors rest against the palate behind the premaxillary teeth.

The form of the crowns

The form of the crowns of all the teeth is similar, but there is great variation in size (Fig. 1). All are single cusped and nowhere is there any degree of minor cusp formation. The nine premaxillary teeth are of uniform, small size and are on a higher plane than those of the maxilla. One is placed in the midline and is remarkable for the bilateral symmetry of its crown. The four teeth on either side of it are inclined slightly away from the midline and the tips of all are curved backwards. The first maxillary tooth is rather larger, and the size increases to the maximum at the fifth and sixth positions, decreasing again posteriorly. A similar gradation in size occurs in the teeth of the dentary. The tips of all the posterior teeth are directed inwards and backwards.

At birth the crowns are long, slender and sharply pointed with the inward and backward curvature pronounced. Succeeding generations of teeth are progressively larger and the crowns become more obtusely pointed, the girth being increased relatively more than the height. This change, however, never seems to advance to the degree seen in *Varanus niloticus* in which the posterior teeth become flat-topped as age increases.

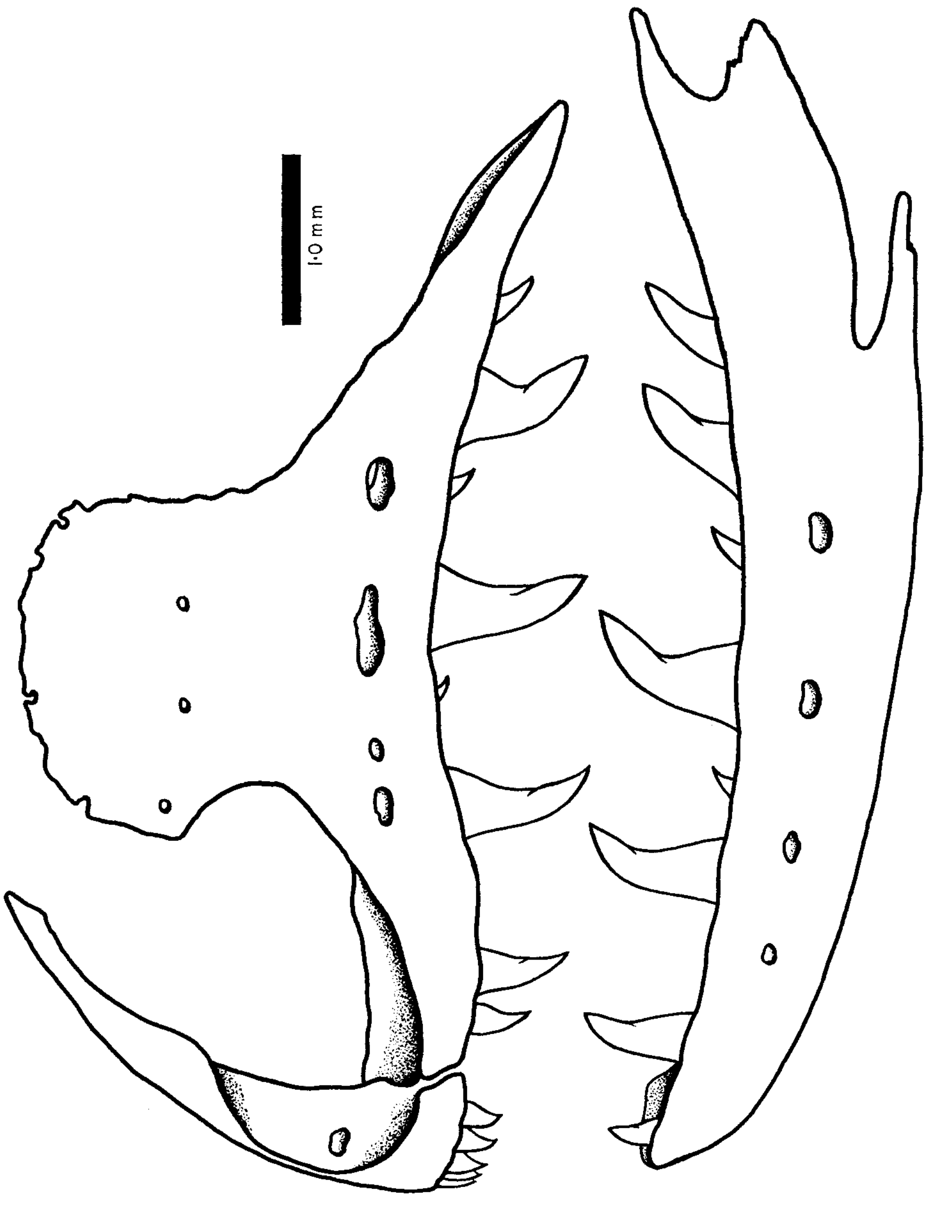


FIG. 1. The teeth and tooth bearing bones of *Anguis fragilis*. Adult male. Premaxilla, maxilla and dentary viewed from the buccal aspect.

A sharp ridge extends down the length of the crowns forming a cutting edge anteriorly and posteriorly and the surface of the tooth shows a system of fine grooves and ridges disposed in the long axis, as described by Leydig (1872). Similar surface features can be seen on the teeth of larger reptiles such as *Crocodilus niloticus* (Poole, 1961).

The only sexual difference in the teeth of *Anguis* lies in their size; in keeping with the size of the head, they are larger in the male than in the female. In the breeding season the male teeth have an important secondary function, when they are used to grip the body of the female during copulation.

Tooth numbers

At birth there are nine teeth on the premaxilla, ten on each maxilla and 11 on each dentary and the length of the whole tooth row is some 2·5 mm. In the second year of life, there are still nine premaxillary teeth, but usually only nine on the maxilla and ten on the dentary. These numbers then remain constant until an advanced age has been reached when, exceptionally, a further reduction to nine teeth on the dentary occurs. The whole tooth row in an aged male specimen is about 7·5 mm long. Reduction in tooth numbers with increasing age is in contrast to the condition found in most reptiles where the tooth rows are greatly extended by additions posteriorly. Changes in tooth numbers are considered further in connection with the replacement pattern.

Tooth attachment and the replacement process

Tooth attachment in *Anguis* is pleurodont, the teeth being ankylosed to the sloping inner aspect of the jaw bones.

Different types of pleurodont tooth-site were recognized and described by McDowell & Bogert (1954), and Edmund (1960) pointed out the two variations of the condition which are most significantly different, the distinction turning largely upon the site of the developing replacement tooth and the effect of the replacement process on the old tooth. In the one type, the replacement tooth develops immediately lingual to the base of the old tooth in which a resorption cavity is formed proportional in size to the growing successor. This is typified in the Iguanidae and designated the "iguanid" method of replacement. The other type is characteristic of all the Anguinomorphan lizards (McDowell & Bogert, 1954), the replacement tooth lying disto-lingual to the base of its predecessor and only moving forward into the latter's position after the old tooth has been shed. This is well demonstrated in the Varanidae and is called the "varanid" method of replacement.

The dentition of *Anguis* is a good example of the varanid type and is strongly reminiscent of the dentition of *Varanus* itself. The bases of the teeth are very broad and a considerable buttress of calcified cementing tissue is laid down round the bases of the functional teeth making an extremely strong attachment. The replacement cycle which occurs at each tooth position is depicted in five stages in Fig. 2. At the base of the completely ankylosed tooth is a foramen which faces disto-lingually and passes through the base of the tooth into the pulp chamber at the junction of the dentine with the cementing tissue. Slightly above and disto-lingual to this foramen lies the replacement tooth which has begun to calcify by the time the functional tooth has become fully ankylosed. Resorption of the old tooth commences in relation to this foramen as the replacement tooth increases in size and moves in the antero-labial direction. The wall of the old tooth is progressively eroded until

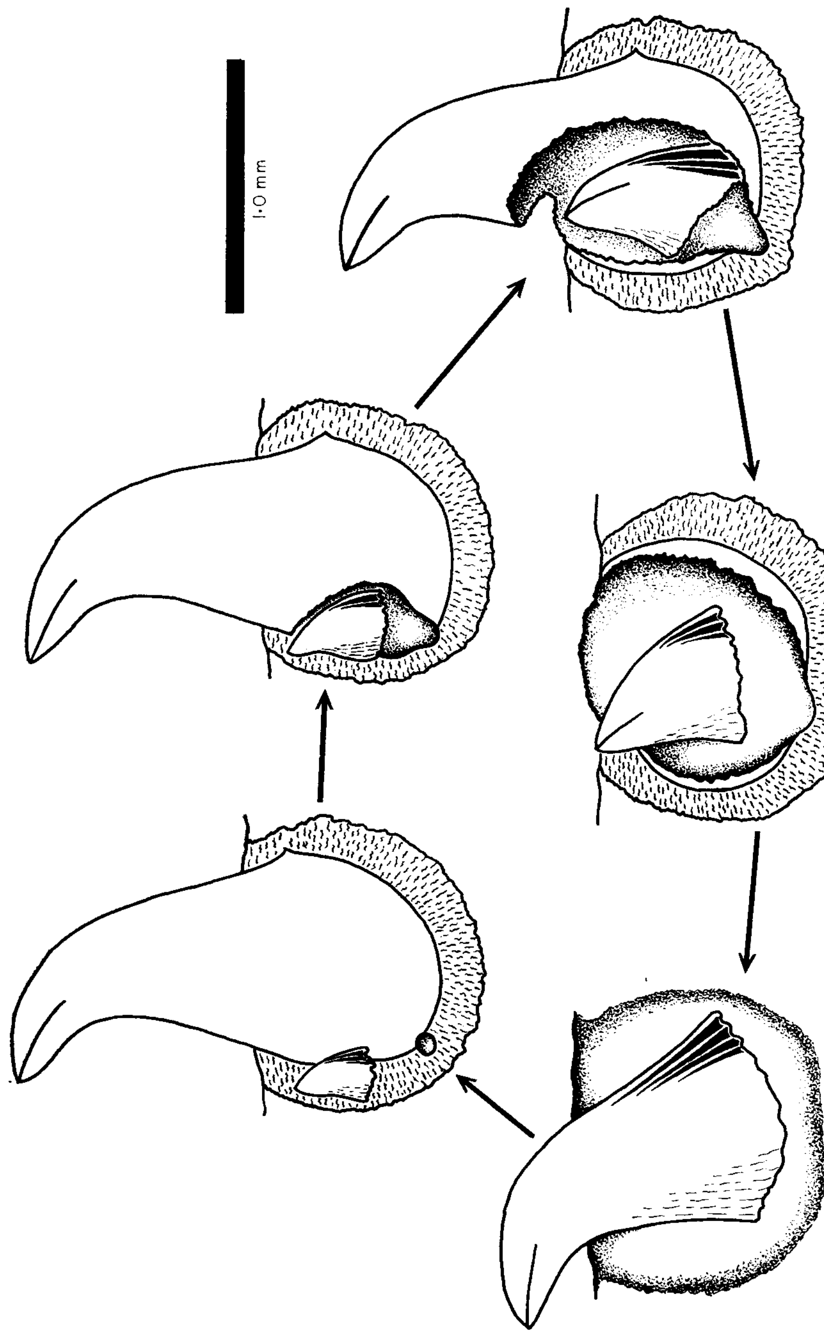


FIG. 2. The replacement cycle which occurs at each tooth position, represented in five stages.

it is penetrated and the pulp chamber invaded. By this time the replacement tooth lies almost within the cavity in the old tooth, the erosion of which then proceeds from the inside of the pulp chamber (Edmund, 1960) until the walls have become so thin that the coronal portion breaks off. A jagged base is sometimes left above gingival level, but this is soon eliminated by further resorption. At this stage the replacement tooth is still only about one-third grown, but continues to increase in size, now occupying the position of the old tooth and maintaining a backward inclination, until it erupts through the soft tissues. Following eruption the movement towards final height and inclination is very rapid, and is effected, together with complete ankylosis, within three days. The short duration of these final stages in tooth replacement is important, since the developing tooth when erupted, yet only anchored by soft tissue, is very liable to injury.

The replacement pattern

In Fig. 3, all the calcified teeth, both functional and replacement, are represented in relation to a diagrammatic dental lamina, and three features of the arrangement of the teeth are pointed out.

The "tooth family" (marked by broken arrows) consists of teeth which arise from the same germinal material at the edge of the dental lamina and replace one another at the same tooth position.

The oblique, continuous lines indicate the Zahnreihen (Edmund, 1960) the upper quadrant being composed of seven, while the lower quadrant of the same specimen was made up of six.

The specimen depicted shows that there are sequences of eruption in the alternately numbered teeth and one such sequence in the even-numbered series is marked with black symbols connected by a dotted line. These teeth would clearly erupt in the order 14, 12, 10, 8, 6, 4, 2, and the whole of the sequence would have erupted before a similar one in the odd-numbered teeth followed at positions 13, 11, 9, 7, 5, 3,1 (shown diagrammatically in Fig. 3 as very small replacement teeth and omitted from the drawing in the interests of clarity). Thus the whole of one alternate series is replaced before replacement of the other commences and the two series function separately. This can also be seen in the records from living specimens (Fig. 4) and is correlated with the fact that the old tooth is lost a long time before eruption of its successor. It is another feature of the varanid method of replacement.

The records in Fig. 4 show some irregularities in the direction of progress of the sequences. On some occasions they run from front to back, and on others from back to front, though on the average, a slight forward progression is maintained. This is a condition close to simple alternate replacement and according to Edmund's hypothesis, denotes a spacing between the Zahnreihen of just over 2·0 tooth positions.

The functional life of all the teeth in *Anguis* appears to be similar irrespective of their size or position in the jaw. For instance, at the beginning of the record in Fig. 4(b) the time from eruption to exfoliation is remarkably constant at seven to eight weeks along the whole tooth row.

This is in contrast to some reptiles where there is marked variation in the functional life of the teeth in different areas of the jaw, correlated with differential spacing between the Zahnreihen and a varying rate of progress of the sequences in the alternate series.

In the Slow worm where the length of life of all the teeth is similar, there is also relative uniformity in the spacing between Zahnreihen and in the form of the replacement sequences.

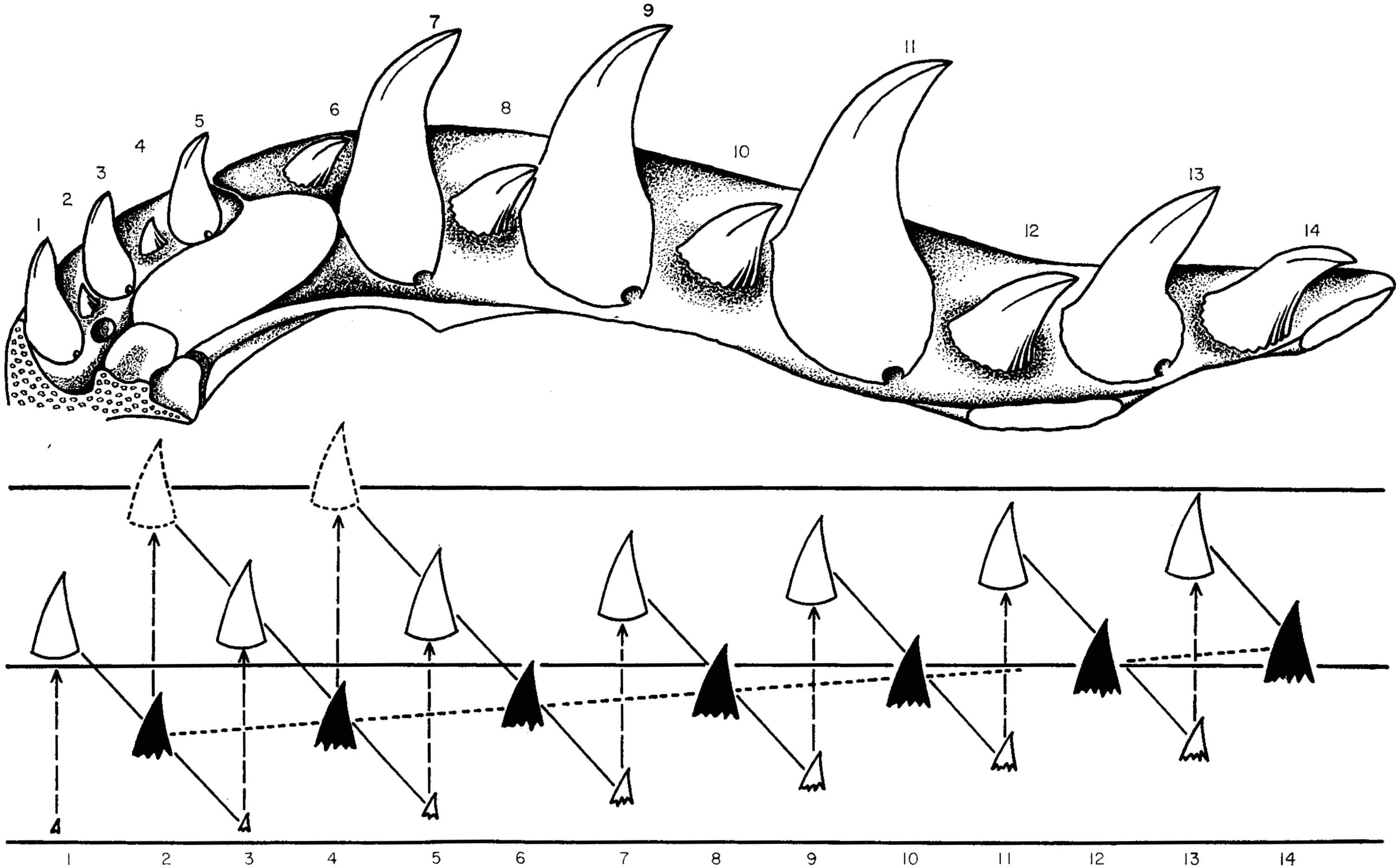


FIG. 3. Above: the left maxilla and the premaxilla inverted to show the inner aspect of the bones, and the teeth numbered from 1 to 14.
Below: Diagrammatic representation of the tooth row. Teeth arising from the same germinal material or "tooth families" are marked by broken arrows, the Zahnreihen by continuous lines, and one replacement sequence in the even-numbered series is indicated by black symbols and a dotted line. The three horizontal lines represent the edge of the lamina where the teeth arise, eruption and exfoliation.

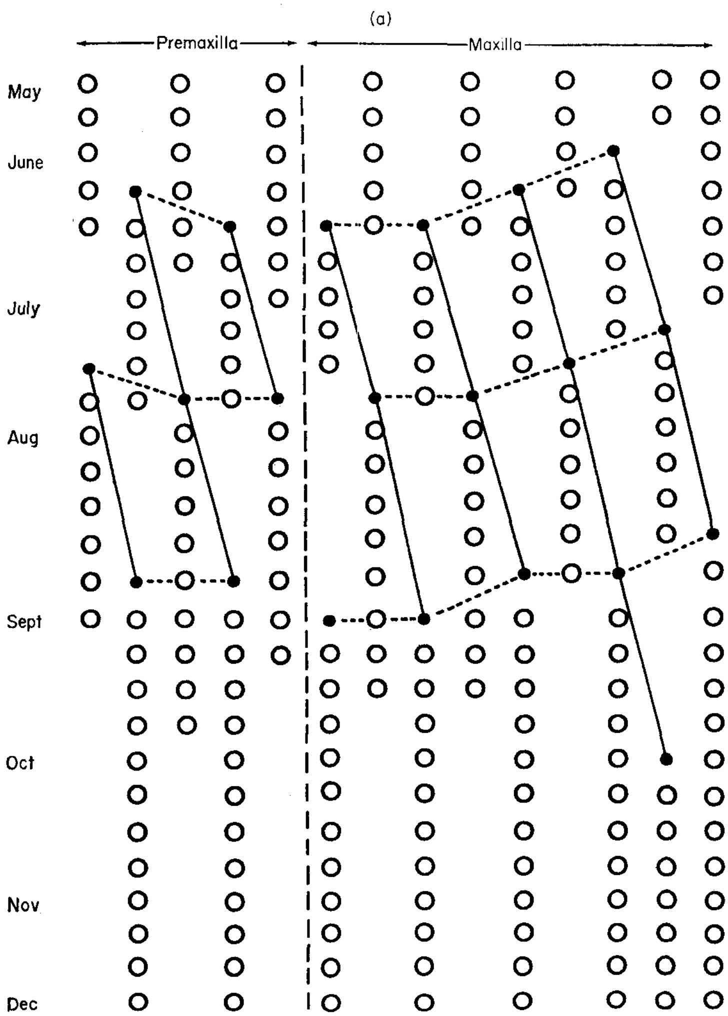


FIG. 4. (a) Female 3 to 4 years old.
See caption overleaf.

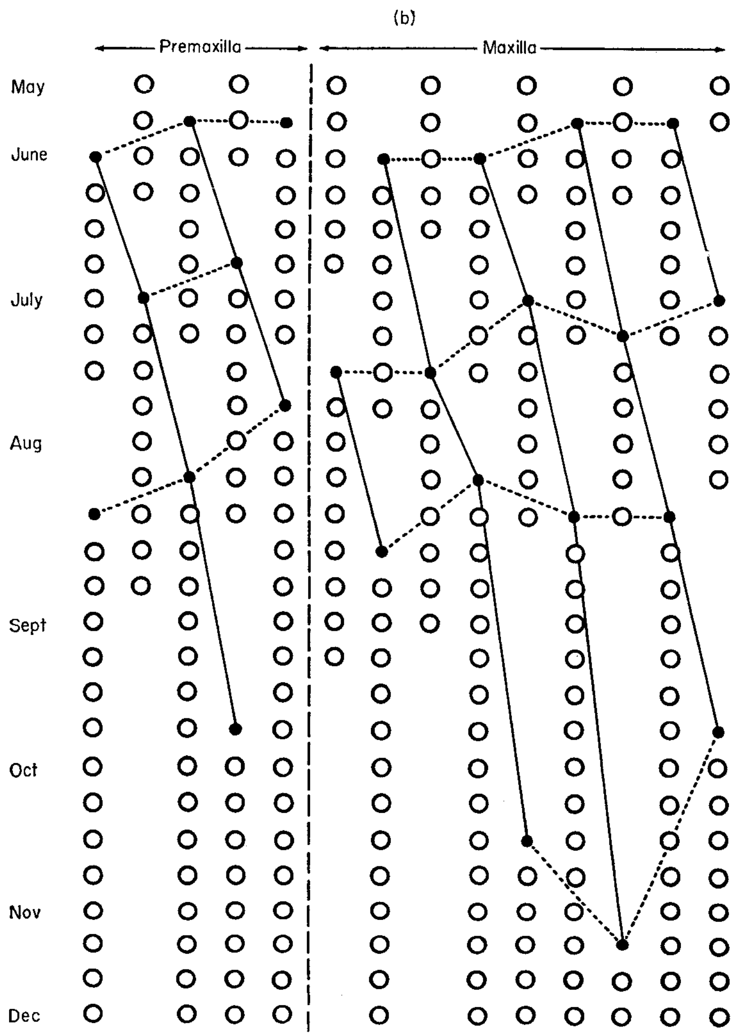
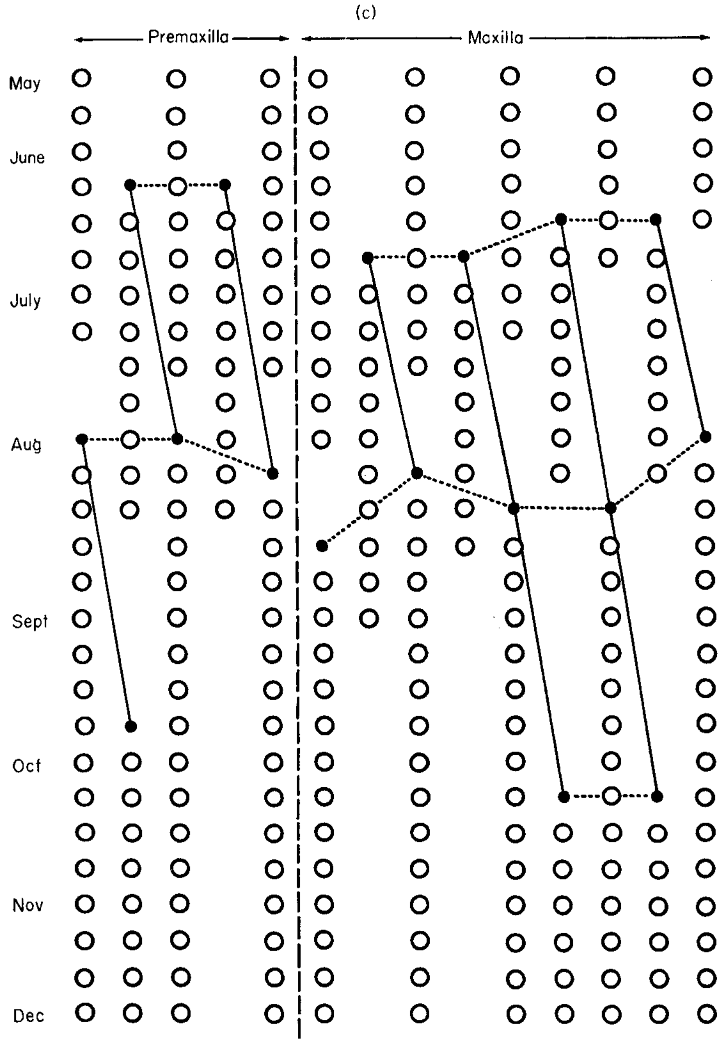


FIG. 4. (b) Female approximately 10 years old.
See caption opposite.



(c) Female of advanced age.

FIG. 4. Records of tooth replacement from living specimens of *Anguis fragilis* collected from May to December 1964.

Open circles represent ankylosed functional teeth, black dots represent newly erupted teeth and unoccupied tooth positions are left blank. Each horizontal row is a record showing the circumstances at every tooth position, i.e., functional tooth present, tooth newly erupted, or vacant space. The records were taken at weekly intervals and arranged consecutively from the top to the bottom of the page. The suture between maxilla and premaxilla is indicated by a broken line. Again Zahnreihen are marked by continuous lines and the replacement sequences by dotted lines.

Length of tooth-life and frequency of replacement in relation to season and age

The facts recorded diagrammatically in Fig. 4 are as follows: In (a) the youngest specimen, the first observed replacement sequence in the even-numbered teeth erupts in the first three weeks of June, and all of these new teeth complete their functional life in four to six weeks (mean 5·0). The next sequence in the odd-numbered teeth starts erupting three weeks after the last eruption in the preceding series and again, all these teeth complete their life and are shed in six to nine weeks (mean 7·3). The first eruption in the next sequence (even) follows four weeks after the last of the "odd" sequences and not one of this series completes its life even in 13 weeks.

In (b) the ten year old specimen, there is general similarity. The first sequence, in this case "odd", erupts during the last week in May and first week in June. These teeth are all replaced and have functional lives of six to seven weeks (mean 6·1). The first eruption of the next sequence (even) takes place three weeks after the last of the previous one, and these teeth all complete their lives in five to eight weeks (mean 6·5). The first eruption in the third sequence is only one week after the last of the preceding series and no tooth in this series completes its life even in 17 weeks.

In (c) the oldest individual, the eruptions in the first observed (even) sequence of replacement occurs in the second to fourth weeks of June, later than the first sequence in either (a) or (b) and overlapping the second sequence in (b). They all complete their functional lives in seven to ten weeks (mean 8·3). The second sequence begins erupting in the first week of August and not one tooth of this series completes its life even in 16 weeks.

Although teeth do erupt as late in the year as November, none of those that erupt after the last week in July completes its life to exfoliation. There are 29 such teeth which remained attached for periods of 11 to 17 weeks. The 32 teeth that erupted between the last week in May and the end of July were exfoliated in four to ten weeks. It seems that buds belonging to the earlier period develop and are shed within relatively few weeks, whereas those of the next sequence are inhibited and do not erupt within the experimental period. Such an abrupt change, occurring almost in mid-summer, does not seem compatible with a seasonal slowing of tooth development. Possibly only a fixed number of tooth changes occur during the season, a certain number of buds being formed in the lamina and then activity ceasing until there is restimulation.

The last eruption was recorded at the beginning of November, and by December the specimens had retired completely into hibernation, so that examination of the teeth was no longer possible. It seems likely that tooth replacement ceases altogether during hibernation.

The suture between maxilla and premaxilla

Edmund (1960) stated of reptiles in general that the sequences of replacement are not interrupted at the suture between maxilla and premaxilla, and this is the case in the specimen of *Anguis* depicted in Fig. 3. In the records from life, however, it can be seen that although the sequences may be construed as continuous in Fig. 4(a), they are certainly not so in Fig. 4(b) and (c), the older individuals. It seems possible that the sequences are continuous across the suture in early life, but that tooth replacement on the two bones becomes independent as age advances. This state of affairs may be correlated with the fact that the dental lamina is interrupted at the suture in some reptiles such as *Lacerta* (Cooper, 1963) and *Vipera berus* (Smith, Bellairs & Miles, 1953).

Reduction in tooth numbers

In most reptilian dentitions the number of teeth increases with age by extension of the tooth rows posteriorly, but this is not the case in *Anguis*. As previously described, there is in fact a reduction in the tooth numbers in older specimens and this is apparently effected by the occasional replacement of two adjacent teeth by one successor. This phenomenon is known to occur in *Lacerta* where it has been observed both in dry material and in life (Cooper, 1963) and a similar condition has been reported in an amphisbaenid lizard by Schmidt (1960). Replacement of two teeth by one could arise either by fusion, or by failure of the one to develop. Sometimes the coalescence is incomplete and two instances of incomplete fusion have been found in the present work. This suggests that two adjacent teeth are fusing rather than the one failing to develop.

Symmetry in replacement

There is bilateral symmetry in replacement of the teeth, waves of eruption passing along the two sides of the jaws at almost exactly the same time. Furthermore, some synchrony seems to exist between upper and lower jaws, since the functional teeth in one jaw are always those which will contact the ones present in the opposing jaw (Fig. 1). This symmetry and synchrony are clearly important in maintaining a dentition which is functional at all times.

Discussion and conclusions

The dentition of *Anguis fragilis* is an unusual one in several respects. Pleurodont attachment and tooth replacement of the varanid type occur widely, but as compared with most reptiles, the number of teeth in *Anguis* is small, and their gross anatomy simple. The pattern of tooth replacement is also relatively uncomplicated and therefore provides a useful example of the underlying principles.

It has already been suggested that the sequences in the alternate (odd and even) series are a measure of the whole replacement pattern, since they are formed by the combination of many factors. In *Anguis* the sequences pass from back to front, but they are nearly "flat" so that replacement of a whole alternate series is effected rapidly, and apart from minor irregularities, the speed of progress of the sequences is constant along the length of the tooth row. This form of wave is commensurate with a spacing between Zahnreihen of just over 2·0, uniform along the tooth row and with equality in length of life of all the teeth.

The observations of Edmund (1960) and Cooper (1963), indicate that there are differences in the form of the sequences and the associated factors in various species, but the extent and significance of these differences could only be ascertained by collecting records of tooth replacement from a wide range of species under similar conditions in order to make direct comparisons.

There has long been curiosity as to whether tooth replacement continues indefinitely in polyphyodont animals and in the factors affecting the process. The records from living specimens of *Anguis* (Fig. 4) suggest that the rate of replacement may slow down with increasing age, the functional life of the teeth becoming longer. There may also be seasonal fluctuations, and replacement probably ceases altogether during hibernation.

There is some evidence that length of tooth life increases with the approach of Autumn. In (a) for instance the average length of life of the teeth that come into service during June (first sequence) is five weeks, whereas that of the new teeth of the second sequence in July is 7·3 weeks. If there is increase in tooth life as season advances, in trying to assess the effects of age on tooth life it is vital to compare only conditions at the same time of year. If averaging is relied on to compensate the season differences, the mean tooth-life in the three specimens is (a) 6·23, (b) 6·36, and (c) 8·3 weeks.

Reduction in the complement of "tooth positions" as age advances has been described and is believed to be due to the occasional coalescence of two adjacent tooth germs. This would of course reduce the number of teeth replaced in a given time.

There could therefore be three elements in the slowing down of replacement, lengthening of functional life of the teeth, reduction in the number replaced in a given time and reduction in the number of "tooth positions". How far these processes of slowing down ever advance is still a matter for conjecture. No specimen examined in the present work has shown evidence of actual cessation of replacement, and no report of such a case has been found. The almost toothless monitors described by Bellairs & Miles (1960, 1961) may have become so from age changes, but there are other possible explanations such as dietary deficiency and disease.

The sample of records from living specimens is too small for statistical treatment, and the technique employed, with observations taken only once a week, admits of a high degree of experimental error. In order to obtain reliable results it would be necessary to take a series of records from a larger number of individuals, at more frequent intervals, and over a number of seasons.

Anguis fragilis can be exceptionally long-lived and has survived as long as 54 years in captivity (Smith, 1965). Very old specimens would therefore be favourable material for the study of age changes and a likely source of further information concerning tooth replacement.

I should like to acknowledge my long-standing indebtedness to Dr D. F. G. Poole of the Dental Research Unit, Medical Research Council, for all the help given and interest shown in the present work.

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