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A review of age determination methods for the Stoat *Mustela erminea*

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ABSTRACT

A sample of 166 Stoats collected from Craigieburn Forest Park, Canterbury, New Zealand, was used to assess the usefulness of seven different methods of age determination. All the methods made use of characters which do change with age, but not all are equally good at defining useful age classes. The recommended approach is to use a combination of skull and baculum measurements to identify young animals, followed by counting of the canine cementum annuli of adults. If the skull or baculum is broken or not available, visual assessment of the status of the nasal sutures, the lateral suprasesamoid tubercle of the femur and the wear of the carnassial teeth are the next best options for distinguishing the young. Kopein's index based on the closure of the pulp cavity of the canines, and the zonation visible in the periosteal bone of the mandible, are unreliable.

INTRODUCTION

From 1973 to 1976 I organized a collection of more than 1600 dead Stoats from 14 study areas, mostly within the National Parks of New Zealand. From the carcasses a range of information on body size, food habits, moult, reproductive status, and some parasites was recorded (King & Moody, 1982; King, 1989a). Analysis of this material was greatly hampered by the lack of any reliable means of age determination. Most of the above parameters are affected by the age distribution of the sample from which they are measured, so it would not have been acceptable to ignore the matter of age; yet to use an unproven method would have served only to introduce unidentifiable errors into our analysis. My colleagues and I (especially J. E. Moody and M. G. Efford) spent an astonishing amount of time trying to resolve this problem. The first purpose of this paper is to help others to avoid having to do the same again.

Our first attempt to obtain a series of known-age specimens started in September 1973 when we began to keep stoats in captivity. By June 1975 we had kept a total of six Stoats for periods of 5-14 months, whose minimum ages ranged from 18 to 20 months. Only one of these was a female and we made no attempt to breed young. None of the six skulls proved to be of any use in preliminary age trials.

THE DSB METHOD

Failing known-age stoats, we then tried to apply the DSB method (named for the three essential items from which information was required: date of death, skull, baculum) worked out from known-age Weasels *Mustela nivalis* by King (1980). The cranial anatomy of Stoats and Weasels is similar, so it was reasonable to assume that age-related characters in the two species should have much in common. The method relies on the

Table 1
Visual assessment of skull shape (% of skulls) by five independent observers (data by courtesy of M. G. Efford)

	Month killed									
	D	J	F	M	A	M	J	Jy	A	S
Unanimous	96	93	61	56	44	40	35	41	17	58
Disagree 1:4	3	7	36	23	25	32	27	38	59	15
Disagree 2:3	1	0	3	20	31	29	37	21	24	27
Number of skulls	69	88	125	74	59	64	40	34	17	26

pronounced changes in the shape and strength of the cranium, which are visible to the eye when a large series of clean skulls is laid out in twelve monthly groups according to the date of death. The single measurement most clearly reflecting the development of the skull is the post-orbital ratio (King, 1980). From clean skulls, the post-orbital ratio is calculated as the inter-orbital width divided by the post-orbital width, both measured with a vernier micrometer to the nearest 0.01 mm. The weight of the baculum (if available), the shape of the bony crests, the closure of the sutures and the texture of the bone, among other characters, are also taken into account. Within each monthly group, the young of the most recent breeding season are separated from the older animals; the aim is eventually to divide the whole sample into 24 groups.

The method is reliable only for as long as the young skulls form an easily distinguishable group. All the Stoats were assumed to have been born on 1 October (for reproductive data, see King & Moody, 1982), and the first month in which undoubted juveniles begin to appear in the population was November. Plots of individual post-orbital ratios (Fig. 1) show that the presumed young are clearly distinguishable until the end of February, but after then it gets progressively more difficult to identify them as they mature towards the adult condition.

Since we had no known-age material, we had no means of checking the accuracy of this process as applied to Stoats. We therefore arranged a trial in which five different people, two experienced in handling mustelid material and three inexperienced, were asked to classify a large series of female skulls. Only the shape, suture fusion, development of crests and bone texture of each skull were taken into account, since the aim was to see whether the five observers could all recognize the same two modes of skull shape in each monthly sample. The 'accuracy' of the five sets of results was estimated from the proportion of skulls in any 1-month group on which the five observers disagreed in the ratio of 3:2, because the number of errors could not be less than the number of disagreements. For Stoats collected from December to February inclusive, fewer than 4% of skulls caused this level of disagreement (Table 1), but in every other month of the year the observers disagreed on at least 20% of the skulls in each group. The disagreements were considerably fewer than would be expected from purely random allocation: the total proportion of unanimous allocations would be 100% if they were all correct, and 6.3% if they were all random, whereas it was 40% in the test. However, this shows only that skull shape is indeed related to age to some extent—as were all the other methods tested here. The question was whether and for how long the two modes of skull shape are visually discrete.

We concluded that the young could be reliably recognized, but only up to the end of February, and that a truncated form of the DSB method could therefore give dependable results for the purposes of our analysis. Three age classes were defined as follows:

(a) young—all Stoats of either sex caught before 28 February of their first year (≤ 4 months old). They are smaller on average than the adults, but are not called 'juvenile': the males are certainly reproductively immature but the females are precocious, and are already pregnant with blastocysts in delay (King, 1989a);

(b) subadult—males with bacula of the typical juvenile shape and development (dry weight < 38 mg) caught between 1 March and 31 August of their first year (5–10 months old);

(c) adult—all males caught after 1 September of their first year (≥ 11 months old), and all females caught after 1 March of their first year (≥ 5 months old).

This classification was sufficient to eliminate the still-growing young animals from samples of adults, but offered no way to distinguish year classes of adults, or even younger from older adults. Since the literature suggested that a significant proportion of individual Stoats could live for 3 years or more, our inability to control more finely for age in these samples was frustrating.

We spent a great deal of time on extensive trials of almost every other possible method of age determination. The trials were confined to a single subsample of 166 stoats from Craigieburn Forest Park, Canterbury. This restriction was necessary because there is very substantial geographical variation in body size of Stoats in New Zealand (King, 1989b), which can make the skeletal characters used for age determination hard to interpret. For example, external features of the skull such as the sagittal crest and the mastoid process, and even internal ones such as periosteal zonation, develop more strongly in larger than in smaller Weasels of the same age (King, 1980). The Craigieburn sample was chosen because it was fairly large, with an even sex ratio (54% males), and collected in 3 years from within a single patch of remnant beech (*Nothofagus* spp.) forest at high altitude (790–1340 m a.s.l.) in the foothills of the Southern Alps. The Stoats in that area are near the top of the range of body sizes recorded in New Zealand [mean (\pm S.E.) body weight: males, 356 ± 7.0 g; females, 222 ± 3.4 g] and the climate of the study area is cool (annual range of mean monthly minimum temperatures -2.8 to 7.2°C), so we hoped that characters influenced by body size and season would be clearly expressed. However, the trials were inconclusive, since we had no known-age material and no objective standard against which to calibrate the results. We concluded that no further progress would be made until known-age material became available. In the meantime, the analysis of the main collection (King & Moody, 1982) proceeded, using the minimum reliable age distinctions as described above.

The opportunity to obtain a series of known-age animals arose in the southern summer of 1979–80. In the beech forests of southern New Zealand, a massive crop of beechmast was produced in the autumn (March) of 1979. During the winter (June–August) of 1979, the density of feral House Mice *Mus musculus* in the beech forests temporarily increased, and in the spring and early summer (late November to December), very large numbers of young Stoats appeared (King, 1983). In two study areas in Fiordland we set live-traps and caught, tagged and released a total of 134 Stoats (113 young of the year, 19 adults and two unknown) over a short but hectic period up to the end of January 1980 (King & McMillan, 1982). Until January the young of the year are still distinctly smaller than adults; also, young males can be distinguished by their small testes, and young females by their undeveloped nipples. Over the following 18

months, 14 marked Stoats, from a possible 107 last seen alive, were recovered. In addition, another eight Stoats were kept in captivity for 15 months in 1979–81. The canine teeth of these 22 animals were sectioned by the standard procedures (Grue & King, 1984). The specimens were labelled in code, so that their origin was unknown to the section reader. Of the 20 that returned clear results, the number of incremental lines corresponded exactly with known age in the 12 marked as young. In the eight marked as adults, the number of lines was equal to or greater than the minimum known number of years of age. We therefore concluded that the counting of canine cemental annuli is a reliable means of estimating the age of adult Stoats in New Zealand.

The calibration of canine cementum annuli against known age removed the practical need to review alternative methods of age determination. On the other hand, it also made such a review possible: and, because the calibration was necessarily done on a rather small sample, it would clearly be useful to be able to check the extent of agreement between cementum annuli and other methods. That is the second purpose of this paper.

MATERIALS

The carcasses used for this trial were collected in Fenn traps, set all year round in tunnels in beech forest and inspected daily. As in all samples collected in properly set Fenn traps, the skulls and teeth of most individuals were undamaged. The skulls, bacula and femurs were removed and cleaned by dermestid beetles (see King & Moody 1982).

METHODS TESTED

A comprehensive review of age determination methods that have been tried on small mustelids was given by King (1980). Many of the general comments made there are still valid, and do not need to be repeated here. However, the reproductive biology and life expectancy of Stoats are different from those of Weasels, and both affect age determination. This paper therefore concentrates on methods applicable to Stoats.

The standard for comparison

The results given by canine sectioning were taken as the standard against which the results given by the other methods were to be compared. Before compiling the sample to be sent for canine sectioning, the young animals of both sexes under 5 months old, and the subadult males under 11 months old, were identified by the DSB method and excluded. One clean jaw from all remaining animals was treated as described by Grue & King (1984). The elimination of young animals and subadult males from sectioning saves a lot of unnecessary histological work, and does not introduce significant error since the characters of the skull and baculum are very reliable means of distinguishing young Stoats up to the ages specified above.

Post-orbital ratio

The post-orbital ratio is one of the key components of the DSB method developed for Weasels, but its value as applied to Stoats has not been tested except indirectly during the trial described above. This paper seeks to document measured changes in the post-orbital ratio with age. A full review of the origin and basis of this method is given by King (1980).

Baculum weight

This method is one of the oldest and most reliable, and is the only one based on empirical evidence. The adult shape and weight of the baculum is attained only after puberty in *M. frenata*, and this development can be prevented by castration (Wright, 1950).

A plot of the individual dry weights of the entire collection of 651 bacula collected from throughout New Zealand (King & Moody, 1982) showed that from December to August there is a distinct group of male Stoats with low (<38 mg) baculum weights. Sectioning of the testes from all males from two subsamples, Craigieburn and Westland, confirmed that these were indeed the pre-pubertal young of the year. From December to February all males with small bacula were classified as young on skull characters, and all males with larger bacula were classified as adult on skull characters. In September and October there is a sudden growth spurt, during which the baculum weight and shape, and body weight and length, of the young males merge with those of the adults.

van Soest & van Bree (1970) showed a good correlation between the baculum weight and number of cementum annuli in 20 males of unknown age from Holland. Likewise, Grue & King (1984) found that the baculum weights of 11 males of known or part-known age from New Zealand continue to drift upwards for 3 or 4 years. Perhaps the secretion of androgens each breeding season has an accumulative effect on the baculum, at least for several years. The present material offers the first opportunity to test for a possible link between weight and age on a larger sample.

Bacula cleaned by dermestids and dried in air to a constant weight were weighed to the nearest 0.01 mg.

Kopein index

The canines of young carnivores have wide internal pulp cavities, which become filled with dentine in older individuals. Kopein (1967) proposed a method of determining age from an index based on the percentage ratio of the external width of the canines to the width of the pulp cavity, both measured at the root of the tooth. From a very large sample of Russian Stoats collected during the winter fur seasons of 1959–64, he defined the age classes as follows:

- (a) first year (young of the previous season, 0.5–1.0 year old)—pulp cavity index of 100 (hollow tooth) down to 20 in males and 100 to 16.5 in females;
- (b) adults (1.5–2.0 years old)—index of 19.5 to 10.5 in males and 16.0 to 9.5 in females;
- (c) old (≥ 2.5 years old)—index of under 10.0 in males and under 9.0 in females.

Kopein claimed that this method was 96–99% reliable, and was confirmed on 26 captive Stoats of known age. It can be done on fresh material (i.e. uncleaned skulls), but requires accurate horizontal cutting of the canine at a consistent position at the root, and fine measurement of the external and pulp cavity widths with a vernier micrometer.

Periosteal zonation

Transverse sections of the jaws of many species show clear growth lines in the periosteal zone (Morris, 1972). They are certainly present in Weasels, and are described and illustrated by King (1980). One jaw of each Stoat was fixed in formol saline, decalcified whole for 4 days in RDO (a proprietary decalcification agent), sectioned with a specially designed hand microtome at about 75 μm and stained with Mayer's haemalum. Klevezal & Kleinenberg (1967) give instructions for interpreting the pattern of growth lines. The first line is often rather wavy, and is called by these authors a 'resorption

line'. It is not clear from their description whether or not this line is to be counted as representing the first year of age, so I recorded it separately.

Stroganov toothwear classes

Stroganov (1937) described the processes of development and abrasion of the carnassial teeth of Stoats, and devised a key to a series of age classes defined from the stages of wear. The classifications can be made quite simply from clean skulls with the aid of a binocular microscope plus the photographs and descriptions provided by Stroganov. The key leads to definitions of six annual age classes, the first subdivided into three stages (young under 4 months old, 4–7 months old, and 7–12 months old).

Lateral suprasamoid tubercle (LSST)

van Soest & van Bree (1970) described and illustrated a small bony tubercle on the distal end of the femur of Stoats. The development of this feature is claimed reliably to separate first-year Stoats from older adults. If true, this could be particularly useful in females, which have no baculum. For this trial, the status of the tubercle was estimated by eye on a three-point scale (absent, developing, present) from the photographs given by van Soest & van Bree (1970).

Nasal sutures

The closing of the nasal sutures is a well-known indicator of maturity in most mammals. Hansson (1968) defined four stages of closure as follows: (I) sutures open; (II) coalescing; (III) coalesced but visible; (IV) invisible. According to Hall (1951), Stoats and Weasels pass through Class I by about 7.5 months and Class IV by about 10 months. However, these estimates were not confirmed from known-age Weasels collected in England by King (1980); in them, both Classes I and II were passed before 3 months of age, and Class III in 3–6 months, which left all animals older than 6 months together in Class IV. For this trial, the state of the sutures in Stoats was estimated by eye on Hansson's four-point scale.

RESULTS

Cementum annuli

Of the 166 Stoats in the Craigeburn subsample, 10 were discarded because of damage or missing data, and 147 were classified without ambiguity into year classes. Because the first-year class is large, it was subdivided into smaller groups, chosen to allow comparison with the groups based on the previously used DSB criteria. The first subgroup corresponds with the 'Young' age class (birth to 4 months), defined from cranial characters in both sexes. In males the second subgroup corresponds with the 'subadult' age class (5–10 months) defined from baculum weight. The remaining first-year males form a small transition group of rapidly maturing young adults killed in September at 11 months old. The remaining first-year females, 5–11 months old, are already mature. Stoats of ≥ 12 months old were all classified into year classes, but the number of individuals in the higher classes was small. In order to obtain samples large enough to compare, the older animals were grouped together as shown at the head of Appendix 1.

The remaining nine Stoats were sectioned but could not be classified because of disagreements between age criteria. These are individually described and discussed below.

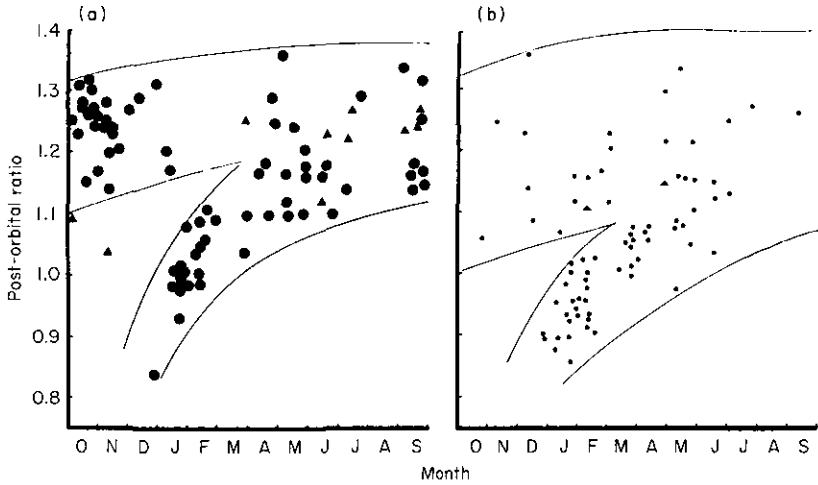


Fig. 1. The post-orbital ratio of individual male (a) and female (b) Stoats plotted against month of death. Birth date assumed to be 1 October. Triangles distinguish individuals affected by skrjabingylosis, a parasitic nematode which distorts the skull in the post-orbital region. The young of the year form a distinct group up to the end of February.

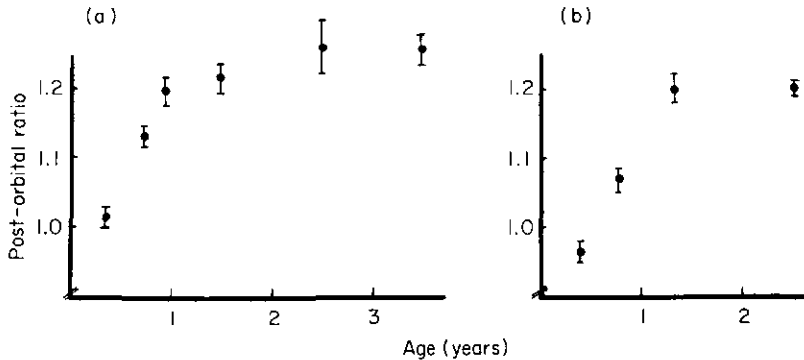


Fig. 2. Development of the post-orbital ratio (mean \pm 1 S.E.) in male (a) and female (b) Stoats with age. Ages of individuals defined from cementum annuli; further details given in Appendix 1.

Post-orbital ratio

The post-orbital ratio is sufficient to identify a distinct group of young males and females up till about 5 months of age. After that, individual ratios for young and adults overlap (Fig. 1). The mean post-orbital ratio rises through the age classes in both males and females (Fig. 2). This observation confirms that the process of skull development, and the reliability of the post-orbital ratio as a single measure of it, are similar in Stoats and in Weasels. It also confirms the suggestion of King (1980) that the narrowing of the post-orbital constriction continues slowly throughout life. However, there is massive overlap of the ranges of individual measurements later in the series, and this too confirms the difficulty experienced by our observers in classifying the older skulls by the DSB method.

Researchers making use of museum material often need to be able to distinguish and set aside young, not yet full-grown individuals, rapidly and without damage. King & Moody (1982) suggested as a simple rule of thumb that a post-orbital ratio of > 1.15 for

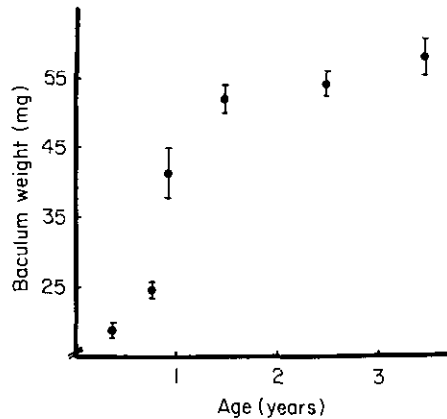


Fig. 3. Development of the baculum (mean \pm 1 S.E.) in male Stoats with age. Ages of individuals defined from cementum annuli; further details given in Appendix 1.

males and > 1.10 for females would be a reasonably reliable criterion to identify adult Stoats. Appendix 1 shows that these suggested values are well below the recorded means for adults of both sexes of ≥ 1 year old. This method can therefore be trusted as an easy and reliable, if somewhat conservative, method of separating out the young skulls. On the other hand, in any growth series there will always be a large group of borderline cases on either side of any suggested breakpoint. As expected, the range of individual measurements of newly maturing adults of both sexes span the breakpoint, and even among the undoubted young individuals there are a few that fall on the wrong side of the line, and vice versa among the undoubted adults.

In theory, since this criterion is based on a ratio rather than on an absolute measurement, it should be independent of geographical variation in body size. However, Stoats of different adult size may grow at different rates. Since the Stoats of Britain and New Zealand are among the largest anywhere (world size distributions mapped in King, 1989a), the appropriate breakpoint figure may well need to be calibrated to body size in other countries where Stoats are smaller.

The post-orbital region is liable to damage by the destructive cranial nematode *Skrjabingylus nasicola*. In advanced cases the distortion of the bone induced by the parasites makes accurate measurement impossible. In New Zealand, relatively few individual Stoats are visibly damaged (twelve are shown in Fig. 1), but in other places, e.g. in parts of North America, the prevalence of skrjabingylosis is high enough to rule out the use of this method.

Baculum weight

The mean baculum weight rises throughout the six age classes defined for males (Fig. 3). The oldest group, males of 3–9 years old, had the highest group mean (58.0 mg) and included the highest individual weight (65.5 mg). These data (Appendix 1) confirm the suggestion that baculum weight does continue to increase slowly throughout life, at least in some males. On the other hand, there is enormous variability within all classes, especially in the adults. The baculum of one 11-month-old male, just entering his first breeding season, had already reached 50.4 mg, whereas that of one male in the oldest group was still 44.5 mg.

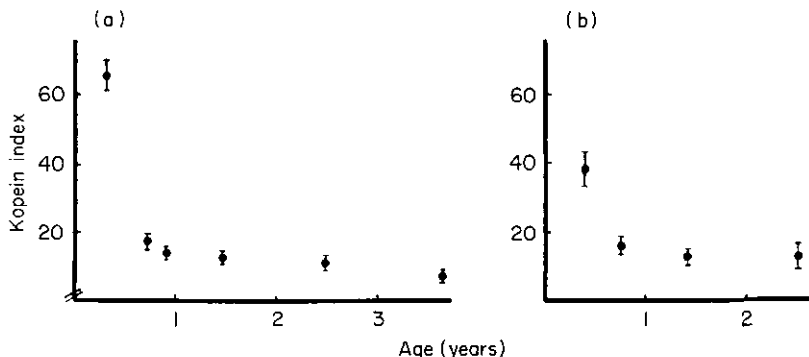


Fig. 4. Infilling of the canine pulp cavity, as measured by the Kopein Index (mean \pm 1 S.E.), in male (a) and female (b) Stoats with age. Ages of individuals defined from cementum annuli; further details given in Appendix 1.

These individual differences probably reflect the social status and reproductive success of each male, because the two are linked and probably have a powerful effect on androgen production and thence on baculum weight. For example, sectioning of the testes of all males from Craigieburn showed that two of 41 adult males were still infertile during the season (September–November) when all other adult males were in full breeding condition. The minimum testis weight associated with fertility was 0.6 g. The testes of both these males weighed well below this threshold and neither contained any spermatozoa. One was 11 months old and with a baculum weight of 30.7 mg (average at that age 41.0 mg; Appendix 1) and rather odd-shaped testes; the other was 13 months old with a baculum weight of 55.0 mg and small but normal-shaped testes, with no apparent anomalies but definitely no spermatozoa.

Kopein index

The pulp cavity is very clearly wider in young Stoats than in older ones. The index proposed by Kopein does reflect the infilling of the pulp cavity by dentine, and the figures fall steeply during the first year and then level off, as expected (Fig. 4). However, the range of figures given by Kopein (1967) was not found, and there was no clear distinction between three year classes as he defined them (Appendix 1). The index clearly identifies the first of the three subdivisions within the first-year class; but so also does the DSB method, which is far more convenient to use and less destructive. This method can safely be neglected.

Periosteal zonation

The results were similar to those obtained previously from Weasels. The lines were clear and often very impressive, but unfortunately their clarity did not make them easy to count and interpret. Let us assume that the lines are annual and laid down in winter, and the 'resorption line' can be discounted. If this is true, then Fig. 5 shows that there was indeed a general relationship between the number of periosteal lines and age as determined from cementum annuli, but it was not very precise. Although 10 of 17 young males and 18 of 27 females caught before the end of their first summer showed, as expected, no lines, yet five of these 17 males and eight of the 27 females already had one or more lines—two of the females, actually under 5 months old, already had three 'annual' lines. Among the 17 males and 18 females 5–11 months old, two of the males and four of the females had no lines at all, although perhaps these had not yet laid them



Fig. 5. Development of incremental lines in the periosteal zone of the mandible in male and female Stoats, shown as the percentage frequency distribution of line counts in each age group. Ages of individuals defined from cementum annuli; further details given in Appendix 2. 0-5 = number of 'annual' lines; r = 'resorption line' of Klevezal & Kleinenberg (1967).

down. The rest of this group all had lines, but only four males and eight females had the expected 'correct' number of them, one.

None of the adults over 1 year old had no lines at all, but among the 1-2-year-olds, three of 14 males and one of nine females had three lines. The oldest adults had the most periosteal lines, but again the relationship between number of lines and years of age was imprecise. Many individuals had 'half' or 'double' lines as illustrated in Weasels by King (1980); 12 sets of sections, of the 151 sets cut, were totally unreadable.

In Weasels, the larger individuals tended to produce more periosteal growth lines than smaller ones, independently of age (King, 1980). To check whether this might be happening in Stoats, I also sectioned jaws from a small subsample of 12 young and subadult males from Westland, all with baculum weights of under 27 mg, plus 12 adult males. The collection area at Westland is at low altitude and has a mild, coastal climate, although it is within one degree of latitude of Craigieburn and only 80 km west across the Southern Alps. Stoats from that area average much smaller than at Craigieburn (body weight of adults: males, 285 ± 7.5 g; females, 195 ± 3.9 g). If body size or climate affect periosteal zonation, the Westland sample might give rather different results. On the contrary; 11 of the 12 jaws from young Westland males gave clear readings, of which only two were zeros. All the rest had one or two clear lines; one, killed in June of his first year with a baculum weight of 23.5 mg, had three. The 12 adults, of unknown age but all with adult bacula and therefore at least 1 year old, had between one and seven lines.

Quantal methods of age determination are usually used alone, and each jaw incorrectly assessed means an undetectable year-class error. Therefore, such a large amount of deviation from a real relationship between periosteal zonation and actual age, extending right through the entire range of ages and body sizes, means that, in Stoats as in Weasels, this method is to be avoided.

The reason why periosteal zonation is unreliable in Stoats and Weasels when it seems to give dependable results in other species is not known, but it might have something to do with the highly geared metabolism typical of small mustelids. Bone is a living tissue, sensitive to numerous influences during its formation and constantly changing its substance even in the adult (Simkiss, 1975). Possibly these processes proceed faster in species that have high metabolic rates.

Resorption from within is known to begin removing periosteal growth lines after the fifth year in Sables *Martes zibellina*, and the sixth year in Mink *Mustela lutreola* and Arctic Foxes *Alopex lagopus* (Klebanova & Klevezal, 1966). Among the jaws of Stoats sectioned for this study were nine in which secondary osteocytes could be seen actively

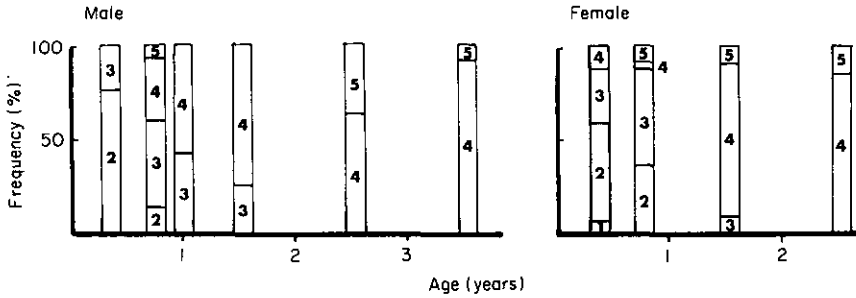


Fig. 6. Development of wear on the carnassial teeth of male and female Stoats, shown as the percentage frequency distribution of wear classes in each age group. Ages of individuals defined from cementum annuli; number in each wear class and chronological span of each age group given in Appendix 3. Wear classes defined from photographs given by Stroganov (1937) and marked on the figure as follows: 1 = Class J4 (under 4 months old); 2 = J7 (4-7 months old); 3 = J12 (7-12 months old); 4 = A2 (second-year adult); 5 = A3 (third-year adult).

restructuring the periosteal bone. In the early stages of this process, single invading osteocytes appeared in the periosteal layer. As restructuring progressed, it gradually obliterated the previous set of periosteal lines and laid down a new set, sometimes at a different angle. The process apparently starts early in Stoats, since the individuals in which this process was observed were 1, 1, 2, 2, 2, 4, 5, 7 and 8.5 years old.

Stroganov's index of toothwear

Under the binocular microscope the categories of wear that Stroganov described are not difficult to recognize. None of the 145 Stoats I classified showed the degree of wear of the teeth corresponding with Stroganov's fourth, fifth or sixth year classes, but these were also rare in his own samples (6%, 3% and < 1% of 616, respectively). Within the range of Stroganov's classes, there was indeed a general relationship between tooth wear and real age, but again it was imprecise (Fig. 6). Among 39 young males under 1 year old, 29 were correctly placed in the first-year class but nine were classed as 2-year-olds and one as a 3-year-old; among 54 females under 1 year old, the corresponding figures were 47, five and two. Among 15 second-year males, 11 were correctly placed but four had been left behind in the senior (7-12-month) subgroup of the previous class. All eight of the 2-3-year-old males were correctly placed, but 10 of the 11 third-year or older males were still classed as 2-year-olds. Similarly, among 11 second-year females, nine were correctly placed and two were out by a single age class, one each way; of seven third-year or older females, six were still classed as 2-year-olds. Four of 34 males over 1 year old were classed with the 7-12-month-olds, and only one of 18 females.

Indices of toothwear commonly overestimate age. This one does that to some extent in the younger age groups, but rather the reverse in the older ones. Actually, the Stroganov index is, as toothwear indices go, a good one. It suffers the inevitable variability of all its kind, but has the advantages that, on the one hand, it can be used on specimens that must not be damaged, and on the other, because the carnassial teeth survive almost any treatment, it can often still be used even if the rest of the skull is completely crushed. It also complements characters such as the DSB method and suture closure, which are useful only for a short period, because it offers the possibility of recognizing the older animals. Clean skulls are easier to examine, but the method could perhaps even be applied to fresh (uncleaned) skulls, if the lower jaw can be cut away. In some studies these considerations could be decisive.

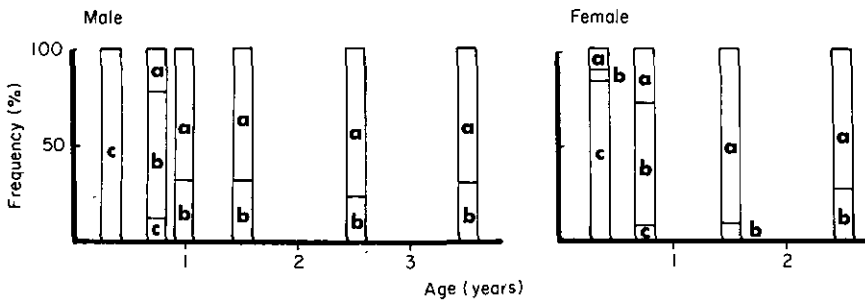


Fig. 7. Development of the lateral suprasamoid tubercle on the femurs of male and female Stoats, shown as the percentage frequency distribution of the three categories of growth in each age group. Ages of individuals defined from cementum annuli; number in each category and chronological span of each age group given in Appendix 4. Categories defined from photographs given by van Soest & van Bree (1970) and are marked as follows: a = fully grown; b = developing; c = absent.



Fig. 8. Closure of the nasal sutures in male and female Stoats, shown as the percentage frequency distribution of the four categories of closure in each age group. Ages of individuals defined from cementum annuli; number in each category and chronological span of each age group given in Appendix 5. Categories defined from description given by Hansson (1968) and are marked as follows: 1 = sutures open; 2 = sutures coalescing; 3 = sutures coalesced but visible; 4 = sutures fully closed and invisible.

Lateral suprasamoid tubercle (LSST)

As in Weasels, the absence of this tubercle is a fairly reliable sign of a young Stoat. In this sample, none of 19 males under 5 months and only three of 30 females that age had the fully developed tubercle; conversely, none of 34 males and none of 18 females over a year old showed the juvenile state (Fig. 7). The transition is of course not instantaneous, so a few young animals with fully developed tubercles begin to appear by the middle of the first year, while a few adults still remained in the intermediate state in their second year or beyond. This transition period makes the tubercle a far less reliable means of identifying females 5–11 months old than the baculum is for males of that age. Its main usefulness is in the first few months of the first year to distinguish juveniles even when the skull is broken or damaged or the baculum lost.

Nasal sutures

As expected, no first-year Stoats caught after February still had open sutures, and in only one subadult male were the sutures still in the process of coalescing (Fig. 8). The transition class, 'coalesced but visible', included individuals of both sexes ranging in age from under 5 months to over 3 years. All of the 29 males with invisible sutures were over

10 months old, but the 20 females in this class included three young under 5 months old and four of 5–11 months.

On these data, therefore, the first two and the fourth categories of nasal suture closure can be used to achieve a remarkably reliable separation of first-year and older males, but they are much less useful for females. Stoats classified into the third category can be of any age whatever. The nasal sutures are therefore in the same position as the lateral suprasesamoid tubercle—neither character turns out to be a reliable substitute for the baculum as a means of identifying females 5–11 months old. The only other suture that might be used, the one between the auditory bulla and the occipital bone, apparently never fully closes even in the adults.

Cementum annuli

Because the known-age material collected in New Zealand had already supported the hypothesis that the cementum annuli are indeed annual, for this trial the ages given by cementum annuli were taken as correct and used as the standard against which the other methods were compared. There is therefore no direct way in which the annuli themselves can be checked from these data. However, because the trials showed that all the other methods tried really are related to age, and fail as independent methods mainly because of the extent of overlap between their classes, then the results given by them and by the annuli should at least harmonize. Any substantial disagreements between the results given by the annuli and by the other methods should be re-examined.

There were in fact nine such cases among the 95 Stoats in the Craigieburn subsample whose jaws had been sectioned. All were undoubted adults, but the sections of their canines clearly showed no cementum annuli at all. Five were males with baculum weights of 47.5, 54.0, 58.8, 59.8 and 61.5 g. Three were females, of which one was pregnant, and two had enlarged nipples; a fourth female had a post-orbital ratio in January of 1.16, closed nasal sutures, a fully developed lateral suprasesamoid tubercle, and carnassial toothwear in Stroganov's second year class. These cases are not only puzzling, but are also some cause for concern if Stoats aged from cementum annuli are to be used with any confidence in population studies. If this sample is representative, the possibility arises that some unknown proportion of adult Stoats have fewer cementum annuli than would be expected from their real age.

The Stoats used for this trial were collected between 1973 and 1976. The study continued in the same area until 1978, extending the total subsample from Craigieburn to 265 Stoats. In a parallel study (1972–81), 390 Stoats were collected from the Eglinton Valley and 250 from the Hollyford Valley, the two areas in Fiordland National Park where most of the known-age material was collected [for the seasonal and annual distribution of the data for all three study areas, see King (1983)]. One or both jaws from all the adults from the three study areas were sectioned. Among the 41 additional adults from Craigieburn that returned clear readings, four males with adult bacula and one female with nipples had no annuli. These bring the total of such cases to 13, of 136 Craigieburn adults sectioned. However, among the 287 Fiordland adults sectioned, the only evidence of a missing line was in two summer-caught females with enlarged nipples. Both must have overwintered at least once, and the annulus representing that winter ought to have been fully developed well before the following summer, but in both it was only just forming.

In all these cases the number of annuli is fewer than expected from the reproductive status or the inferred reproductive history of the animal. The deposition of the annuli and the reproductive activities of individuals are the visible signs of invisible and

separate annual cycles in the physiology or metabolism of the body. The disagreement between them must arise because one or the other has for some reason failed to register the passing of an annual cycle. Which of the two is likely to be more variable? On present data the reproductive cycles of Stoats of both sexes seem to be extremely closely controlled by photoperiod (King 1989a). Where the two do not agree, the chances are that it will be the cementum annuli that are the less precisely geared to season. If it was the first annual cycle that was not registered in the teeth, it will be detected from the incongruity between the annuli and the reproductive status; after at least one cycle has been recorded, later 'misses' cannot be identified. The consequent underestimation of the true age of the individual will be undetectable.

The calibration of the cementum lines was done mostly from 1979–80 Fiordland material, and in the complete (1972–81) collection from the same study area the incidence of missing lines was rare (two of 287 adults, or 0.7%). This suggests that population data derived from the Fiordland Stoats may be reasonably reliable. The reason why missing lines were detected so much more often (13 of 136, or 10% of adults) in the subsample from Craigieburn is a mystery.

CONCLUSIONS

The results of these trials showed that all the characters tested were indeed related to age, but over different spans of time and with different degrees of reliability. They can be classified in order of decreasing usefulness as follows.

The best of methods tested

Cementum annuli have been calibrated from New Zealand material of known or part-known age; they produce classifications of individuals that are almost always compatible with those from other methods; and they lead to population age structures that are entirely consistent with the biology of the species. These features are sufficient to recommend cementum annuli as the method of choice for classifying adults. The identification by DSB criteria of young animals and their exclusion from processing is a reliable way of saving a lot of unnecessary histological work.

Baculum weight is a completely reliable criterion distinguishing pre-pubertal young males up to 11 months old from adults of 12 months old or more. The transition period is very short and for 10 months of the year there is no overlap in baculum weight groups. Among adults the mean baculum weight continues to rise through all year classes, but huge individual variation precludes the use of baculum weight to distinguish younger adults from older ones.

The *LSST*, *nasal sutures*, and *post-orbital ratio* can be used to distinguish the youngest (<5 months) animals from the adults of >12 months old. There is some variation but these methods are reasonably reliable if applied together and restricted to these groups. They are especially useful in the negative (e.g. no adults have open sutures or totally undeveloped LSST), and also when the skull is either already damaged during collection or is complete and must not be damaged by processing, provided the specimens are dated. The large transition group (Stoats aged 5–12 months old) cannot be classified by these criteria. In general, characters that have to be judged (LSST, sutures) are more difficult to apply consistently than those that can be measured (post-orbital constriction), but none of these methods is likely to give seriously misleading results, especially if the sample is large and well dated.

Periosteal zonation, the *width of cemental cavity* (*Kopein index*) and *carnassial tooth-wear* (*Stroganov index*) are all methods that claim to be able to distinguish several

year classes from a single character. All were found to be unreliable, and cannot be recommended. Of the three, the Stroganov index is the least misleading and the only one that does not involve destructive technical preparation. It could be used in combination with the methods listed immediately above because, unlike them, it is potentially able to identify the older adults.

This study has ranked potential age determination methods applicable to Stoats, and provided some estimate of the reliability of each. It confirms the widely held (but seldom *proven*) view that cementum annuli are generally reliable indicators of age in adult mammals. However, the extensive comparisons undertaken here have also identified a curious geographical twist to this otherwise satisfactory conclusion. At Craigieburn, one of three study areas discussed here, a rather alarming number of individual Stoats apparently failed to lay down a cementum annulus in their first year. The reasons for this are unknown, but the consequence is that there could be undetectable year-class errors in samples of adult Stoats from this area. The problem might have gone unnoticed except that the Craigieburn Stoats happened to be the ones chosen for the intensive examination of age-determination methods, in relation to previous data on reproductive biology, reported in this paper. The larger samples from the other two study areas in Fiordland appear to be more reliable.

This observation is a source of concern if material classified this way is to be used for population analysis. On the other hand, it is hardly a new problem. For example, Grue & Jensen (1973) sectioned the canines of 135 wild Red Foxes of known age; 125 were correctly classified by cementum annuli, five were out by one year and the other five were unreadable. Rudge (1976) tried three different methods of classifying the teeth of 55 known-age Sheep, and found that the best of them was still wrong by more than one year in 23% of cases. These inconsistencies arise because biological material is inherently variable, and some degree of error in single-character methods of age determination is to be expected. Some means of identifying the unreliable specimens should ideally be part of any procedure, but is possible only if there is some prospect of spotting disagreements between two or more independent methods. Future users of the cementum annuli method are advised carefully to check the results given by the tooth sections against those given by the best or most appropriate of the alternative methods listed above. The proper procedure for dealing with any discrepancies that may be detected depends on the objectives of the study.

The best of methods not requiring cleaned skulls

Cleaning of skulls can be laborious and time-consuming, unless an active colony of dermestid beetles is available. Methods of age determination which avoid the necessity of cleaning the skulls have an advantage, if all other considerations are equal.

Sectioning of canine teeth does not require the jaws to be cleaned first, since the teeth are extracted before processing. The method of first choice, cementum annuli, is therefore still available. Identification of young animals that do not need sectioning is also still possible. The post-orbital ratio can still be measured; when the skin on top of the head is peeled back, the bone in the inter-orbital and post-orbital areas can readily be scraped clear of muscle with a scalpel. The Stroganov toothwear index does not require the skull to be cleaned, although it is probably easier to use if it is. The baculum and the femur are both much more easily cleaned than the skull, so supporting evidence from the baculum weight and LSST can be made available with minimum effort.

The best of methods not requiring histology

There are two common situations in which the best possible method, cementum annuli, cannot be used at all. The first arises because histological equipment is expensive, and the procedure demands some skill and experience. For anyone for whom this option is not open for merely practical reasons, the best alternative, if funds permit, is to engage a commercial laboratory to do the work, provided there is some means of checking that it is done correctly. The second common situation is when the material to be examined belongs to a museum whose authorities are unwilling to give permission for destructive sectioning. In this case, the only possible course is to use the truncated form of the DSB method as described above and used by King & Moody (1982). The Stroganov toothwear index could possibly be used with caution to identify the older adults.

Methods for use on living Stoats

Determination of the age of living Stoats from external characters of size and reproductive condition is possible only up to midsummer (late January in New Zealand). Mermod & Debrot (1978) and Debrot & Mermod (1978) have proposed that living young could be distinguished from adults by cranial X-rays until after any external signs cease to be reliable. The X-ray plates show the clearly different shape of young skulls, and also the open pulp cavities of their canine teeth. But the data presented here show that the young form a clearly distinct group on these criteria only until late summer (the end of February in New Zealand; see Appendix 1). The extension of time during which the young can be identified with certainty from X-ray plates (as opposed to external characters) is short, only about a month; and the use of X-rays requires complex and expensive equipment and a strong probability that every animal would have to be transported from the field to the X-ray machine and later returned. Whether such disruption and expense are considered worthwhile would depend on the aims of the study.

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APPENDICES

Appendix 1

Distribution of variation with age in measurable characters

	Cementum age						
	Males Females	0-4 months	5-10 months	11 months	1 year	2 years	3-9 years
Males							
Post-orbital ratio							
Mean \pm S.D.		1.02 \pm 0.064	1.14 \pm 0.045	1.20 \pm 0.054	1.21 \pm 0.086	1.27 \pm 0.032	1.23 \pm 0.077
Range		0.84-1.11	1.04-1.21	1.14-1.27	1.04-1.36	1.22-1.30	1.12-1.32
n		18	15	6	13	7	11
Baculum weight							
Mean \pm S.D.		18.5 \pm 2.89	24.4 \pm 3.16	41.0 \pm 9.96	51.5 \pm 6.84	53.8 \pm 3.78	58.0 \pm 6.82
Range		10.6-22.7	18.7-30.7	30.7-50.4	37.8-63.9	47.6-59.8	44.5-65.5
n		17	15	6	15	8	7
Kopein index							
Mean \pm S.D.		40.3 \pm 7.42	19.2 \pm 3.78	16.8 \pm 1.63	16.2 \pm 2.26	15.5 \pm 1.70	14.3 \pm 2.49
Range		55.6-25.5	28.9-12.0	18.5-14.3	20.8-12.0	18.9-13.7	18.1-11.0
n		15	15	7	14	7	11
Females							
Post-orbital ratio							
Mean \pm S.D.		0.97 \pm 0.070	1.08 \pm 0.064		1.20 \pm 0.078	1.21 \pm 0.115	
Range		0.86-1.16	0.98-1.27		1.11-1.36	1.05-1.34	
n		29	22		10	6	
Kopein index							
Mean \pm S.D.		29.7 \pm 11.29	18.8 \pm 3.58		17.1 \pm 1.99	16.0 \pm 2.73	
Range		59.3-14.6	30.8-13.9		19.5-13.8	18.9-12.2	
n		27	23		9	7	

Appendix 2

Frequency distributions of periosteal lines. *R* = resorption line (see text)

	Cementum age						
	Males Females	0-4 months 0-4 months	5-10 months 5-11 months	11 months —	1 year 1 year	2 years 2-5 years	3-9 years —
Males							
No. lines							
0		10	1	1	0	0	0
R only		2	3	0	0	0	0
R±1		5	3	1	4	1	2
2		0	3	0	6	2	0
3		0	3	1	3	2	3
4		0	0	1	1	1	1
5		0	0	0	0	0	5
Not counted		2	2	3	1	2	0
<i>n</i>		19	15	7	15	8	11
Females							
No. lines							
0		18	4		0	0	
R only		2	3		0	0	
R±1		3	8		3	0	
2		3	2		5	5	
3		2	3		1	2	
4		0	0		0	0	
5		0	0		0	0	
Not counted		2	6		2	0	
<i>n</i>		30	24		11	7	

Appendix 3

Frequency distributions of Stroganov toothwear classes

	Cementum age						
	Males Females	0-4 months 0-4 months	5-10 months 5-11 months	11 months —	1 year 1 year	2 years 2-5 years	3-9 years —
Males							
Stroganov classes							
J4		0	0	0	0	0	0
J7		13	2	0	0	0	0
J12 (= A1)		4	7	3	4	0	0
A2		0	5	4	11	5	10
A3		0	1	0	0	3	1
?		2	0	0	0	0	0
<i>n</i>		19	15	7	15	8	11
Females							
Stroganov classes							
J4		2	0		0	0	
J7		16	9		0	0	
J12 (= A1)		8	12		1	0	
A2		4	1		9	6	
A3		0	2		1	1	
?		0	0		0	0	
<i>n</i>		30	24		11	7	

Appendix 4
Development of the lateral suprasedamoid tubercle (LSST)

	Males Females	Cementum age					3-9 years
		0-4 months	5-10 months	11 months	1 year	2 years	
		0-4 months	5-11 months	—	1 year	2-5 years	—
Males							
LSST class							
a		0	2	4	10	6	7
b		0	10	3	5	2	3
c		19	3	0	0	0	0
?		0	0	0	0	0	1
<i>n</i>		19	15	7	15	8	11
Females							
LSST class							
a		3	7		10	5	
b		2	15		1	2	
c		25	2		0	0	
?		0	0		0	0	
<i>n</i>		30	24		11	7	

Appendix 5
Closure of the nasal sutures

	Males Females	Cementum age					3-9 years
		0-4 months	5-10 months	11 months	1 year	2 years	
		0-4 months	5-11 months	—	1 year	2-5 years	—
Males							
Class							
I		6	0	0	0	0	0
II		10	1	0	0	0	0
III		2	13	5	4	1	1
IV		0	0	2	10	7	10
?		1	1	0	1	0	0
<i>n</i>		19	15	7	15	8	11
Females							
Class							
I		5	0		0	0	
II		9	0		0	0	
III		13	20		3	1	
IV		3	4		7	6	
?		0	0		1	0	
<i>n</i>		30	24		11	7	