Tooth wear patterns in black rats (*Rattus rattus*) of Madagascar differ more in relation to human impact than to differences in natural habitats

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Abstract
Dietary characteristics and environmental variables are important selective factors directing ecological diversification in rodents. On Madagascar, the introductions and spread of the commensal black rat (*Rattus rattus*) can be seen as example cases to study dietary niche occupation and dietary adaptation in an insular environment. We investigate how tooth wear as a measure of dietary adaptation of black rats differs between four distinct habitats (village, manioc fields, spiny forest, and rainforest) with different dietary resources. We use the 3D surface texture analysis (3DST, using 30 parameters according to ISO 25178) as a measure of dietary abrasiveness. 3DST is applied on the occlusal surface of the upper first molar of 37 black rat specimens. The rainforest sample displays less rough and less voluminous surface textures compared to the village samples as indicated by smaller values for height parameters (Sa, Sp, Sq), inverse areal material ratio (Smc), and volume parameters (Vm, Vmc, Vmp, Vv, and Vvc). We therefore rank sampling areas from highest to lowest abrasiveness (village>manioc fields/spiny forest>rainforest). The rats from villages and rainforest differ to such an extent that one could have interpreted them to belong to different species. This indicates a high degree of variability in terms of ingesta abrasiveness. Furthermore, the pronounced difference between rats from human habitations compared to rats from associated fields or natural vegetation is interpreted to clearly indicate shifts in dietary niche occupation in relation to human impact.

Introduction
Dietary characteristics and environmental variables are important selective factors directing ecological diversification in small mammals. In murine rodents, mechanical characteristics of the diet, that is hard vs. soft foods, have been found to influence mandible shape in experimental set-ups in domestic house mice (Renaud and Auffray 2010), and rats (Yamada and Kimmel 1991; Maki et al. 2002; Levrini et al. 2003). Such experimentally obtained phenotypic plasticity suggests the possibility of rapid evolution in natural populations under increased selective pressure. For wild water shrew (*Neomys fodiens*) populations, Rychlik et al. (2006) described intraspecific covariation between mandible shape and environmental variables such as altitude, temperature, and rainfall. In wild house mice (*Mus musculus*), Boell and Tautz (2011) found a large differences in mandible shape between 15 wild populations, which they attributed to adaptive evolution, especially pronounced in newly colonized islands of the Kerguelen Archipelago, where mice had arrived less than 200 years ago (Hardouin et al. 2010). A recent study by Pergams et al. (2015) showed rapid morphological changes in cranial measurements in the black rat (*Rattus*
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**Material and Methods**

**Sampling sites**

In the arid area, rats were collected in and around Tsimanampetsotsa National Park in southwestern Madagascar about 85 km south of the village Tulear. Sampling sites were the spiny forest of Tsimanampetsotsa National Park around the research camp Andranovao (24°01'S; 43°44'E), in manioc fields surrounding Miarintsoa, and in the village of Miarintsoa located on the Mahafaly plateau composed of laterite soil east of the Tsimanampetsotsa National Park (Fig. 1A–C). Annual precipitation is around 400 mm at Andranovao and increases from west to east (Ratovonamana et al. 2013). These samples are referred to as “spiny forest,” “manioc fields” and “village,” respectively. In the humid area, rats were collected in the littoral rainforest of Mandena some 12 km north-east of Tolagnaro at an altitude of 0–20 m above sea level (24°57'S, 47°00'E) in the forest fragments M16 and M15. The forest is growing on sand with a few centimeters of litter, has a thick understory, and is evergreen and up to 15 m high (Ramanamanjato and Ganzhorn 2001). Annual precipitation is around 1600 mm (Vincelette et al. 2007). These samples are referred to as “rainforest samples” (Fig. 1D).

**Material**

Black rats (*Rattus rattus* Linnaeus, 1758) were caught using Sherman Live Traps. Traps had been set on the...
ground and about 1.5 m in the vegetation between the end of the wet season in April 2013 until the end of the dry season in November 2014. Traps were baited with banana in the evening and checked the next morning. Rats were euthanized after capture. Captured specimens were determined by external morphological characters to ensure they belonged to *Rattus rattus* and were not mistaken for *R. norwegicus*. In *R. rattus*, tail length almost always exceeds the length of the body, while the tail is shorter than the body in *R. norwegicus*. Skull specimens were cleaned manually in Madagascar and macerated at the University of Hamburg. Overall, 37 individuals were suitable for dental surface texture analysis. The other specimens were either too young with no tooth wear on the focal tooth position M1 yet, or the M1 was damaged or missing. We consistently chose only specimens with all permanent teeth present and in full wear and dismissed senile individuals with highly worn teeth in order to have a consistent age structure in the sample.

**Methods**

3D surface texture analysis (3DST) of enamel wear facets has successfully been applied in dietary reconstruction of extant (Calandra et al. 2012; Schulz et al. 2013a,b) and extinct species (Rozzi et al. 2013; Winkler et al. 2013a,b). In controlled feeding experiments with rabbits (Schulz et al. 2013b), it is shown that dietary variability within a single species is accessible via 3DST. We apply the 3DST approach according to Schulz et al. (2010) using the 30 ISO 25178 parameters to describe tooth wear of the upper first molar (M1) at the mesial to lingual part of the hypocone (Fig. 2). A description of all 3DST parameters is given in the appendix. (Appendix Table A1)

**Statistics**

Previous studies (Schulz et al. 2010, 2013a,b; Calandra et al. 2012) have shown that 3DST data are usually non-normally distributed and heteroscedastic. We therefore adopt an approach developed by Wilcox (2003, 2005), applying the robust Welch–Yuen heteroscedastic omnibus test (Welch 1938; Yuen 1974) coupled with a heteroscedastic pairwise comparison test (analogous to Dunnett’s T3 test; Dunnett 1980) to detect significant differences between trimmed means. We used a 15%
Table 1. Descriptive statistics (mean and SD = standard deviation) of surface texture parameters according to ISO (25178-2) showing a significant difference between habitats.

| Habitat       | n  | Sa [μm] | Smc [μm] | Sp [μm] | Sq [μm] | Vm [μm^2/μm^2] | Vmc [μm^3/μm^2] | Vmp [μm^3/μm^2] | Vv [μm^3/μm^2] | Vvc [μm^3/μm^2] |
|---------------|----|---------|----------|---------|---------|----------------|----------------|----------------|---------------|---------------|
| Manioc fields | 3  | Mean    | 0.238    | 0.362   | 1.162   | 0.299          | 0.013          | 0.268          | 0.013         | 0.377         |
|               |    | SD      | 0.109    | 0.161   | 0.830   | 0.129          | 0.007          | 0.128          | 0.007         | 0.168         |
| Spiny forest  | 3  | Mean    | 0.248    | 0.373   | 1.053   | 0.319          | 0.013          | 0.270          | 0.013         | 0.387         |
|               |    | SD      | 0.107    | 0.193   | 0.655   | 0.142          | 0.009          | 0.112          | 0.009         | 0.204         |
| Rainforest    | 12 | Mean    | 0.173    | 0.258   | 0.801   | 0.229          | 0.009          | 0.189          | 0.009         | 0.267         |
|               |    | SD      | 0.072    | 0.093   | 0.437   | 0.103          | 0.005          | 0.075          | 0.005         | 0.097         |
| Village       | 19 | Mean    | 0.269    | 0.411   | 1.248   | 0.350          | 0.014          | 0.294          | 0.014         | 0.426         |
|               |    | SD      | 0.060    | 0.097   | 0.446   | 0.078          | 0.005          | 0.067          | 0.005         | 0.101         |

Table 2. Test statistics from Welch–Yuen test with 15% trimming. Values in bold indicate a significant difference (P ≤ 0.05). Ft = test statistics, nu1 and nu2 = 1st and 2nd degree of freedom, respectively, P = significance level.

| Parameter | Ft  | P   | nu1 | nu2 |
|-----------|-----|-----|-----|-----|
| S10z      | 1.496 | 0.311 | 2   | 4.928 |
| SSp       | 1.897 | 0.242 | 2   | 5.121 |
| SSv       | 0.084 | 0.920 | 2   | 5.403 |
| Sa        | 5.418 | 0.050 | 3   | 4.996 |
| Sal       | 2.540 | 0.144 | 3   | 6.662 |
| Sda       | 1.279 | 0.370 | 3   | 5.445 |
| Sdq       | 0.491 | 0.701 | 3   | 5.963 |
| Sdr       | 0.465 | 0.717 | 3   | 6.081 |
| Sdv       | 1.092 | 0.433 | 3   | 5.023 |
| Sha       | 2.681 | 0.135 | 2   | 7.220 |
| Shv       | 3.335 | 0.121 | 2   | 4.968 |
| Sku       | 0.893 | 0.489 | 3   | 7.194 |
| Smc       | 5.678 | 0.046 | 3   | 4.945 |
| Smr       | 1.690 | 0.282 | 3   | 5.096 |
| Sp        | 2.950 | 0.137 | 3   | 5.016 |
| Spc       | 0.626 | 0.626 | 3   | 5.500 |
| Spd       | 1.852 | 0.236 | 3   | 6.146 |
| Sq        | 4.922 | 0.059 | 3   | 5.009 |
| Ssk       | 4.558 | 0.061 | 3   | 5.451 |
| Std       | 0.355 | 0.787 | 3   | 7.277 |
| Str       | 3.679 | 0.077 | 3   | 6.390 |
| Sv        | 0.342 | 0.796 | 3   | 6.446 |
| Sxp       | 3.914 | 0.084 | 3   | 5.239 |
| Sz        | 0.920 | 0.489 | 3   | 5.582 |
| Vm        | 4.618 | 0.066 | 3   | 4.996 |
| Vmc       | 5.629 | 0.047 | 3   | 4.993 |
| Vmp       | 4.618 | 0.066 | 3   | 4.996 |
| Vv        | 5.894 | 0.043 | 3   | 4.949 |
| Vvc       | 6.285 | 0.039 | 3   | 4.921 |
| Vvv       | 2.752 | 0.144 | 3   | 5.394 |

Discussion

Black rats from the rainforest have significantly different surface textures compared to black rats from the villages,
with rainforest rats showing less rough surface textures than village rats (Fig. 3). The Malagasy black rats from villages and rainforest differ to such an extent in their dental surface texture patterns that without knowledge about their distinctly different habitats one could have interpreted them to belong to different species. This indicates a high degree of variability in terms of ingesta abrasiveness that can be found in an opportunistic and variable feeding species as the black rat. However, when surface texture patterns are the only proxy system for dietary composition, one should keep in mind how variable a single species may be. In particular, when extinct species are concerned, texture patterns are among the few proxy systems available. We also conclude that the surface textures always reflect both, the dietary components as well as the quality of the habitat. In contrast to a generalistic species, a specialist may not be able to cope with habitats highly different in quality.

Several studies on large mammals such as ungulates and primates have related surface texture signatures to the mechanical properties and abrasiveness of the diet (Schulz et al. 2010, 2013a,b; Calandra et al. 2012). Assuming that these results can be transferred to small mammals as well, larger height and volume parameter values would translate into higher levels of ingesta abrasion and would therefore support our hypothesis. All village rats show more abrasion-dominated wear patterns (Fig. 3) as expected for the more open and arid areas. This is supported by data from Bender and Irwin (2014), who found that in open areas, even on trails inside the rainforest, more grit is accumulated on leaves. On the contrary, the food consumed by rats in the closed environment of the rainforest is interpreted to
be less abrasive. The rainforest is a more humid habitat in which less dust is supposed to accumulate on plant parts which can be utilized as forage items by black rats. Ingesta of rainforest rats also contain abrasives to a certain amount. Because of high precipitation levels, the influence of external abrasives should be neglectable. However, phytoliths (silica bodies inside the plant cells,) which are very common in monocotyledonous plants but also occur in some dicots (Piperno 2006), are thought to be a potential source of abrasion (for a review, see Damuth and Janis 2011; Rabenold and Pearson 2011, 2014). As the Malagasy rainforests house very few monocotyledonous plants, the influence of phytoliths, if present at all, must be confined to dicots. Our results thus show that the case of the Malagasy black rat can be interpreted as the ecological scenario where external abrasives are the overriding, if not exclusive abrasives, as hypothesized in Müller et al. (2014). Rats from manioc fields and spiny forest show intermediate parameter values with a larger overlap to the village sample. By trend, this indicates partly more abrasion in the arid environment. Therefore, our study highlights that surface texture analysis is a powerful tool to differentiate between closed and open environments.

However, the foods consumed in villages still exceed those ingesta foraged from manioc fields and spiny forest in abrasiveness. We assume that this is linked to the available anthropogenically influenced diet with a high load of external abrasives. Intuitively one could assume that human waste (especially from meals) might have softer internal composition because of cooking. We suppose rats to feed on waste dumps around the buildings from the ground or in storage chambers. Therefore, one possible explanation is that rats primarily feed on human food waste covered with high loads of external grit and dust. And even if the internal food parts might be softer, the external abrasives dominate the surface texture signal.

As we do not have data on food composition of introduced rats in the different habitats, we refrain from speculation about possible reasons for the different wear patterns. From an evolutionary point of view, the results are astonishing, as they indicate only small or even no differences between rats from very different natural habitats (spiny forest versus rainforest). Rather, the data show a pronounced difference between rats from human habitats compared to rats from associated field or natural vegetation with similar abiotic conditions. If differential tooth wear can indeed facilitate speciation, it is to be expected that human-dwelling individuals become isolated from their congeners in natural habitats. This might facilitate speciation in relation to human impact rather than to even extreme differences in natural habitats.
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Conflict of Interest

None declared.

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Appendix 1:

Table A1. Description, standard, and units of the applied parameters according to ISO 25178.

| Acronym | Description                                          | Unit       | Standard | Parameter group |
|---------|------------------------------------------------------|------------|----------|-----------------|
| S10z    | Ten-point height of the surface                      | µm         | ISO 25178| Feature         |
| S5p     | Five-point peak height                               | µm         | ISO 25178| Feature         |
| S5v     | Five-point valley height                             | µm         | ISO 25178| Feature         |
| Sa      | Arithmetical mean height                             | µm         | ISO 25178| Height          |
| Sal     | Autocorrelation length                               | µm         | ISO 25178| Spatial         |
| Sda     | Closed dale area                                     | µm²        | ISO 25178| Feature         |
| Sdq     | Root mean square gradient (slope) of the scale limited surface | No unit    | ISO 25178| Hybrid         |
| Sdr     | Developed interfacial area ratio of the scale limited surface (indicator of complexity) | %          | ISO 25178| Hybrid         |
| Sdv     | Closed dale volume                                   | µm³        | ISO 25178| Feature         |
| Sha     | Closed hill area                                     | µm²        | ISO 25178| Feature         |
| Shv     | Closed hill volume                                   | µm³        | ISO 25178| Feature         |
| Sku     | Kurtosis                                             | No unit    | ISO 25178| Height          |
| Smc     | Inverse areal material ratio (P = 10%)               | µm         | ISO 25178| Functional      |
| Smr     | Surface bearing area ratio                           | %          | ISO 25178| Functional      |
| Sp      | Maximum peak height                                  | µm         | ISO 25178| Height          |
| Spc     | Arithmetic mean peak curvature                       | 1/µm       | ISO 25178| Feature         |
| Spd     | Density of peaks                                     | 1/µm²      | ISO 25178| Feature         |
| Sq      | Root mean square height of the surface               | µm         | ISO 25178| Height          |
| Ssk     | Skewness                                             | No unit    | ISO 25178| Height          |
| Std     | Texture direction of the surface                     | °          | ISO 25178| Spatial         |
| Str     | Texture aspect ratio                                 | No unit    | ISO 25178| Spatial         |
| Sv      | Maximum pit height                                  | µm         | ISO 25178| Height          |
| Sxp     | Peak extreme height                                 | µm         | ISO 25178| Functional      |
| Sz      | Maximum height of the surface                        | µm         | ISO 25178| Height          |
| Vm      | Material volume                                      | µm³/µm²    | ISO 25178| Functional      |
| Vmc     | Material volume of the core                          | µm³/µm²    | ISO 25178| Functional      |
| Vmp     | Peak material volume                                 | µm³/µm²    | ISO 25178| Functional      |
| Vv      | Void volume of a given height                        | µm³/µm²    | ISO 25178| Functional      |
| Vvc     | Void volume of the core                              | µm³/µm²    | ISO 25178| Functional      |
| Vvv     | Void volume of the valley                            | µm³/µm²    | ISO 25178| Functional      |
### Appendix 2:

Table A2. Test statistics for significant parameters from Cliff’s method. *p.hat* = test statistics, *p.ci.lower* = lower 95% confidence interval, *p.ci.upper* = upper 95% confidence interval, *P* = significance level, *p.crit* = critical significance level, adjusted for family-wise error.

| Group 1          | Group 2          | *p.hat* | *p.ci.lower* | *p.ci.upper* | *P*  | *p.crit* |
|------------------|------------------|---------|--------------|--------------|------|----------|
| **Sa**           |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.556   | 0.136        | 0.909        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.278   | 0.068        | 0.669        | 0.300| 0.013    |
| Manioc fields    | Village          | 0.632   | 0.176        | 0.932        | 0.670| 0.025    |
| Spiny forest     | Rainforest       | 0.250   | 0.063        | 0.624        | 0.210| 0.010    |
| Spiny forest     | Village          | 0.632   | 0.176        | 0.932        | 0.670| 0.017    |
| Rainforest       | Village          | 0.864   | 0.633        | 0.959        | 0.003| 0.008    |
| **Smc**          |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.444   | 0.091        | 0.864        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.306   | 0.081        | 0.686        | 0.360| 0.013    |
| Manioc fields    | Village          | 0.649   | 0.196        | 0.933        | 0.610| 0.017    |
| Spiny forest     | Rainforest       | 0.306   | 0.081        | 0.686        | 0.360| 0.010    |
| Spiny forest     | Village          | 0.632   | 0.176        | 0.932        | 0.670| 0.025    |
| Rainforest       | Village          | 0.877   | 0.693        | 0.958        | <0.000| 0.008    |
| **Sp**           |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.444   | 0.091        | 0.864        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.333   | 0.095        | 0.705        | 0.430| 0.010    |
| Manioc fields    | Village          | 0.684   | 0.178        | 0.956        | 0.590| 0.017    |
| Spiny forest     | Rainforest       | 0.417   | 0.138        | 0.761        | 0.690| 0.025    |
| Spiny forest     | Village          | 0.719   | 0.216        | 0.960        | 0.490| 0.013    |
| Rainforest       | Village          | 0.838   | 0.617        | 0.943        | 0.004| 0.008    |
| **Sq**           |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.667   | 0.203        | 0.940        | 0.580| 0.017    |
| Manioc fields    | Rainforest       | 0.306   | 0.089        | 0.665        | 0.320| 0.013    |
| Manioc fields    | Village          | 0.649   | 0.196        | 0.933        | 0.610| 0.025    |
| Spiny forest     | Rainforest       | 0.250   | 0.063        | 0.624        | 0.210| 0.010    |
| Spiny forest     | Village          | 0.632   | 0.176        | 0.932        | 0.670| 0.050    |
| Rainforest       | Village          | 0.846   | 0.620        | 0.949        | 0.004| 0.008    |
| **Vm**           |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.556   | 0.136        | 0.909        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.194   | 0.057        | 0.493        | 0.045| 0.010    |
| Manioc fields    | Village          | 0.614   | 0.218        | 0.901        | 0.650| 0.017    |
| Spiny forest     | Rainforest       | 0.306   | 0.089        | 0.665        | 0.320| 0.013    |
| Spiny forest     | Village          | 0.614   | 0.174        | 0.923        | 0.710| 0.025    |
| Rainforest       | Village          | 0.846   | 0.608        | 0.951        | 0.006| 0.008    |
| **Vmc**          |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.444   | 0.091        | 0.864        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.222   | 0.041        | 0.658        | 0.240| 0.013    |
| Manioc fields    | Village          | 0.632   | 0.176        | 0.932        | 0.670| 0.017    |
| Spiny forest     | Rainforest       | 0.250   | 0.067        | 0.606        | 0.180| 0.010    |
| Spiny forest     | Village          | 0.614   | 0.177        | 0.922        | 0.700| 0.025    |
| Rainforest       | Village          | 0.873   | 0.658        | 0.961        | 0.002| 0.008    |
| **Vmp**          |                  |         |              |              |      |          |
| Manioc fields    | Spiny forest     | 0.556   | 0.136        | 0.909        | 0.860| 0.050    |
| Manioc fields    | Rainforest       | 0.194   | 0.057        | 0.493        | 0.045| 0.010    |
| Manioc fields    | Village          | 0.614   | 0.218        | 0.901        | 0.650| 0.017    |
| Spiny forest     | Rainforest       | 0.306   | 0.089        | 0.665        | 0.320| 0.013    |
| Spiny forest     | Village          | 0.614   | 0.174        | 0.923        | 0.710| 0.025    |
| Rainforest       | Village          | 0.846   | 0.608        | 0.951        | 0.006| 0.008    |

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| Group 1  | Group 2      | $p_{\text{hat}}$ | $p_{\text{ci.lower}}$ | $p_{\text{ci.upper}}$ | $P$  | $p_{\text{crit}}$ |
|---------|--------------|------------------|------------------------|------------------------|------|------------------|
| Vv      | Manioc fields | 0.444            | 0.091                  | 0.864                  | 0.860| 0.050            |
|         | Spiny forest  | 0.306            | 0.081                  | 0.686                  | 0.360| 0.013            |
|         | Rainforest    | 0.649            | 0.196                  | 0.933                  | 0.610| 0.017            |
|         | Village       | 0.306            | 0.081                  | 0.686                  | 0.360| 0.010            |
|         | Rainforest    | 0.632            | 0.176                  | 0.932                  | 0.670| 0.025            |
|         | Village       | 0.873            | 0.676                  | 0.958                  | 0.001| 0.008            |
| Vvc     | Manioc fields | 0.444            | 0.091                  | 0.864                  | 0.860| 0.050            |
|         | Spiny forest  | 0.306            | 0.081                  | 0.686                  | 0.360| 0.013            |
|         | Rainforest    | 0.614            | 0.177                  | 0.922                  | 0.700| 0.025            |
|         | Village       | 0.306            | 0.081                  | 0.686                  | 0.360| 0.010            |
|         | Rainforest    | 0.649            | 0.178                  | 0.940                  | 0.640| 0.017            |
|         | Village       | 0.882            | 0.702                  | 0.959                  | <0.000| 0.008            |