



MICROWEAR IN MODERN SQUIRRELS IN RELATION TO DIET

Sherry Nelson, Catherine Badgley, and Emily Zakem

ABSTRACT

Dental microwear consists of microscopic damage features on the occlusal surfaces of tooth enamel and reflects physical properties of the diet, as well as enamel structure and post-mortem history of the tooth. Microwear analysis has been used to infer the diets of extinct mammals through comparison of features on fossil teeth with those on teeth of living mammals with known diets. A method for documenting microwear of large mammals using a light microscope was developed as an alternative to approaches based on scanning electron microscopy. We adapted this method for investigating microwear features on squirrel teeth. Both modern and fossil squirrels occur in diverse terrestrial habitats and eat a range of herbivorous to omnivorous diets.

We compared microwear features from upper molars of several modern species of frugivorous tree squirrels and omnivorous ground squirrels. We also examined fossil sciurids from the Miocene Siwalik sequence of Pakistan and a Pliocene locality in the central plains of the United States. We found significant differences in microwear features among modern squirrels of different diets and habitats, suggesting that microwear features can be used to infer the diets or preferred habitats of extinct species. Microwear features were preserved on some of the fossil specimens. A comparison of Pliocene *Spermophilus rexroadensis* to modern *Spermophilus* suggests a diet similar to that of the modern species examined. Microwear of Miocene *Eutamias* differed from the pattern in any of the living squirrels examined. The approach presented here holds strong potential for illuminating the trophic ecomorphology of small-mammal fossils.

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INTRODUCTION

We present here the initial results of a new approach to gathering ecological and paleoecological information from small mammals. We adapted methods of microwear analysis, originally developed for the study of dental microwear in large mammalian herbivores, to the teeth of modern and fossil squirrels (Sciuridae). Our results demonstrate that microwear features commonly present on large mammalian teeth are also observed on teeth of modern and fossil squirrels. Furthermore, a preliminary analysis of microwear on the teeth of eight extant species suggests that different microwear features are associated with different diets.

Modern mammals provide a set of potential models for the feeding habits of fossil mammals, although some fossil mammals have teeth unlike those of any modern species. An important approach to the inference of paleodiets involves documenting microwear on mammalian cheek teeth, where most chewing of food occurs. Dental microwear features are microscopic pits and scratches in tooth enamel, and they mainly reflect the physical properties of the foods most recently eaten by an animal. Different diets produce different wear features in modern mammals, and for this reason, microwear analysis has been used to infer paleodiets for a wide range of herbivorous fossil mammals (Walker et al. 1978; Teaford and Walker 1984; Grine and Kay 1988; Solounias et al. 1988; Solounias and Hayek 1993; Morgan 1994; Nelson 2003).

We have focused on squirrels for ecological and practical reasons. Squirrels are taxonomically and ecologically diverse today and throughout much of their evolutionary history (Mercer and Roth 2003). The extant species occupy a wide range of biomes, from tundra to semi-desert to rainforest. They include canopy dwellers, ground-dwellers, and burrowing species. Also, squirrel molars are more similar to the general tribosphenic molar pattern than are the molar teeth of most other rodents, thus facilitating comparison of microwear data from squirrels with prior results from a diverse set of larger mammals. In addition, squirrels have a substantial fossil record, and we have particular interest in some of the Neogene sequences in which fossil squirrels occur. The broader purpose of this study is to develop and assess methods that will be suitable for a wide range of rodents and other small mammals.

Small mammals are those that weigh ~1 kg or less as adults. Almost three-fourths of the roughly 4600 species of living mammals (Wilson and Reeder 1993) qualify as small mammals. Most small mammals are rodents and bats; others include shrews, moles, hedgehogs, tenrecs, tree shrews, small primates, and small marsupials. In most undisturbed modern mammalian faunas, over half of the species are small mammals. Also, the fossil record of small mammals is rich and increasingly well documented (Luckett and Hartenberger 1985; Black and Dawson 1989; Korth 1994; Qiu and Li 2003), despite taphonomic biases against small-mammal remains, as well as the intensive efforts required to recover those remains.

This subject is fitting as a tribute to Will Downs. Will devoted a substantial portion of his professional life to recovering and preparing remains of small vertebrates, including some of the fossil specimens in this study. He enlarged our knowledge about the evolution and ecological diversity of small mammals. He also encouraged and facilitated new empirical and theoretical approaches in vertebrate paleontology, so it is an honor to dedicate this paper to him.

This paper describes microwear analysis as adapted for squirrel teeth and evaluates initial results from this approach. For modern and fossil specimens, our goals were to 1) determine the feasibility of casting small-mammal teeth so as to be suitable for microwear analysis, 2) determine if microwear features were present on the casts, 3) develop a replicable approach for documenting microwear features on squirrel teeth, and 4) investigate the relationship between dietary habits and microwear features among selected modern taxa for the purpose of inferring dietary habits from fossil specimens. In the next section, we present information about the modern and fossil squirrels examined in this study, and review previous approaches to microwear analysis of mammalian teeth. A summary of our approach follows, including preparation of specimens and documentation of microwear features. Results are presented as photographs and statistical summaries of microwear features from modern and fossil specimens. We interpret the microwear data in relation to the dietary habits of selected modern squirrels. The results suggest several directions for future work. An appendix lists all of the specimens studied and data gathered for this study.

BACKGROUND

Modern Squirrels

The family Sciuridae includes 273 living species, distributed on all continents except Australia and Antarctica (Wilson and Reeder 1993). In terms of general ecology and body proportions, modern squirrels are divided into three groups: tree squirrels, flying (gliding) squirrels, and ground squirrels (Gurnell 1987). Tree squirrels, including *Sciurus carolinensis*, the common gray squirrel of eastern North America, and several species of *Callosciurus*, the large canopy dwellers of South Asian rainforests, are typically slender-bodied with long bushy tails. While tree squirrels can walk, run, and dig on the ground with ease, they spend most of their time in trees, climb head up or head down, and maneuver among small branches with great agility. Flying squirrels have a patagium—a membrane of fur-covered skin—that provides an airfoil for gliding across gaps from tree to tree. This group includes the two species of *Glaucomys* (northern and southern flying squirrels) common in North America, and several species of *Petaurista*, the giant flying squirrels of eastern and southeastern Asia. These species are similar in body form to tree squirrels and are obligate forest dwellers. The ground squirrels include the marmots (*Marmota*), prairie dogs (*Cynomys*), chipmunks (*Tamias*), and others. Species in this group have stocky bodies and shorter tails in relation to body length. These species occur in a wide range of non-forested habitats, including grasslands, woodlands, rocky terrain, mountain tundra, and semi-desert (Nowak 1999). Ground squirrels dig burrows and rely on an assortment of terrestrial food resources. Trophically, most squirrels are omnivorous, with a strong reliance on plant foods. The diets of tree squirrels and flying squirrels are dominated by fruits and nuts, whereas the diets of ground squirrels are dominated by seeds, nuts, roots, green vegetation, as well as fruits (Nowak 1999). A recent phylogenetic analysis of squirrels, based on nuclear and mitochondrial DNA sequences, showed that flying squirrels form a monophyletic clade, but tree squirrels and ground squirrels have a complex evolutionary history involving parallel radiations in different continental regions (Mercer and Roth 2003).

Dental Microwear

Analyses of dental microwear features, such as microscopic pits and scratches on enamel surfaces, have been used in investigations of diet in extinct mammals (Walker et al. 1978; Teaford and Walker 1984; Grine and Kay 1988; Solounias et al.

1988; Solounias and Hayek 1993; Nelson 2003). Wear features on occlusal surfaces reflect mainly the physical properties of the foods most recently eaten by an animal. Other features may represent tooth-on-tooth wear (Rensberger 1978). In addition, teeth may acquire post-mortem modification, including cracking, chipping, and coating with precipitates from sediment matrix; these features may obscure original microwear features. Turnover in microwear features can be rapid, in some cases as little as 24 hours (Teaford and Oyen 1989). Microwear studies of modern species have demonstrated significant differences between folivorous and frugivorous primates, soft-fruit diets and hard-fruit diets in primates, browsing and grazing ungulates, and even seasonal dietary variation in sympatric hyraxes (Walker et al. 1978; Teaford and Walker 1984; Grine and Kay 1988; Solounias et al. 1988; Solounias and Hayek 1993).

Early microwear studies focused on dental factors that might confound dietary interpretations, including microwear patterns resulting from different molar positions and dental facets (Gordon 1982). These studies showed that within a species, different facets and molar positions can yield consistent differences in microwear, including feature ratios, densities, and dimensions, resulting from differences in masticatory biomechanics (Gordon 1982). However, the differences among facets from teeth of the same species are generally much smaller than differences between homologous facets from different species (Teaford and Walker 1984). Also, homologous mandibular and maxillary facets show the same microwear because upper and lower facets break down food by reciprocal action. Thus, these studies indicated that interspecific comparisons on the same facet are reliably informative about differences in diet. Another concern was that differences in microwear features across species may be due to differences in enamel microstructure, or prismatic packing and crystallite orientation (Maas 1991). In a comparison of lemur, sheep, human, and crocodile, Maas (1991) demonstrated that quantitative striation width, which had not been found to discriminate among different diets using a scanning electron microscope (Teaford 1986; Solounias et al. 1988; Ryan and Johanson 1989; Teaford and Robinson 1989), is likely influenced by differences in enamel microstructure. In our rodent analyses, we avoided major differences in enamel microstructure by comparing squirrels to other squirrels. Furthermore, we only used microwear features that have been shown to differentiate diets in large mammals using the light-microscope technique.

Most previous microwear analyses have focused on reconstructing the diets of large mammals. Studies of fossil primates, including early hominids (Grine and Kay 1988) and Miocene hominoids (summarized by King 2001), have generally compared fossil taxa to modern apes and monkeys, and attributed general frugivore/folivore/hard-object diets to the fossil taxa. Typically, primate frugivores have higher proportions of pits relative to scratches; folivores, more scratches than pits; hard-object feeders, the most pits (Teaford and Walker 1984). Likewise, microwear analyses of modern and fossil bovids have differentiated between grazers, characterized by many scratches, and browsers, characterized by fewer scratches (Solounias et al. 1988; Solounias and Hayek 1993; Morgan 1994; Solounias and Moelleken 1994).

Microwear on small-mammal teeth has received comparatively little attention. Rensberger (1978) documented microwear on particular molar facets of several rodents (including one squirrel), with emphasis on variation in microwear features in relation to different phases of chewing. Microwear on a molar of *Sciurus griseus* was dominated by striations, which Rensberger attributed to detritus. Other SEM and light microscope microwear analyses of striations, or scratches, however, have found them to be a feature related to diet and, specifically, to food components such as phytoliths or hard-shelled fruits (Teaford and Walker 1984; Grine and Kay 1988; Solounias et al. 1988; Solounias and Hayek 1993; Solounias and Semprebon 2002; Godfrey et al. 2004; Semprebon et al. 2004).

The Light-Microscope Method

These earlier microwear analyses have provided valuable information on dietary preferences of fossil species. These analyses were performed using scanning-electron microscopes and were therefore expensive and time-consuming, thus limiting sample sizes. Solounias and Semprebon (2002), however, developed a new technique using a light microscope to build a large "library" of modern ungulate microwear. This technique has the advantages of being simpler, quicker, and less expensive than using an electron microscope, thus facilitating analysis of much larger sample sizes. Furthermore, because their technique involves analyzing larger surface areas, it obtains a better representation of a dental facet rather than a fraction of it. This method depends upon identification of features such as pits and scratches on the enamel surface without detailed measurements. Features examined under the light microscope complement those examined in SEM studies, but

are not equivalent; features examined at 35X are much larger than those examined at 500X (Semprebon et al. 2004). The light-microscope method has been validated for both single-observer and inter-observer reproducibility (Semprebon et al. 2004). Using this method to document microwear of 50 extant ungulates, Solounias and Semprebon (2002) accurately distinguished among browsers, grazers, and mixed feeders. Frugivorous bovids, tragulids, and pigs were characterized by large numbers of pits and coarse scratches, similar to those seen among frugivorous primates.

Using this light-microscope technique, Nelson (2003) analyzed fossil teeth from a wide range of large mammalian herbivores from the Miocene record of Pakistan. The Neogene Siwalik sequence of Pakistan is a long terrestrial record spanning most of the last 20 m.y.r., providing the opportunity to document changes in species' diets over 10^5 to 10^6 years for some temporal intervals (Barry et al. 2002). Many taxa, including a hominoid, suids, tragulids, and some bovids, were as frugivorous as their modern rainforest counterparts (Nelson 2003). Fruit availability appeared to decrease over time, with many frugivores becoming extinct. For species that persisted through the interval of greatest extinction of frugivores, the fruit dietary component was replaced by browse among the remaining frugivores, and browse was replaced by C_4 graze (grass) in others. This study contributed to the inference that monsoon forest was replaced by more open habitat in northern Pakistan during the Late Miocene (Quade and Cerling 1995; Barry et al. 2002; Flynn 2003; Nelson 2003).

We applied the light-microscope technique to evaluate the presence of microwear in fossil sciurids. Rodents constitute a significant portion of the Cenozoic mammalian fossil record and yet little is known about their diets. Fossil rodents and other small mammals potentially offer a more fine-grained reconstruction of paleoenvironmental changes than do larger mammals, as do small mammals in many modern communities (Eisenberg 1981). Thus, an approach based on small mammals should usefully complement previous microwear studies involving large mammals. We present an initial determination of whether modern and fossil rodent teeth can be successfully molded and cast for microwear, and of whether microwear features can differentiate among species known to differ in their diets.

MATERIALS AND METHODS

We selected these taxa for this preliminary study because they represent squirrels from differ-

Table 1. Geographic and ecological information for extant species documented for microwear on upper molars; references are for information about habitat and diet.

Species	Number of specimens studied	Country of origin of specimens	Habitat	Dietary habits	References
<i>Callosciurus finlaysonii</i>	9	Thailand	Lowland and montane forest	Fruits, nuts, seeds, flowers, vegetation, insects, eggs	Nowak 1999, Corlett 1998
<i>Callosciurus prevostii</i>	7	Malaysia	Dense rainforest	Fruit mainly, insects	Lekagul and McNeely 1988
<i>Ratufa</i> sp.	2	India, Malaysia	Forest canopy	Fruits, nuts, bark, insects, eggs	Nowak 1999, Corlett 1998
<i>Ratufa affinis</i>	1	Malaysia	Forest canopy	Fruits, seeds, leaves bark, insects, eggs	Payne et al. 1985, Nowak 1999, Corlett 1998
<i>Ratufa bicolor</i>	3	India, Malaysia	Forest canopy	Fruit preferentially, nuts, bark, insects, eggs	Nowak 1999, Corlett 1998
<i>Ratufa indica</i>	1	India	Forest canopy	Fruit preferentially, nuts, bark, insects, eggs	Nowak 1999, Corlett 1998
<i>Spermophilopsis leptodactylus</i>	1	Iran	Sandy desert	Fruits, seeds, bulbs, leaves, insects	Nowak 1999
<i>Spermophilus annulatus</i>	11	Mexico, Jalisco and Colima	Tropical deciduous forest; rocky areas	Fruits, nuts; also insects	Best 1995
<i>Spermophilus variegatus</i>	18	Mexico, USA	Rocky canyons	Seeds, fruits, nuts, leaves; also invertebrates, meat	Oaks et al. 1987

ent habitats and regions and offer numerous upper teeth for examination. All specimens of the modern species are housed in the Mammal Division of the Museum of Zoology, University of Michigan. The modern species studied have medium to large body size (ca. 150 g to 3 kg) and molar size (ca. 6 to 10 mm² in cross-sectional area), providing reasonable occlusal areas in which to document microwear.

We anticipated a systematic difference in microwear features between the southeast Asian tree squirrels, which rely heavily on fruits from the canopy (Corlett 1998), and the ground squirrels, which rely on a wide variety of ground-based forage (see references in Table 1). Because the number of specimens available for *Ratufa* was low and Lekagul and McNeely (1988) reported similar diets for *Ratufa affinis* and *Ratufa bicolor*, we combined the data for *Ratufa* species in graphs and statistical analyses. Whereas most species in Table 1 have fruits as a prominent component of the diet, the plant sources of these fruits vary considerably from dense rainforest (*Callosciurus prevostii*) to sandy desert (*Spermophilopsis leptodactylus*). The tree squirrels obtain most of their fruit from the forest canopy, whereas the ground squirrels forage on the ground and below ground. We selected modern specimens from eight species of tree squirrels and ground squirrels (Table 1). The specimens include two species of *Callosciurus* and three spe-

cies of *Ratufa*—large tree squirrels that inhabit southeast Asian rainforests today. We also examined specimens of three ground squirrels—two species of *Spermophilus* and the single species of *Spermophilopsis*, which inhabit drier environments. The fossils include teeth attributed to the chipmunk genus *Eutamias* from the Miocene of Pakistan, as well as teeth attributed to *Spermophilus rexroadensis* from the Pliocene of Kansas.

We examined fossil sciurid specimens from Miocene sediments of Pakistan and Pliocene deposits from the central plains of the United States. The Miocene Siwalik record of northern Pakistan has provided a rich record of rodent evolution (Jacobs et al. 1989; Barry et al. 2002). Sciurids are a minor but persistent component of Siwalik small-mammal assemblages; their presence is documented in fossil assemblages ranging in age from 18 to 7 Ma (Flynn 2003). The two Siwalik fossils belong to unnamed species of the chipmunk genus *Eutamias*. Fossils of *Spermophilus rexroadensis* are from the upper Pliocene (late Blancan) of Meade County, Kansas (Hibbard 1941; Bell et al. 2004).

Microwear was measured on the metaconule of upper second molars for all modern species, and on upper first or second molars for the fossil species; for one specimen of fossil *Eutamias*, an upper third molar was measured (Figures 1-3). The advantage of sampling the metaconule was that it

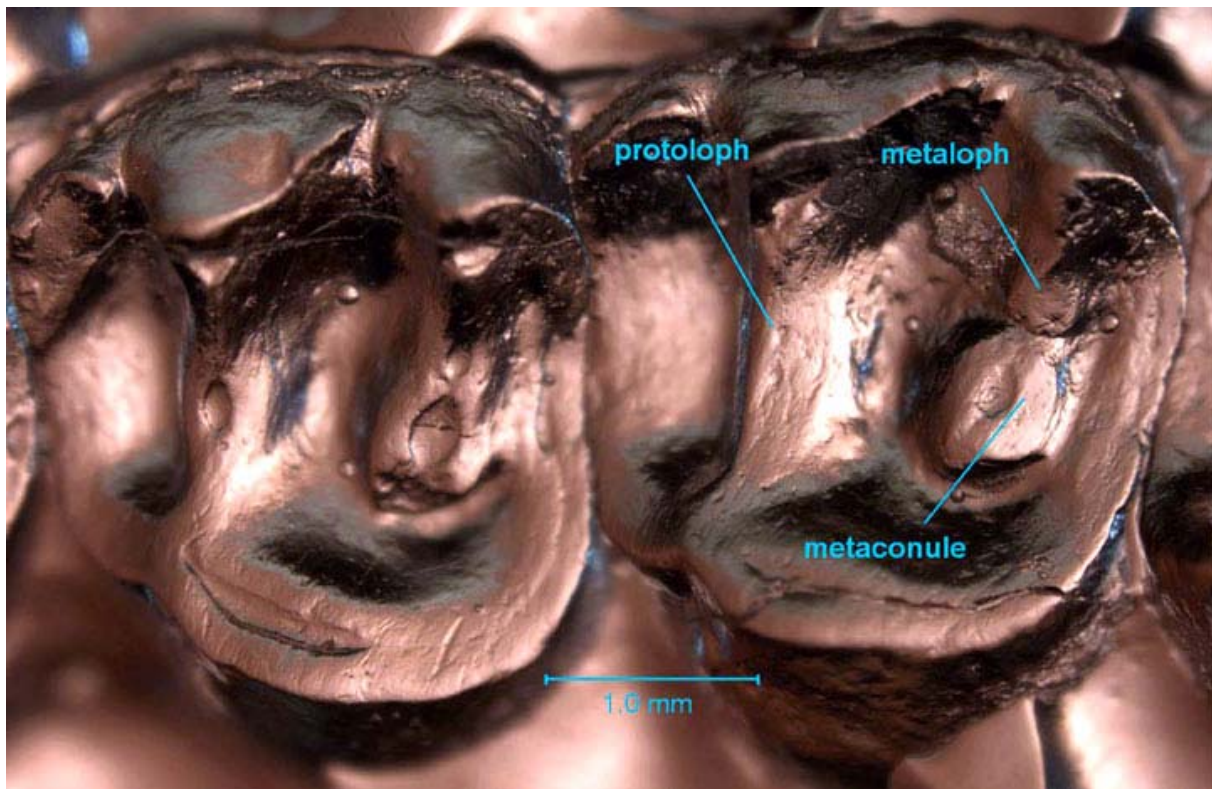


Figure 1. First and second upper molars of *Spermophilus variegatus* (UMMZ 89994) at 20X demonstrate the dental anatomy of squirrel molars. Anterior is to the left, lingual is down. The protoloph and metaloph are major transverse crests on the upper molars of sciurids. Dental microwear was documented in an area 0.30 mm by 0.30 mm on the metaconule of the M² for specimens of modern taxa and the metaconule of the M¹ or M² for fossils. For one specimen of fossil *Eutamias*, an M³ was used.

provided a relatively large, flat, occlusal surface toward the center of the tooth where most food processing takes place. Over a range of wear stages, the metaconule consistently showed microwear features on squirrel teeth.

Microwear was measured on clear epoxy casts of modern and fossil specimens. All specimens were first cleaned with acetone and cotton swabs. Once dry, each tooth was molded twice using a high-precision, polyvinylsiloxane dental-impression material by President Jet[®] (Coltene/Whaledent). The first mold was discarded as a final cleaning step. The second mold was surrounded by a ribbon of putty to contain the casting material. Casts were made using clear epoxy resin (Epo-quick[®] resin and hardener from Buehler). Once the epoxy was mixed and poured, the molds were placed in a vacuum chamber to remove air bubbles and were then left to cure for two days.

Casts were subsequently examined under a stereo light microscope at 70X magnification using the Solounias and Semprebon (2002) technique. We examined a 0.3 mm X 0.3 mm area on the metaconule of each specimen (Figure 1). On each

tooth, we documented eight microwear variables: two continuous variables—the number of pits and the number of scratches; and four categorical variables—the presence/absence of at least four gouges, at least four large pits, and cross scratches; and the texture of the scratches, i.e., whether they were predominantly coarse, fine, or a mix of coarse and fine. Pits were defined as those features that are generally circular. Small pits were shallow and hence highly refractive. Large pits were deeper, wider, and reflected less light. Gouges were very irregularly shaped large pits. Scratches were features with greater lengths than widths and with parallel sides. Cross scratches were those running at angles to one another. Scratches were categorized as fine (narrow and shallow) or coarse (wider and deeper). These are the microwear variables measured by Solounias and Semprebon (2002). The Appendix contains data for the six microwear variables for 53 modern specimens and six fossil specimens.

The number of pits and the number of scratches exhibited the greatest variation among specimens (Appendix) and thus showed the great-

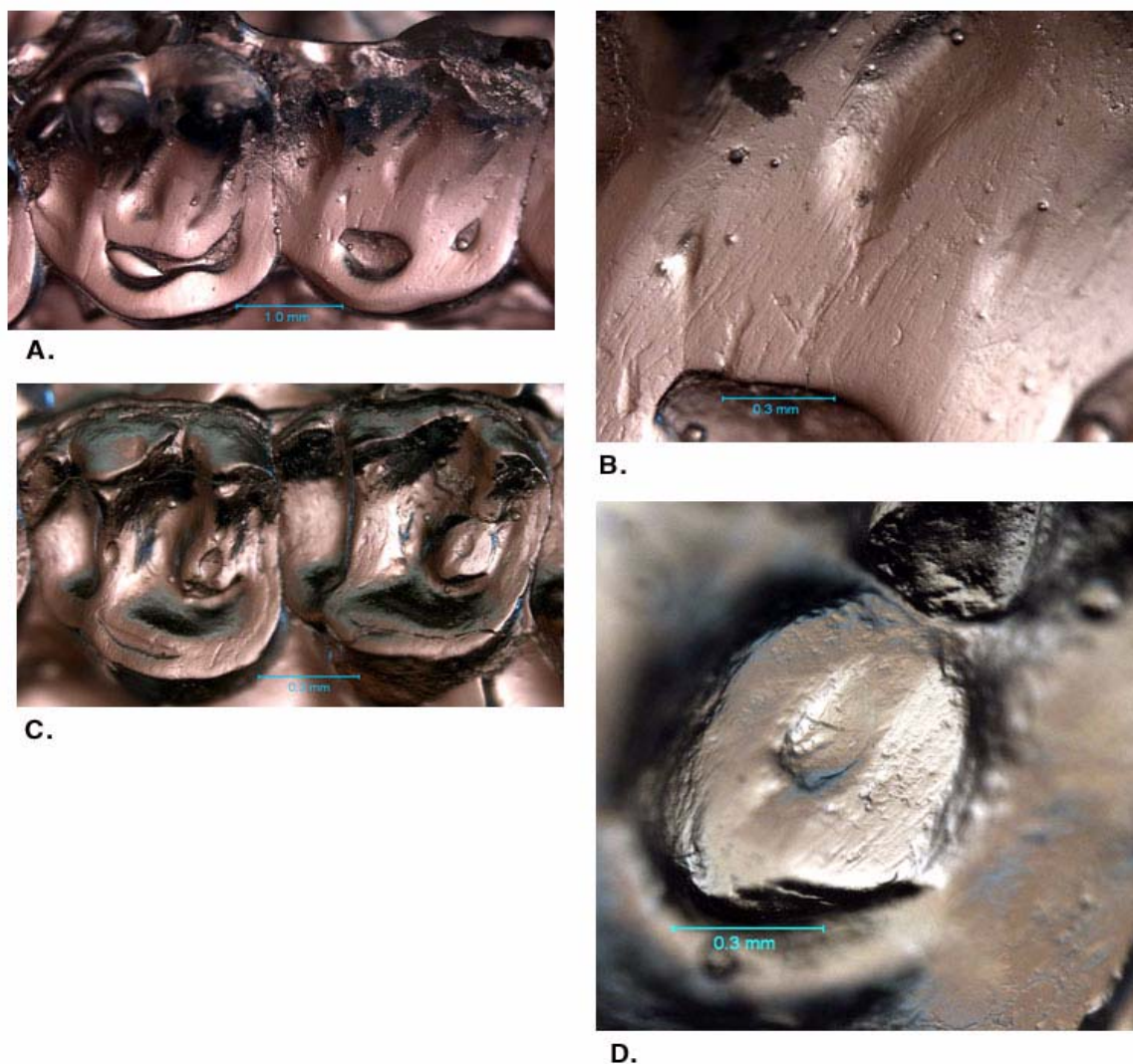


Figure 2. Microwear on molars of modern squirrels. (A) *Callosciurus finlaysonii*, M¹⁻² at 20X; UMMZ 59792. (B) Detail from (A) at 70X. Scale bar is 0.30 mm. (C) *Spermophilus variegatus*, M¹⁻² at 20X; UMMZ 89994. (D) Detail from (C) at 70X. Scale bar is 0.30 mm.

est potential for discriminating differences in diet. We determined the mean and standard deviation of the number of pits and the number of scratches (Figure 4A) and plotted the differences in average number of pits and scratches for all of the modern species (Figure 4B). Rather than assume that these variables are normally distributed, we used nonparametric tests to assess whether the differences among species were statistically significant. We used a Kruskal-Wallis test to determine whether the average number of pits and scratches differed significantly among modern species. The Kruskal-Wallis test is a nonparametric analogue of the one-way analysis of variance (Lowry 2004b). In

this test, the raw data are converted to ranks across the entire data set; then differences in the means of ranks among samples are compared. The test statistic is approximated by the *chi*-square distribution. In addition, we used the Mann-Whitney test, corrected for ties, to determine whether the tree squirrels and ground squirrels differed individually or as a group based on the number of pits (Table 2A) and the number of scratches (Table 2B). The Mann-Whitney test is the nonparametric analogue of the *t*-test and indicates whether two samples differ significantly according to the ranks of their variates (Lowry 2004a). The test statistic can

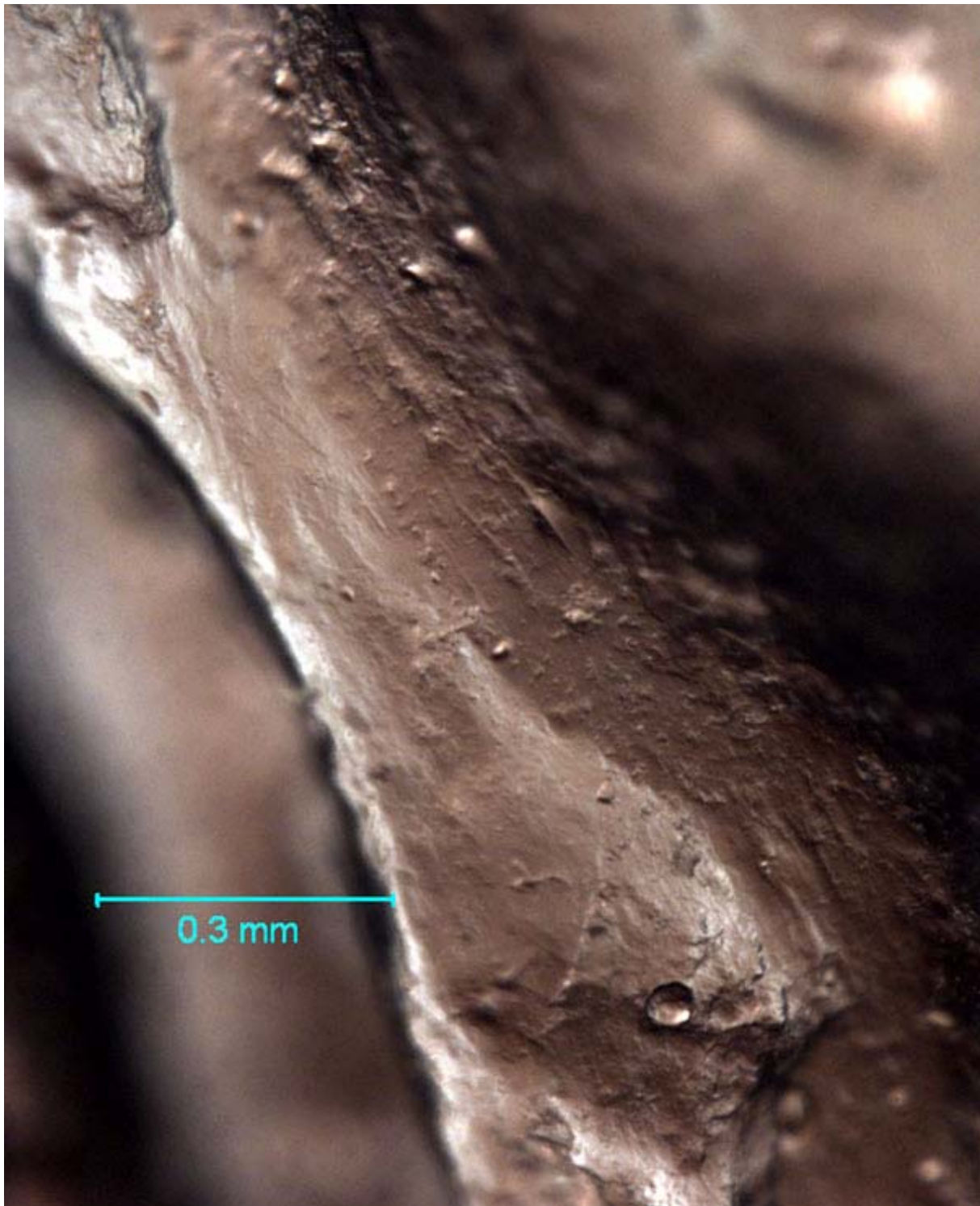


Figure 3. Microwear on upper first or second molar of *Spermophilus rexroadensis*, UMMP 43862 at 70X. Scale bar is 0.30 mm.

be converted into a z-ratio for the unit-normal distribution.

RESULTS AND DISCUSSION

Microwear features were visible on many (73%) of the modern specimens examined. Figure 2 illustrates microwear features on molars of a representative tree squirrel (*Callosciurus finlaysonii*,

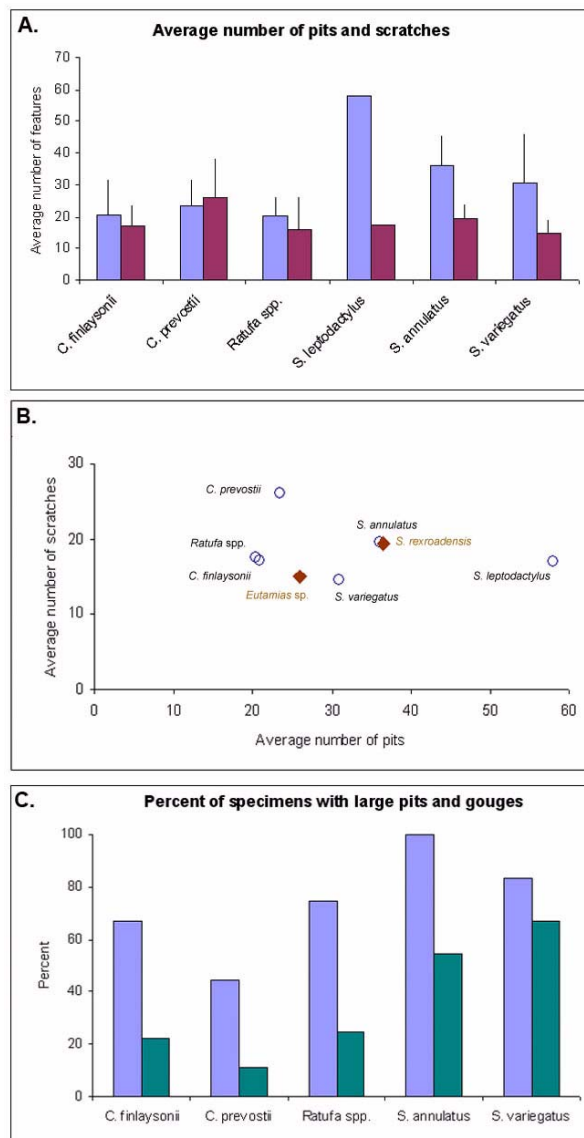


Figure 4. Microwear data for modern frugivorous tree squirrels and herbivorous to omnivorous ground squirrels. (A) Mean and 1 standard deviation of the number of pits (blue) and number of scratches (red) for each species (with all species of *Ratufa* combined due to small sample sizes). (B) Scatterplot of mean values in (A). Ground squirrels show a higher frequency of pits per unit area than do frugivorous tree squirrels. Means also shown for two fossil ground squirrel species (brown). (C) Percent of specimens with large pits (blue) and gouges (green). Ground squirrels more often show coarse features than do frugivorous tree squirrels.

2A-B) and a representative ground squirrel (*Spermophilus variegatus*, 2C-D). Microwear features visible in the photographs include fine and coarse scratches as well as large pits. (Small pits are highly refractive under the light microscope and are difficult to photograph without causing a glare that

masks other features.) These photos and the data of the Appendix demonstrate that microwear features can be documented from the epoxy casts of squirrel teeth. All microwear features were also visible on some of the fossil specimens (e.g., Figure 3).

The modern species show variable counts of pits and scratches (Figure 4). In Figure 4A, the species overlap considerably in the number of scratches, but the teeth of *Spermophilus* show a greater number of pits than do teeth from *Callosciurus* and *Ratufa*. This contrast is seen more clearly in Figure 4B, in which the average number of pits of the ground squirrels occupies the right side of the scatter-plot. In terms of the average number of pits, the ground squirrels exhibit a greater range of differences than do the tree squirrels. It is intriguing that the greatest number of pits was found on one specimen of *Spermophilopsis leptodactylus*, the species that occupies the most arid environment of the modern ground squirrels sampled.

Statistical analysis indicates that the extant tree squirrels and ground squirrels studied differ significantly in the frequency of pits. For the two continuous variables, the Kruskal-Wallis test shows a significant difference in numbers of pits and a marginally significant difference in numbers of scratches among all modern squirrel species (pits: $\chi^2 = 18.94$; $p = 0.002$; scratches: $\chi^2 = 10.40$; $p = 0.064$). This test demonstrates that the variation plotted in Figure 4A-B is statistically significant. According to the Mann-Whitney test, none of the frugivorous tree squirrels differ significantly from one another with respect to number of pits; nor do the ground squirrels differ from one another (Table 2A). However, all three tree squirrels differ significantly from *S. annulatus*, *Ratufa* differs significantly from *S. variegatus*, and the difference between *C. finlaysonii* and *S. variegatus* is marginally significant (at $p = 0.079$). Tree squirrels do not differ significantly from *Spermophilopsis leptodactylus*, most likely due to a sample size of one for the desert squirrel. With respect to numbers of scratches, some differences are seen among tree squirrels, among ground squirrels, and between tree-ground squirrel pairs (Table 2B). Also, the tree squirrels differ significantly from the ground squirrels in the frequency of specimens with large pits and gouges (large pits: $\chi^2 = 5$, $p < 0.05$; gouges: $\chi^2 = 8$, $p < 0.005$; Figure 4C, Table 2C). Altogether, these results suggest that the most diagnostic difference between the more frugivorous tree squirrels and the omnivorous ground squirrels is a higher number of pits in ground squirrels, possibly due to persistent grit in the forage of ground squirrels. The fact that *S. leptodactylus*, the desert

Table 2. Mann-Whitney comparisons of microwear features for modern squirrels and one fossil squirrel. A. Comparison of pit counts. Ground squirrels have higher numbers of pits compared to frugivorous tree squirrels, possibly due to more grit in the ground-squirrel diets. B. Comparison of scratch counts. Some species differ significantly from one another, but no consistent differences are seen between ground and tree squirrels

A..

	C. finlaysonii	C. prevostii	Ratufa spp.	S. annulatus	S. variegatus	S. rexroadensis
C. finlaysonii		NS	NS	Z=-2.52; p=0.012	Z=-1.75; p=0.079	Z=-2.09; p=0.037
C. prevostii			NS	Z=-2.54; p=0.011	NS	Z=-2.27; p=0.023
Ratufa spp.				Z=-3.49; p=0.001	Z=-2.58; p=0.010	Z=-2.65; p=0.008
S. annulatus					NS	NS
S. variegatus						NS

B.

	C. finlaysonii	C. prevostii	Ratufa spp.	S. annulatus	S. variegatus	S. rexroadensis
C. finlaysonii		Z=-1.81; p=0.071	NS	NS	NS	NS
C. prevostii			NS	NS	Z=-2.79; p=0.005	NS
Ratufa spp.				NS	NS	NS
S. annulatus					Z=-2.34; p=0.019	NS

squirrel from Iran, had the highest number of pits supports this inference, but further work is needed, since this species was represented by only one specimen.

The distinction in microwear patterns between the frugivorous tree squirrels and the ground squirrels differs from the microwear patterns observed in large mammals. Across primates, suids, and bovids, frugivorous species are generally characterized by a higher number of pits than scratches relative to folivorous species (Teaford and Walker 1984; Solounias and Semprebon 2002). This difference is probably due to the feeding by frugivores on hard-shelled fruits and seeds. The higher number of pits and high frequency of large pits and gouges in the ground squirrels studied (Figure 4) suggest that their diet is more abrasive, possibly due to more grit, seed-eating, or chitin from insect exoskeletons. In their analysis of the microwear of 50 extant ungulates, Solounias and Semprebon (2002) found that the average number of scratches was more useful in distinguishing among browsers, mixed feeders, and grazers than was the average number of pits. Our initial results for squirrels suggest that the average number of pits and coarse features will be useful in distinguishing frugivorous tree squirrels from ground squirrels. These results are compatible with Rensberger's (1978) suggestion that striations on squirrel enamel are due to detritus but leave open the issue of whether the microwear features result from mastication of food or detritus on food items.

Solounias and Semprebon (2002) developed new categorical microwear variables (e.g., presence of large pits, etc.; see Appendix) to provide greater resolution of dietary categories among ungulates. For each species, they noted the percentage of specimens with particular microwear features. Species that routinely encounter grit in their diets, either because they live in dry habitats (pronghorn, camel) or because they root for food (bush pig), showed a high frequency of coarse features on their molars. Figure 4C suggests that these variables may be similarly useful among squirrel species. These patterns can be tested with an expanded dataset of squirrel species and individuals per species.

For the fossil squirrels, we found that isolated teeth can be successfully molded and cast to capture microwear features and that these features are preserved on some of the specimens. We examined four specimens of *Spermophilus rexroadensis* from the Pliocene of Kansas and found that their microwear features most closely resemble those of modern ground squirrels (Figure 4B). Furthermore, this fossil species, like the modern *Spermophilopsis*, differs significantly from modern tree squirrels in number of pits (See Table 2A). Two *Eutamias* specimens from the Miocene of Pakistan also yielded microwear. Based on the average number of pits, *Eutamias* plots in between the modern frugivorous tree squirrels and ground squirrels. At present, our limited sample of modern species does not permit us to interpret this pattern. Microwear analysis of modern and fossil squirrels

is ongoing with larger samples and additional species.

Six of 23 fossil specimens examined, or 26%, had visible microwear features. The other teeth had either excessive wear, large dentine pits on the original teeth, problematic casts, or a sugary texture that obscured microwear. Wear and dentine pits are properties of the original teeth; modern teeth may have the same properties. The solution to these challenges is to examine additional specimens if they are available. The solution to problematic casts (e.g., air bubbles) is to recast the specimens; in some instances the improved cast did show microwear. The sugary texture occurred on fossil teeth from both the Miocene of Pakistan and the Pliocene of Kansas, as well as on small-mammal fossils from other sequences (Beranek, personal commun., 2004; Semprebon, personal commun., 2004). The frequency of this surficial texture on fossil teeth suggests the need to consider the post-mortem processes that might alter primary microwear features on small-mammal teeth. We briefly note two processes—acid etching and mineral precipitates.

During the retrieval of fossils, small-mammal remains are sometimes processed during screen-washing with a dilute acid solution to soften and break down a calcareous sediment matrix. In future work on fossil teeth, we need to consider whether acid might etch the surface of teeth or enlarge microscopic pits already present. Fortunately, the Siwalik collection includes rodents from both acid-washed and non-acid-washed localities, allowing us to examine this influence in future work. A similar concern with fossil-modern rodent comparisons is that many fossil rodents may have been the victims of predation, whereas modern museum specimens were collected in live-traps. Andrews (1990) compared the digestive modifications of small-mammal teeth by avian and mammalian predators. The effects of predator digestion ranged from none to near complete dissolution of the tooth. Among owls and hawks, digestion of rodent and insectivore molars ranged from 0 to 53% (Andrews 1990, table 3.12). Among canid, mustelid, and viverrid predators, digestion of rodent and insectivore molars ranged from 10 to 70%. The intensity of digestion was greater on isolated molars than on molars in dentaries. Andrews (1990) did not examine the effects of digestion at the scale of microwear features. Since the macroscopic effects of predator digestion were quite striking, the microscopic effects also need study in relation to microwear features. Acid etching may also have occurred in the soil—as a result of organic acids released by roots or microbes—after fossil speci-

mens were buried, suggesting another line of investigation.

As fossil teeth lie in a sediment matrix for thousands to millions of years, they are susceptible to chemical interaction with sediment pore waters, including those that cement the sediment matrix during diagenesis. Calcareous, siliceous, and other common cementing agents may deposit a thin veneer of cement on fossils and other sedimentary particles. This process may explain the sugary texture seen on fossil teeth from different times and locations. We will investigate chemical and mechanical methods for removing this precipitate in ongoing work.

CONCLUSIONS

Epoxy casts of squirrel teeth suitable for documenting microwear were constructed, and microwear features were visible on 73% of the modern specimens. Microwear was also visible on 26% of the fossil specimens, and preliminary analysis of teeth from Pliocene *Spermophilus rexroadensis* suggested that its diet was similar to that of modern species of *Spermophilus*. Our study suggests that the Solounias and Semprebon (2002) light-microscope technique works with squirrel teeth. Despite the small number of modern species and small sample sizes for some of them, we found statistically significant differences in microwear features of frugivorous tree squirrels and herbivorous ground squirrels. These data suggest that the overall approach can be developed for both characterizing the dietary variability of extant taxa and inferring the dietary habits of fossil squirrels.

In order to develop this method to its potential, several future efforts are needed. First, a greater number of modern species, each with a sample size of 20 specimens (to capture individual variation and for robust statistical tests), is needed for the reference library. It will be especially valuable to include species whose dietary habits have been documented in detail. Because it is unclear which physical properties of food and detritus create which microwear features, it would also be useful to experiment with captive animals fed on controlled diets followed by casting of their teeth. Second, other groups of rodents with teeth of different sizes and shapes should be evaluated to determine if epoxy casts reproduce microwear. Third, as Gordon (1982) documented for primates, different facets of the same tooth and different molars in the same jaw should be examined to determine how consistent microwear features are in the teeth of the same individual; similar studies would be useful for samples from within and among populations to

characterize ecophenotypic variability in microwear. For fossil rodents, large samples of teeth should be examined to characterize the variability present within and among fossil localities. The effects of different taphonomic histories and of acid-washing on fossil enamel, as mentioned above, need to be evaluated. Once the application of this method to fossil teeth is better understood, then it will be possible to study variation in microwear features over the duration of small-mammal lineages, as Nelson (2003) did for large mammals. In combination with microwear data for large mammals and other paleoecological approaches, the study of small-mammal microwear will expand our knowledge of mammalian ecomorphology in ecosystems of the present and the past.

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Appendix. Microwear data for 53 modern and 6 fossil squirrel teeth. For modern specimens, all counts were made in an area 0.30 x 0.30 mm on the metaconule of M². Specimen numbers for modern specimens are from the University of Michigan Museum of Zoology, Mammal Division. On fossil specimens, counts were made over an area of the same size on the metaconule of M¹ or M² or on the metaloph for one M³. Specimen numbers for fossils are from the University of Michigan Museum of Paleontology (UMMP) or the Harvard-Geological Survey of Pakistan Project (YGSP). For large pits, gouges, and cross scratches, presence of the feature is indicated by a "1" and absence is indicated by a "0." The scratch texture of each tooth is designated as 1=fine, 2=coarse, and 3=mixed fine and coarse.

Extant species, specimen no. Extant species	Country of origin	All Pits	Large Pits	Gouges	All Scratches	Cross Scratches	Scratch Texture
<i>Callosciurus Finlaysonii</i>							
59786	Thailand	4	0	0	9	1	2
59788	Thailand	17	1	0	15	0	1
59790	Thailand	9	0	0	17	0	3
59791	Thailand	34	1	0	8	0	1
59792	Thailand	29	1	1	18	0	3
59793	Thailand	32	1	0	23	0	3
59801	Thailand	12	1	0	27	0	3
59827	Thailand	21	0	0	14	1	1
59828	Thailand	29	1	1	24	0	3
<i>Callosciurus prevostii</i>							
59805	Malaysia	33	1	0	52	1	3
85382	Malaysia	10	0	0	16	1	3
117142	Malaysia	31	0	0	26	1	1
121045	Malaysia	26	1	0	24	1	3
121046	Malaysia	16	0	1	18	1	3
121047	Malaysia	27	1	0	18	0	3
121048	Malaysia	21	1	0	29	0	3
<i>Ratufa</i> sp.							
59807	Malaysia, Malacca	19	0	0	8	1	1
81079	India, Londa	24	1	0	17	0	3
<i>Ratufa affinis</i>							
85394	Malaysia, Malacca	26	1	0	3	0	1
<i>Ratufa bicolor</i>							
66439	India, Bengal	9	0	0	23	1	1
102237	India, Assam	22	1	0	21	1	3
117152	Malaysia	18	1	1	16	0	3
<i>Ratufa indica</i>							
81080	India	25	1	1	35	0	3
<i>Spermophilopsis leptodactylus</i>							
118457	Iran, Khorassan	58	1	0	17	1	3
<i>Spermophilus annulatus</i>							
80978	Mexico, Jalisco	54	1	0	27	1	3
80979	Mexico, Jalisco	33	1	1	16	1	1
80980	Mexico, Jalisco	39	1	0	14	1	3
80981	Mexico, Jalisco	31	1	1	24	1	3
80982	Mexico, Jalisco	31	1	1	17	1	3
80983	Mexico, Jalisco	28	1	1	23	1	3
80985	Mexico, Jalisco	29	1	0	12	1	3
94630	Mexico, Jalisco	38	1	0	20	1	3
94631	Mexico, Jalisco	32	1	1	22	1	3

Appendix (continued).

Extant species, specimen no. Extant species	Country of origin	All Pits	Large Pits	Gouges	All Scratches	Cross Scratches	Scratch Texture
99970	Mexico, Colima	53	1	0	19	0	1
99971	Mexico, Colima	29	1	1	22	1	3
Spermophilus variegatus							
75250	Mexico, Sonora	29	1	1	24	0	3
75251	Mexico, Sonora	23	1	1	11	1	3
75252	Mexico, Sonora	27	1	0	12	1	1
79328	USA, Texas	11	0	0	17	1	3
79329	USA, Texas	38	1	1	15	1	1
79330	USA, Texas	19	1	1	11	0	3
79331	USA, Texas	26	1	0	8	0	3
79332	USA, Texas	14	0	0	10	0	1
79334	USA, Texas	31	0	1	15	0	3
79335	USA, Texas	31	1	1	23	0	3
79336	USA, Texas	53	1	1	13	0	3
89466	Mexico, Michoacan	37	1	0	16	1	1
89994	USA, Texas	51	1	1	21	0	3
89996	Mexico, Durango	26	1	0	8	1	1
89997	Mexico, Durango	46	1	1	31	0	3
91880	Mexico, Michoacan	29	1	1	9	0	1
91881	Mexico, Michoacan	37	1	1	12	0	3
94629	Mexico, Michoacan	29	1	1	9	0	3
Fossils							
Spermophilus rexroadensis							
UMMP 43862.2, M ^x	Kansas, Pliocene	34	1	1	27	1	3
UMMP 43862.3, M ^x	Kansas, Pliocene	28	1	0	20	1	3
UMMP 43862.5, M ^x	Kansas, Pliocene	44	1	1	19	0	1
UMMP 43862.7, M ^x	Kansas, Pliocene	40	1	1	20	1	3
<i>Eutamias</i> sp.							
YGSP 17326, M ²	Pakistan, Miocene	33	0	0	10	1	3
YGSP 17327, M ³	Pakistan, Miocene	19	1	0	20	1	2