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Abstract

The genetic basis of androstenone anosmia has been well-studied due to androstenone's putative role as a human sex pheromone and its presence in pork meat. Polymorphisms

have been identified on the olfactory receptor gene OR7D4, which significantly affect perception of androstenone pleasantness and intensity in several western populations.

This study aims to investigate androstenone sensitivity and the influence of OR7D4 polymorphisms in non-western populations. Androstenone perception was tested in 132 individuals from Madagascar using a double 3-Alternative Choice test with two concentrations of androstenone (0.17 µg/ml and 1.7 µg/ml).

We found that Malagasy populations described this molecule in a similar way to European populations, and 21% of the sample was not able to smell androstenone. In contrast to previous studies, there was no significant evidence of the influence of rs61729907: C>T (R88W) and rs5020278: C>T polymorphisms (T133M) on androstenone sensitivity in Malagasy populations. We found, however, a significant effect of the polymorphism rs61732668 (P79L), and a significant difference in androstenone perception between populations in different locations across Madagascar.

This study indicates the existence of population specific factors to androstenone sensitivity, suggesting that population history has a role in shaping an individual's smell and flavor preferences, and food preferences in general.

Introduction

There is extreme variation in the olfaction abilities of humans (Menashe et al. 2003, Keller et al. 2012, Olender et al. 2012). An emerging research interest focuses on the genetic causes of the diverse perceptions of smell and flavor, because they are likely to be related to food preferences (Bremner et al. 2003, Menashe et al. 2007, Abbott 2012, Knaapila et al. 2012, McRae et al. 2012, Pierron et al. 2012, Jaeger et al. 2013, Mainland et al. 2014, Lunde et al. 2012, McRae et al 2013).

Sensitivity to androstenone (5α-androst-16-en-3-one) has been particularly well-studied due to its putative role as a human sex pheromone (Wysocki and Beauchamp 1984, Gross-Isseroff et al. 1992, Karl 1993, Araneda and Firestein 2004, Zhou et al. 2014). High frequencies of androstenone specific anosmia have, however, been reported in some European populations (Annor-Frempong et al. 1997, Mainland et al. 2002, Bremner et al. 2003, Keller et al. 2007, Bekaert et al. 2011). Moreover, among androstenone-sensitive individuals perceptions vary: some individuals are indifferent to the smell (neutral perception), others describe it as a pleasant woody, musky or floral smell, while others express a high aversion to the smell, describing it as unpleasant, urinous, or sweaty (Keller et al. 2007, Lundstrom et al. 2006). Recently, androstenone sensitivity became a societal concern due to the debate regarding animal welfare and the practice of male pig castration (Annor-Frempong et al. 1997, Blanch et al. 2012); androstenone being one of the main molecules causing the unpleasant boar taint in non-castrated pigs (Lunde et al. 2012).

For several decades, androstenone anosmia was known to be a heritable trait (Wysocki and Beauchamp 1984, Gross-Isseroff et al. 1992), however, no causal polymorphisms were identified. More recently, sensitivity to androstenone (not only anosmia) was linked to two polymorphisms: rs61729907: C>T and rs5020278: C>T, on the olfactory receptor gene OR7D4, which explain about 39% of the valence of pleasantness and intensity ratings of androstenone (Keller et al. 2007).

These two non-synonymous single-nucleotide-polymorphisms (SNPs) are in linkage disequilibrium and result in two amino acid substitutions: R88W and T133M i.e., three genotypes exist: RT/RT, RT/WM and WM/WM. This association has been replicated in Australian and American populations of European descent (Knaapila et al. 2012). There is also a correlation with genotype and the aversion to androstenone in pork meat from non-castrated pigs in Norwegian population (Lunde et al. 2012).

Since these results only concern western countries, the present study aims to quantify the influence of OR7D4 genotypes on androstenone perception in populations with different ways of life and genetic backgrounds. We tested androstenone perception in four different Malagasy populations who present high genetic and cultural diversity inherited from an ancient admixture between African and Indonesian populations (Razafindrazaka et al. 2010, Pierron et al. 2014).

Materials and Methods

Populations

We sampled and tested men, aged between 18–45 years, who had four grandparents within a 50 km radius of the sampling location. Individuals came from one of four distinct regions in Madagascar; 28 individuals were from Antananarivo, 28 from Sainte-Marie Island, 29 from the Mahajanga region and 33 from the Belo-sur-Tsiribihina region. Men and women have been repeatedly found to have different detection thresholds for androstenone (Bekaert et al., 2011; Blanch et al., 2012; Bremner et al., 2003; Lunde et al., 2009; Weiler et al., 2000; Wysocki and Beauchamp, 1984). We chose to focus on men, because smell perception in women might be affected by their hormonal cycle and use of oral contraceptives (Renfro and Hoffmann 2013); our aim was to only study the effect of OR7D4 polymorphisms.

Sampled individuals whose four grandparents were not from a 50 km radius of the sampling location were excluded from the region group. Although the results are not useful in inter-region Malagasy studies, these data are relevant to inter-country studies (i.e., USA versus Madagascar) and thus, we decided to pool the individuals into one heterogeneous group labeled “Others”.

No clinical examinations were performed, however: (i) all individuals were asked whether they suffered from any cold-related or olfactory problems (temporary or permanent); and (ii) global impaired olfaction was tested by performing a double 3-Alternative Choice test using Bourgeonal (CAS 18127-01-0) at 1/200 (high concentration used by Keller et al. 2007) in paraffin oil. Bourgeonal was used for this test due to its general positive hedonic value. All individuals reported to smoke fewer than five cigarettes a day. Individuals stating olfactory problems were excluded from the experiment and all the other individuals were positive for the 3AFC test. Procedures were approved by the Madagascar National Ethical Committee of the Health Minister: PROJET GENOMIX "Comité d’Éthique auprès du Ministère de la Santé Publique - Agence du Medicament de Madagascar".

Individuals were given detailed information on the study and all gave written consent prior to the study. DNA was collected from saliva using the Oragen Kit. Genotyping was performed by amplifying the coding region of the OR7D4 gene (chr19:9324555-9325532, GRCh37/hg19, 978bp) using the primers that we designed using primer-BLAST (Ye et al. 2012): 5’ CAGCAGACACAACAGCTACAT 3’; 5’ AGTTCTGAGGCCCTGATTTGTC 3’ and the amplicon was sequenced using the same primers.

Sensitivity Testing

Two concentrations of androstenone (5α-ANDROST-16-EN-3-ONE, Sigma, CAS 18339-16-7) were used for the odor sensitivity tests: the standard concentration of 0.17 μg/ml and a high concentration of 1.7 μg/ml. Crystals of androstenone were diluted in distilled water and sonicated for 30 mins by Bioruptor (Diagenode). Undissolved crystals were not removed.

Sensitivity was tested using a double 3-Alternative Choice test, similar to the test described by Lunde et al. (Lunde et al. 2012). For each test, the subjects were asked to assess verbally the perceived intensity (1-very low, 2-low, 3-moderate or 4-high) of the odor. The value of reported intensity corresponds to the mean of the two answers for the same concentration. Mean values below three were later grouped into a “low” category for statistical purposes. Subjects were also asked to assess verbally the hedonic valence (unpleasant, neutral, and pleasant) and to give a description of the perceived odor. No visual scale was used for either the intensity or hedonic valence.

Individuals were classified as “sensitive” when they were able to detect the standard concentration, “hyposensitive” when they were only able to detect the high concentration, and “anosmic” when they were not able to detect either concentrations.

Statistical Methods

Statistics on Genetic data were computed using PLINK and R softwares (R Core Team 2014, Purcell et al. 2007). For each SNP, the departure from the Hardy-Weinberg equilibrium was computed by Chi-squared test and Likelihood-ratio using HardyWeinberg R package (Graffelman et al. 2008). The genotype effect on phenotype was computed using allelic test Chi-squared and logistic regression on genotypes on PLINK. Correlation between genotype and perceived intensity or hedonic value was computed by Komogorov-Smirnov test alternative hypothesis using R software.

Results

Among the 132 individuals, about 60% (n = 79) were androstenone-sensitive at 0.17 µg/ml, 19% were hyposensitive at 1.7 µg/ml and 21% were anosmic. These percentages cannot be reliably compared with other studies, given the significant differences in methodologies for testing androstenone-sensitivity. Nevertheless, the percentage of anosmics in Malagasy populations appears to be at the lower value of the reported range, which is from 18% in USA to 74% in Norway (Bekaert et al., 2011; Blanch et al., 2012; Bremner et al., 2003; Lunde et al., 2009; Weiler et al., 2000; Wysocki and Beauchamp, 1984).

We sequenced the OR7D4 locus for 128 individuals (four DNA extractions were not successful) in order to genotype the polymorphism R88W and T133M. We found that 83 individuals were homozygous for the ancestral form RT/RT. Forty-one individuals were heterozygous RT/WM for the two polymorphisms and four individuals were homozygous for WM/WM (Table 1; Table 2). Considering the Malagasy sample as a whole, the allele frequency of the derived form was 19% and there was no significant departure from the Hardy-Weinberg equilibrium (Chi-squared test: 0.155, p value = 0.69; Likelihood-ratio test = 0.16, p value = 0.69). The observed frequencies for both SNPs (R88W and T133M) were closer to the frequencies observed in Europe (FIN : 22%, GBR : 18%, IBS : 18%, TSI : 22%) and in Asia (CHB : 20%, CHS : 24%, JPT : 25%) than the frequencies observed in Africa (YRI : 7%, ASW : 4%, LWK : 3%) (Genomes Project et al., 2012).

The minor allele WM reached its maximum frequency in the Antananarivo population (33%); although a high proportion of heterozygotes was observed almost no homozygotes for WM/WM were observed. However there was no significant divergence from the Hardy-Weinberg equilibrium (Chi-squared test: 2.513, p value = 0.11; Likelihood-ratio test = 2.80, p value = 0.09). With a larger sample we might find this population to be out of equilibrium; Antananarivo is the capital city and the population might be socially structured (despite all individuals and their four grandparents living within a 50 km radius).

We tested the influence of the WM/WM, RT/RT and RT/WM genotypes on the perception of androstenone and observed that being anosmic was not strongly influenced by the WM allele. Indeed (i) subjects carrying the WM/WM genotype were androstenone-sensitive; (ii) 24% of the RT/RT genotype were androstenone anosmic (Table 1; Figure 1); and (iii) statistical tests failed to show any significant effect on anosmia (allelic test Chi-squared :1.68, p value = 0.19; logistic regression on genotypes: -1.31, p value = 0.19) or on sensitivity (allelic test chi-square : 0.49, p value = 0.49; logistic regression on genotypes: -0.71, p value = 0.48).

Since the OR7D4 genotype has been proposed to influence subjective perception (Keller et al. 2007), we asked the participants to rate the intensity and the hedonic value of the odor at the 0.17 μg/ml concentration. No correlation was observed between the genotype and the perceived intensity or the hedonic value (Figure 1, Komogorov-Smirnov, D = 0.333 p value = 1, alternative hypothesis: one-sided).

Regarding the hedonic value (Table 3), it is worth noting that among the non-anosmics 76% of the RT/RTs found androstenone unpleasant, and only 20% found it pleasant. For the heterozygotes for RT/WM, the spilt was more balanced with about half the individuals reporting androstenone as pleasant/neutral and half as unpleasant (Table 3). However, the difference between heterozygotes and homozygotes was not statistically significant (Chi-squared = 2.82, df = 1, p value = 0.093).

The low percentage of homozygotes for WM/WM genotypes (carried by only four individuals) indicated that WM/WM is not a major contributor to androstenone anosmia in the Malagasy. However, it should be noted that our results do not exclude the influence of OR7D4 on androstenone sensitivity in general.

Since the WM/WM genotype is not the main factor explaining androstenone anosmia in Malagasy populations, we investigated the influence of other non-synonymous polymorphisms across the OR7D4 gene. We did not observe any new non-synonymous polymorphisms specific to Malagasy populations (Table 2), however, some individuals presented a derived allele for the P79L SNP (rs61732668, allele frequency = 7%) and the S84N SNP (rs5020280, allele frequency = 2%). We observed little variation between sampling locations in the frequency of the derived allele at the S84N SNP (occurring at a frequency of 0% in the Antananarivo, 2% in the Belo and Sainte Marie, and 3% in the Mahajanga samples) and at the P79L SNP (occurring at a frequency of 3% in the Mahajanga, 6% in the Antannarivo and Belo, and 12% in the Sainte-Marie samples). Based on a dataset of 1000 genomes (Genomes Project et al., 2012), it appears that both SNPs are very rare in Europe and Asia (allele frequency less than 1% in any population), but they are more frequent in Africa (P79L rs61732668 : ASW: 11%, LWK : 11%, YRI: 13%) , (S84N - rs5020280 : ASW: 7%, LWK : 4%, YRI: 3%). The observed frequencies appear to be congruent with the Malagasy context of admixture between African and Asiatic populations.

In vitro experiments suggest that S84N carriers would be more sensitive to androstenone (Keller et al. 2007), because we found only four heterozygotes it was not possible to test this hypothesis. Nevertheless, it is interesting to note that none of these heterozygotes are anosmic (two sensitive and two hyposmic) which is consistent with (Keller et al. 2007). In contrast, we found that the polymorphism P79L was significantly associated with androstenone anosmia (allelic test Chi-squared : 6.343, p value = 0.011, logistic regression on genotypes 2.5, p value = 0.012), confirming Keller et al. findings. Of the 18 individuals heterozygous for the P79L SNP only two individuals reported androstenone as an unpleasant odor, therefore, a significant association was found with the hedonic rating (allelic test chi-square : 7.078, p value = 0.008, logistic regression on genotypes -1.962, p value = 0.05). None of the five individuals heterozygous for P79L and WM were sensitive to androstenone (four anosmics and one hyposmic), which is consistent with the in vitro experiment suggesting a deleterious effect of the P79L polymorphism (Keller et al. 2007).

Finally, we assessed other factors, such as geographical origin. Interestingly, we found a significant difference between the location of individuals and their intensity rating and hedonic valence (Figure 2). For example, all individuals sensitive to androstenone living in Mahajanga described the smell as unpleasant, which was significantly different from other sampled populations (Fisher Exact test, $p = 8 \times 10^{-4}$).

The Malagasy descriptions of the odor were the same as those classically found in other populations (Keller et al. 2007, Knaapila et al. 2012, Lunde et al. 2012); androstenone was described as a urinous, animal and sweaty smell, rather than as a pleasant or floral smell (Table 4; Table 5).

Discussion

Despite the fact that R88W and T133M polymorphisms on OR7D4 gene have been shown to influence androstenone perception in four different western populations (Keller et al. 2007, Knaapila et al. 2012, Lunde et al. 2012), we did not find any significant influence in the Malagasy populations studied. Our results do not fit with most of the published data and suggest the existence of population specific factors to androstenone sensitivity. Indeed we found that the P79L polymorphism has a significant effect on androstenone anosmia despite its low frequency in the populations.

Interestingly, the presence of the P79L polymorphism might be linked to the African heritage of the Malagasy population (because it is almost absent in Asia and in Europe). This possibility has to be investigated for future work and open new questions regarding the influence of population history, such as African and Asian genetic admixture on the phenotype of Malagasy individuals.

At a population level, all individuals sensitive to androstenone living in Mahajanga described the smell of androstenone as unpleasant, while more than 20% of each other populations described it as neutral or pleasant. This result could be related to the fact that the Mahajanga population rarely consumes pork. In this region low pork consumption is due to ancestral and religious taboos related to their population history and their closer connection (genetic and cultural) to the Islamic world, including more direct and contemporary connections with the Comoro islands. Again these results suggest new questions regarding the linkage between population history and androstenone sensitivity.

More generally this suggests that the observed heterogeneity of androstenone aversion in populations inhabiting the same island could be linked to the genetic and cultural heterogeneity of Madagascar, due to its singular settlement history (Razafindrazaka et al. 2010, Pierron et al. 2014).

More genetic and anthropological studies, based on a larger sample from more regions, are needed to determine the cultural and genetic components of androstenone sensitivity. In the future, environmental factors must also be considered to determine the differences between these populations, for example, students from Antananarivo have been raised in a more polluted environment compared with other populations living in remoter areas. Indeed the inhalation of toxic substances harm the olfactory system and penetrating the the olfactory bulb via the olfactory epithelium and then can change the odor perception(Calderon-Garciduenas, L. et al. 2015, Sorokowska et al . 2015). This study, however, raises new questions about the role of population history on individuals' perceptions of smell and flavour, and its influence on food preferences.

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Literature Cited

Abbott, A. 2012. Science on the Silk Road: Taste for adventure. Nature. 488(7411): 269–271.

Annor-Frempong, I.E., G.R. Nute, F.W. Whittington and J.D. Wood 1997. The problem of taint in pork: 1. Detection thresholds and odour profiles of androstenone and skatole in a model system. Meat Sci. 46(1): 45–55.

Araneda, R.C. and S. Firestein 2004. The scents of androstenone in humans. J Physiol. 554(Pt 1): 1.

Bekaert, K.M., F.A. Tuyttens, L. Duchateau, H.F. De Brabander, M. Aluwe, S. Millet, F. Vandendriessche and L. Vanhaecke 2011. The sensitivity of Flemish citizens to androstenone: influence of gender, age, location and smoking habits. Meat Sci. 88(3): 548–552.

Blanch, M., N. Panella-Riera, P. Chevillon, M.F. Furnols, M. Gil, J.M. Gil, Z. Kallas and M.A. Oliver 2012. Impact of consumer's sensitivity to androstenone on acceptability of meat from entire male pigs in three European countries: France, Spain and United Kingdom. Meat Sci. 90(3): 572–578.

Bremner, E.A., J.D. Mainland, R.M. Khan and N. Sobel 2003. The prevalence of androstenone anosmia. Chem Senses. 28(5): 423–432.

Calderon-Garciduenas, L., Kulesza, R. J., Doty, R. L., D'Angiulli, A., and Torres-Jardon, R. (2015). Megacities air pollution problems: Mexico City Metropolitan Area critical issues on the central nervous system pediatric impact. Environ Res 137C, 157–169.

Graffelman, J. and Morales, J. 2008. Graphical tests for Hardy-Weinberg equilibrium based on the ternary plot. Human Heredity 65(2):77–84.

Gross-Isseroff, R., D. Ophir, A. Bartana, H. Voet and D. Lancet 1992. Evidence for genetic determination in human twins of olfactory thresholds for a standard odorant. Neurosci Lett. 141(1): 115–118.

Jaeger, S.R., J.F. McRae, C.M. Bava, M.K. Beresford, D. Hunter, Y. Jia, S.L. Chheang, D. Jin, M. Peng, J.C. Gamble, K.R. Atkinson, L.G. Axten, A.G. Paisley, L. Tooman, B. Pineau, S.A. Rouse and R.D. Newcomb 2013. A mendelian trait for olfactory sensitivity affects odor experience and food selection. Curr Biol. 23(16): 1601–1605.

Karl, G. 1993. 5-alpha-androst-16en-3alpha-on: A Male Pheromone? A brief report. Ethology and Sociobiology. 14: 201–208.

Keller, A., M. Hempstead, I.A. Gomez, A.N. Gilbert and L.B. Vosshall 2012. An olfactory demography of a diverse metropolitan population. BMC Neurosci. 13: 122.

Keller, A., H. Zhuang, Q. Chi, L.B. Vosshall and H. Matsunami 2007. Genetic variation in a human odorant receptor alters odour perception. Nature. 449(7161): 468–472.

Knaapila, A., G. Zhu, S.E. Medland, C.J. Wysocki, G.W. Montgomery, N.G. Martin, M.J. Wright and D.R. Reed 2012. A genome-wide study on the perception of the odorants androstenone and galaxolide. Chem Senses. 37(6): 541–552.

Lunde, K., B. Egelandsdal, E. Skuterud, J.D. Mainland, T. Lea, M. Hersleth and H. Matsunami 2012. Genetic variation of an odorant receptor OR7D4 and sensory perception of cooked meat containing androstenone. PLoS One. 7(5): e35259.

Lunde, K., E. Skuterud, A. Nilsen, and B. Egelandsdal (2009). A new method for differentiating the androstenone sensitivity among consumers. Food Quality and Preference 20, 304–311.

Lundstrom, J.N., S. Seven, M.J. Olsson, B. Schaal and T. Hummel 2006. Olfactory event-related potentials reflect individual differences in odor valence perception. Chem Senses. 31(8): 705–711.

Mainland, J.D., E.A. Bremner, N. Young, B.N. Johnson, R.M. Khan, M. Bensafi and N. Sobel 2002. Olfactory plasticity: one nostril knows what the other learns. Nature. 419(6909): 802.

Mainland, J.D., A. Keller, Y.R. Li, T. Zhou, C. Trimmer, L.L. Snyder, A.H. Moberly, K.A. Adipietro, W.L. Liu, H. Zhuang, S. Zhan, S.S. Lee, A. Lin and H. Matsunami 2014. The missense of smell: functional variability in the human odorant receptor repertoire. Nat Neurosci. 17(1): 114–120.

McRae, J.F., J.D. Mainland, S.R. Jaeger, K.A. Adipietro, H. Matsunami and R.D. Newcomb 2012. Genetic variation in the odorant receptor OR2J3 is associated with the ability to detect the "grassy" smelling odor, cis-3-hexen-1-ol. Chem Senses. 37(7): 585–593.

McRae, J. F., Jaeger, S. R., Bava, C. M., Beresford, M. K., Hunter, D., Jia, Y., Chheang, S. L., Jin, D., Peng, M., Gamble, J. C., Atkinson, K. R., Axten, L. G., Paisley, A. G., Williams, L., Tooman, L., Pineau, B., Rouse, S. A., and Newcomb, R. D. (2013). Identification of regions associated with variation in sensitivity to food-related odors in the human genome. Curr Biol 23, 1596–600.

Menashe, I., T. Abaffy, Y. Hasin, S. Goshen, V. Yahalom, C.W. Luetje and D. Lancet 2007. Genetic elucidation of human hyperosmia to isovaleric acid. PLoS Biol. 5(11): e284.

Menashe, I., O. Man, D. Lancet and Y. Gilad 2003. Different noses for different people. Nat Genet. 34(2): 143–144.

Morlein, D., L. Meier-Dinkel, J. Moritz, A.R. Sharifi and C. Knorr 2013. Learning to smell: repeated exposure increases sensitivity to androstenone, a major component of boar taint. Meat Sci. 94(4): 425–431.

Olender, T., S.M. Waszak, M. Viavant, M. Khen, E. Ben-Asher, A. Reyes, N. Nativ, C.J. Wysocki, D. Ge and D. Lancet 2012. Personal receptor repertoires: olfaction as a model. BMC Genomics. 13: 414.

Ottino, Paul, 1982, "Myth and History, the Malagasy Andriambahoaka and the Indonesian legacy", in History in Africa, 9, University of Wisconsin, pp. 221–250

Pierron, D., N.G. Cortes, T. Letellier and L.I. Grossman 2012. Current relaxation of selection on the human genome: Tolerance of deleterious mutations on olfactory receptors. Mol Phylogenet Evol.

Pierron, D., H. Razafindrazaka, L. Pagani, F.X. Ricaut, T. Antao, M. Capredon, C. Sambo, C. Radimilahy, J.A. Rakotoarisoa, R.M. Blench, T. Letellier and T. Kivisild 2014. Genome-wide evidence of Austronesian-Bantu admixture and cultural reversion in a hunter-gatherer group of Madagascar. Proc Natl Acad Sci U S A. 111(3): 936–941.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ & Sham PC 2007. PLINK: a toolset for whole-genome association and population-based linkage analysis. American Journal of Human Genetics, 81 (3):559–75.

R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Razafindrazaka, H., F.X. Ricaut, M.P. Cox, M. Mormina, J.M. Dugoujon, L.P. Randriamarolaza, E. Guitard, L. Tonasso, B. Ludes and E. Crubezy 2010. Complete mitochondrial DNA sequences provide new insights into the Polynesian motif and the peopling of Madagascar. Eur J Hum Genet. 18(5): 575–581.

Renfro, K.J. and H. Hoffmann 2013. The relationship between oral contraceptive use and sensitivity to olfactory stimuli. Horm Behav. 63(3): 491–496.

Sorokowska, A., Sorokowski, P., and Frackowiak, T. (2015). Determinants of human olfactory performance: a cross-cultural study. Sci Total Environ 506–507, 196–200.

The 1000 Genomes Project Consortium 2012. An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65.

Weiler, U., I. F. M. Font , K. Fischer, H. Kemmer, M.A. Oliver, M. Gispert, A. Dobrowolski, and R. Claus 2000. Influence of differences in sensitivity of Spanish and German consumers to perceive androstenone on the acceptance of boar meat differing in skatole and androstenone concentrations. Meat Sci. 54: 297–304.

Wysocki, C.J. and G.K. Beauchamp 1984. Ability to smell androstenone is genetically determined. Proc Natl Acad Sci U S A. 81(15): 4899–4902.

Wysocki, C.J., K.M. Dorries and G.K. Beauchamp 1989. Ability to perceive androstenone can be acquired by ostensibly anosmic people. Proc Natl Acad Sci U S A. 86(20): 7976–7978.

Ye, J., G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen and T.L. Madden 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 13: 134.

Zhou, W., X. Yang, K. Chen, P. Cai, S. He and Y. Jiang 2014. Chemosensory communication of gender through two human steroids in a sexually dimorphic manner. Curr Biol. 24(10): 1091–1095.</LC>

Table 1: Sensitivity of the Malagasy populations to androstenone according to OR7D4 genotype

Genotype	% Sensitive (number)	% Hyposensitive (number)	% Anosmic (number)
RT-RT	63% (49)	61% (14)	74% (20)
RT-WM	33% (26)	35% (8)	26% (7)
WM-WM	4% (3)	4% (1)	0% (0)

Table 2: List of individuals' phenotypes and genotypes according to their geographic origin.

		Genotypes				Concentration 0.17 mg/ml			Concentration 1.7 mg/ml	
Regions	Subjects	**P79L**	**S84N**	**R88W**	**T133M**	Sensitive (0.17 mg/ml)	Intensity (1-4)	Pleasant (1) Neutral (2) Unlpeasant (3) Unable to smell (0)	Sensitive (1.7 mg/ml)	Sensitive (1) Hyposensitive (2) Anosmic (3)
Antananarivo	ETU1	*NA*	*NA*	*NA*	*NA*	0	0	0	1	2
Antananarivo	ETU119	*NA*	*NA*	*NA*	*NA*	0	0	0	0	3
Antananarivo	ETU2	PP	SS	RR	TT	1	3	3	1	1
Antananarivo	ETU3	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU4	PL	SS	RR	TT	1	4	3	1	1

Antananarivo	ETU6	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU7	PP	SS	RR	TT	1	4	2	1	1
Antananarivo	ETU8	PP	SS	RR	TT	0	0	0	0	3
Antananarivo	ETU9	PP	SS	RR	TT	1	2	3	1	1
Antananarivo	ETU10	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU11	PP	SS	RR	TT	1	3	3	1	1
Antananarivo	ETU12	PP	SS	RW	TM	1	3.5	1	1	1
Antananarivo	ETU13	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU14	PP	SS	RW	TM	1	3.5	3	1	1
Antananarivo	ETU15	PP	SS	RW	TM	1	4	1	1	1
Antananarivo	ETU16	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU17	PP	SS	RW	TM	1	2	2	1	1
Antananarivo	ETU18	PP	SS	RW	TM	0	0	0	0	3
Antananarivo	ETU19	PP	SS	RW	TM	1	4	2	1	1
Antananarivo	ETU20	PP	SS	RR	TT	0	0	0	1	2

Antananarivo	ETU21	PL	SS	RW	TM	0	0	0	0	3
Antananarivo	ETU118	PP	SS	RR	TT	1	2	3	*NA*	1
Antananarivo	ETU120	PP	SS	RR	TT	1	2	3	1	1
Antananarivo	ETU122	PL	SS	RR	TT	0	0	0	0	3
Antananarivo	ETU123	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU125	PP	SS	WW	MM	1	1	2	1	1
Antananarivo	ENST4	PP	SS	RW	TM	1	2	2	1	1
Sainte-Marie	OLF37	*NA*	*NA*	*NA*	*NA*	1	4	3	1	1
Sainte-Marie	OLF43	*NA*	*NA*	*NA*	*NA*	1	4	3	1	1
Sainte-Marie	OLF22	PP	SS	RR	TT	0	0	0	0	3
Sainte-Marie	OLF23	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF25	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF26	PP	SS	RW	TM	1	2	3	1	1
Sainte-Marie	OLF28	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF29	PP	SS	RW	TM	1	4	1	1	1

Sainte-Marie	OLF30	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF31	PP	SS	RR	TT	0	0	0	1	2
Sainte-Marie	OLF32	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF33	PL	SS	RW	TM	0	0	0	0	3
Sainte-Marie	OLF34	PP	SS	RW	TM	0	0	0	0	3
Sainte-Marie	OLF35	PP	SS	RW	TM	1	2	1	1	1
Sainte-Marie	OLF36	PP	SS	RW	TM	1	1	1	1	1
Sainte-Marie	OLF38	PL	SS	RR	TT	0	0	0	1	2
Sainte-Marie	OLF39	PP	SS	RR	TT	1	2	3	1	1
Sainte-Marie	OLF40	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF41	PP	SS	RR	TT	0	0	0	1	2
Sainte-Marie	OLF42	PP	SS	RR	TT	1	3	1	1	1
Sainte-Marie	OLF44	PP	SS	RR	TT	1	3	3	1	1
Sainte-Marie	OLF45	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF46	PP	SS	RR	TT	1	4	*NA*	1	1

Sainte-Marie	OLF47	PP	SS	RW	TM	1	3	2	1	1
Sainte-Marie	OLF48	PL	SS	RW	TM	0	0	0	1	2
Sainte-Marie	OLF49	PP	SS	RR	TT	1	2.5	*NA*	1	1
Sainte-Marie	OLF50	PP	SN	RR	TT	1	4	3	1	1
Sainte-Marie	OLF51	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF52	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF53	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF54	PP	SS	RW	TM	1	4	3	1	1
Mahajanga	OLF55	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF56	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF57	PP	SS	RR	TT	1	2	3	1	1
Mahajanga	OLF58	PP	SS	RR	TT	1	1	3	1	1
Mahajanga	OLF59	PP	SS	RW	TM	1	2	3	1	1
Mahajanga	OLF60	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF61	PP	SS	RW	TM	0	0	0	1	2

Mahajanga	OLF62	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF63	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF64	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF65	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF66	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF67	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF68	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF69	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF70	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF71	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF72	PP	SS	RW	TM	1	4	3	1	1
Mahajanga	OLF73	PP	SS	RW	TM	1	2	3	1	1
Mahajanga	OLF74	PP	SS	WW	MM	1	3	3	1	1
Mahajanga	OLF75	PP	SS	RR	TT	1	1	*NA*	1	1
Mahajanga	OLF76	PL	SS	RR	TT	0	0	0	0	3

Mahajanga	OLF77	PP	SS	RW	TM	1	3.5	3	*NA*	1
Mahajanga	OLF78	PL	SS	RW	TM	0	0	0	0	3
Mahajanga	OLF79	PP	SN	RW	TM	0	0	0	1	2
Mahajanga	OLF80	PP	SN	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF83	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF84	PP	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF85	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF86	PL	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF87	PP	SS	RR	TT	0	0	0	1	2
Belo	OLF88	PP	SS	RR	TT	0	0	0	0	3

surTsiribihina							
Belo surTsiribihina	OLF89	PL SS RR TT	0	0	0	1	2
Belo surTsiribihina	OLF90	PP SS RR TT	0	0	0	1	2
Belo surTsiribihina	OLF91	PP SS RR TT	1	4	3	1	1
Belo surTsiribihina	OLF92	PP SS RR TT	1	2	3	1	1
Belo surTsiribihina	OLF93	PP SS RR TT	0	0	0	0	3
Belo surTsiribihina	OLF94	PP SS RR TT	0	0	0	1	2
Belo surTsiribihina	OLF95	PP SS RR TT	0	0	0	0	3

Belo surTsiribihina	OLF96	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF97	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF98	PP	SS	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF99	PP	SS	RW	TM	1	3	1	1	1
Belo surTsiribihina	OLF100	PP	SS	RW	TM	1	4	1	1	1
Belo surTsiribihina	OLF101	PP	SS	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF102	PP	SS	RR	TT	1	4	3	1	1
Belo	OLF103	PP	SS	RR	TT	1	4	1	1	1

surTsiribihina							
Belo surTsiribihina	OLF104	PP SS RR TT	1	4	1	1	1
Belo surTsiribihina	OLF105	PL SS RR TT	0	0	0	0	3
Belo surTsiribihina	OLF106	PP SS RR TT	0	0	0	0	3
Belo surTsiribihina	OLF107	PP SS RR TT	1	1.5	3	1	1
Belo surTsiribihina	OLF108	PP SS RR TT	1	2	3	1	1
Belo surTsiribihina	OLF109	PP SN RR TT	0	0	0	1	2
Belo surTsiribihina	OLF110	PP SS RW TM	0	0	0	1	2

Belo surTsiribihina	OLF111	PP	SS	RW	TM	0	0	0	0	3
Belo surTsiribihina	OLF112	PL	SS	RR	TT	1	2	1	1	1
Belo surTsiribihina	OLF113	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF114	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF115	PP	SS	RR	TT	0	0	0	0	3
Others	OLF81	PP	SS	RR	TT	1	2	3	1	1
Others	OLF82	PP	SS	WW	MM	1	NA	3	1	1
Others	ENST1	PP	SS	RW	TM	1	4	3	1	1
Others	ICMAA1	PP	SS	RR	TT	1	4	3	1	1
Others	ICMAA2	PP	SS	RR	TT	1	4	3	1	1

Others	ICMAA3	PP	SS	RR	TT	1	4	2	1	1
Others	ICMAA5	PP	SS	RR	TT	1	4	3	1	1
Others	ENST2	PL	SS	RW	TM	0	0	0	0	3
Others	ENST3	PL	SS	RR	TT	1	4	3	1	1
Others	ICMAA4	PP	SS	RW	TM	0	0	0	1	2
Others	ETU116	PP	SS	RR	TT	0	0	0	0	3
Others	ETU117	PP	SS	WW	MM	0	0	0	1	2
Others	ETU121	PL	SS	RR	TT	0	0	0	0	3
Others	ETU124	PP	SS	RR	TT	0	0	0	0	3

Table 3: Hedonic rating according to genotype and population

	Anosmic	pleasant	neutral	Unpleasant	**Total**
RT-RT total	**43% (n=34)**	**11% (n=9)**	**3% (n=2)**	**44% (n=35)**	**1**
RT-RT Antananarivo	30% (n=3)		10% (n=1)	60% (n=6)	10
RT-RT Belo surTsiribihina	56% (n=15)	19% (n=5)		26% (n=7)	27
RT-RT Mahajanga	44% (n=8)			56% (n=10)	18
RT-RT Others	33% (n=3)		11% (n=1)	56% (n=5)	9
RT-RT Sainte-Marie	31% (n=5)	25% (n=4)		44% (n=7)	16
RT-WM total	**37% (n=15)**	**17% (n=7)**	**12% (n=5)**	**34% (n=14)**	**1**
RT-WM Antananarivo	33% (n=5)	13% (n=2)	27% (n=4)	27% (n=4)	15
RT-WM Belo surTsiribihina	33% (n=2)	33% (n=2)		33% (n=2)	6
RT-WM Mahajanga	33% (n=3)			67% (n=6)	9
RT-WM Others	67% (n=2)			33% (n=1)	3
RT-WM Sainte-Marie	38% (n=3)	38% (n=3)	13% (n=1)	13% (n=1)	8

WM-WM total	**25% (n=1)**		**25% (n=1)**	**50% (n=2)**	**1**
WM-WM Antananarivo			100% (n=1)		1
WM-WM Mahajanga				100% (n=1)	1
WM-WM Others	50% (n=1)			50% (n=1)	2

Table 4: Description of androstenone according OR7D4 genotype

	GENOTYPES			
Descriptions	**RT-RT**	**RT-WM**	**WM-WM**	**Total**
Urinous	**8**	**5**	**_**	**13**
Sweaty	**5**	**1**	**1**	**7**
Animal	**6**	**2**	**_**	**8**
Alcohol	**4**	**_**	**_**	**4**
Pills	**4**	**_**	**_**	**4**
Purgent	**3**	**1**	**_**	**4**
Unpleasant vegetal	**1**	**4**	**_**	**5**
Pleasant perfume, floral and fruit	**4**	**3**	**_**	**7**
Mineral water	**_**	**1**	**_**	**1**
Undefined	**15**	**9**	**2**	**26**
Total	**50**	**26**	**3**	**79**

Table 5: Description of androstenone according sampling region

	Regions					
Descriptions	**Others**	**Belo sur Tsiribihina**	**Mahajanga**	**Sainte-Marie**	**Antananarivo**	**Total**
Urinous	**1**	**_**	**2**	**3**	**7**	**13**
Sweaty	**_**	**1**	**3**	**3**	**_**	**7**
Animal	**1**	**1**	**3**	**3**	**_**	**8**
Alcohol	**1**	**2**	**1**	**_**	**_**	**4**
Pills	**_**	**_**	**2**	**2**	**_**	**4**
Purgent	**_**	**1**	**_**	**1**	**2**	**4**
Unpleasant vegetal	**_**	**1**	**2**	**1**	**1**	**5**
Pleasant perfume, floral and fruit	**_**	**3**	**_**	**3**	**1**	**7**
Mineral water	**_**	**1**	**_**	**_**	**_**	**1**

Undefined	**5**	**6**	**5**	**5**	**7**	**28**
Total	**8**	**16**	**18**	**21**	**18**	**81**

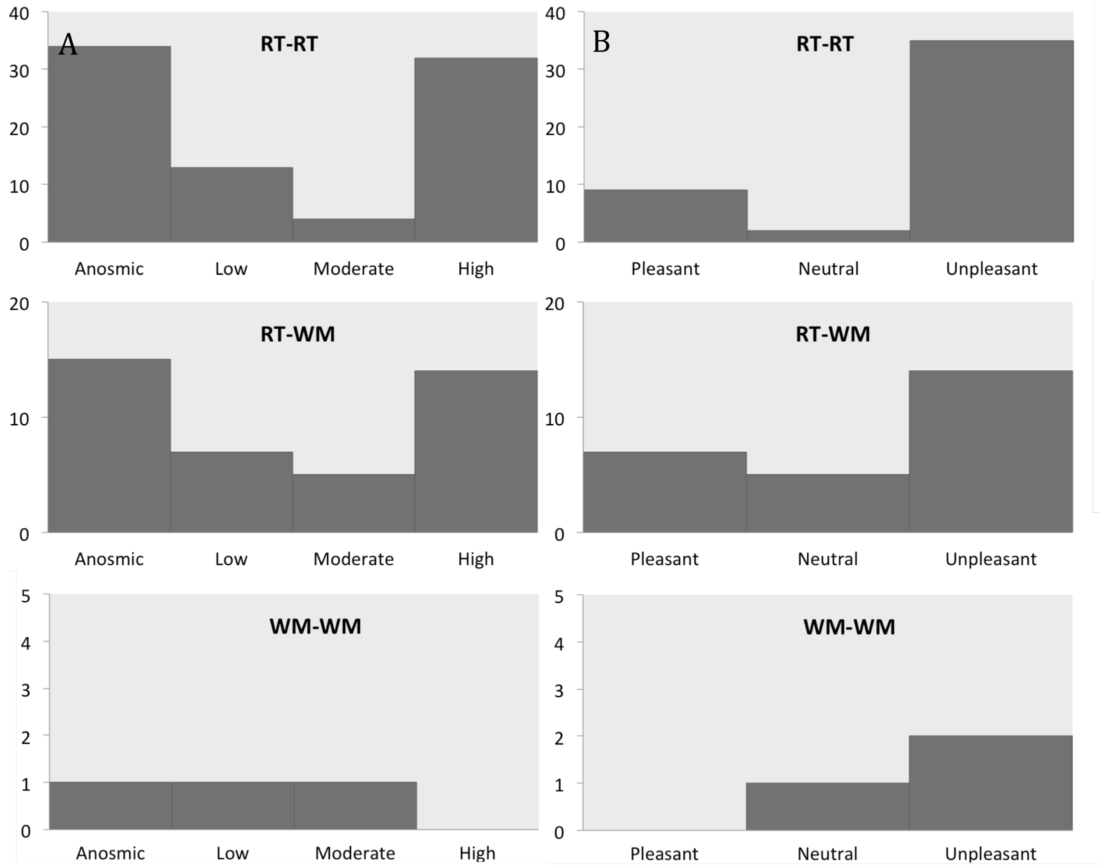

Figure 1: Intensity rating (A) and hedonic valence (B) of androstenone according to OR7D4 genotype. *(Rating and valence on the abscissa and Nb of Individual on ordinate).*

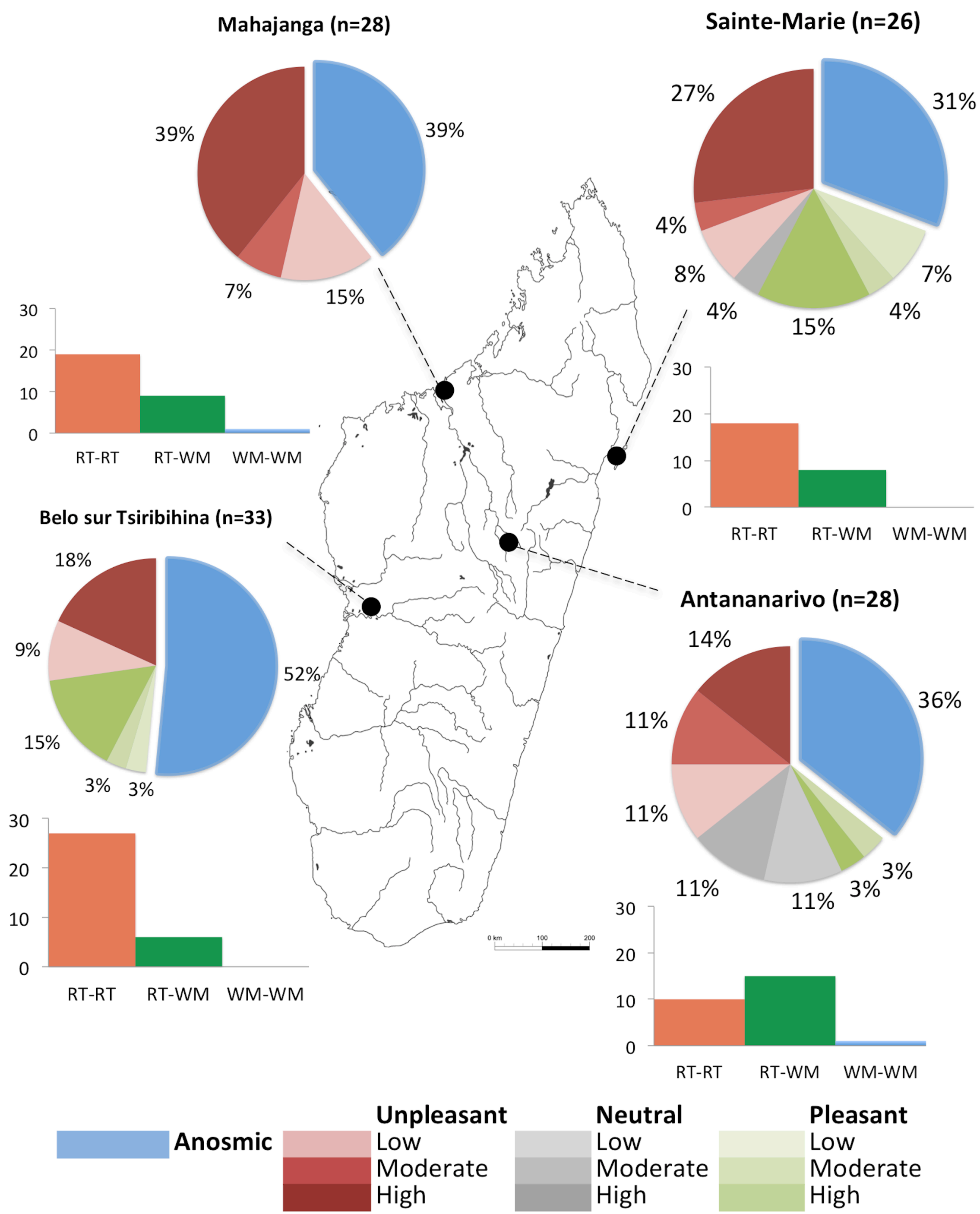

Figure 2: Intensity rating and hedonic valence of androstenone according to the geographic location of the populations in Madagascar.

Supplemental Table 1: Genotype List / Phenotype

		Genotypes				Concentration 0.17 mg/ml			Concentration 1.7 mg/ml	
Regions	Subjects	**P79L**	**S84N**	**R88W**	**T133M**	Sensitive (0.17 mg/ml)	Intensity (1-4)	Pleasant (1) Neutral (2) Unlpeasant (3) Unable to smell (0)	Sensitive (1.7 mg/ml)	Sensitive (1) Hyposensitive (2) Anosmic (3)
Antananarivo	ETU1	*NA*	*NA*	*NA*	*NA*	0	0	0	1	2
Antananarivo	ETU119	*NA*	*NA*	*NA*	*NA*	0	0	0	0	3
Antananarivo	ETU2	PP	SS	RR	TT	1	3	3	1	1
Antananarivo	ETU3	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU4	PL	SS	RR	TT	1	4	3	1	1
Antananarivo	ETU5	PP	SS	RW	TM	1	4	2	1	1
Antananarivo	ETU6	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU7	PP	SS	RR	TT	1	4	2	1	1
Antananarivo	ETU8	PP	SS	RR	TT	0	0	0	0	3
Antananarivo	ETU9	PP	SS	RR	TT	1	2	3	1	1
Antananarivo	ETU10	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU11	PP	SS	RR	TT	1	3	3	1	1
Antananarivo	ETU12	PP	SS	RW	TM	1	3.5	1	1	1
Antananarivo	ETU13	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU14	PP	SS	RW	TM	1	3.5	3	1	1
Antananarivo	ETU15	PP	SS	RW	TM	1	4	1	1	1
Antananarivo	ETU16	PP	SS	RW	TM	1	4	3	1	1
Antananarivo	ETU17	PP	SS	RW	TM	1	2	2	1	1
Antananarivo	ETU18	PP	SS	RW	TM	0	0	0	0	3

Antananarivo	ETU19	PP	SS	RW	TM	1	4	2	1	1
Antananarivo	ETU20	PP	SS	RR	TT	0	0	0	1	2
Antananarivo	ETU21	PL	SS	RW	TM	0	0	0	0	3
Antananarivo	ETU118	PP	SS	RR	TT	1	2	3	*NA*	1
Antananarivo	ETU120	PP	SS	RR	TT	1	2	3	1	1
Antananarivo	ETU122	PL	SS	RR	TT	0	0	0	0	3
Antananarivo	ETU123	PP	SS	RW	TM	0	0	0	1	2
Antananarivo	ETU125	PP	SS	WW	MM	1	1	2	1	1
Antananarivo	ENST4	PP	SS	RW	TM	1	2	2	1	1
Sainte-Marie	OLF37	*NA*	*NA*	*NA*	*NA*	1	4	3	1	1
Sainte-Marie	OLF43	*NA*	*NA*	*NA*	*NA*	1	4	3	1	1
Sainte-Marie	OLF22	PP	SS	RR	TT	0	0	0	0	3
Sainte-Marie	OLF23	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF25	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF26	PP	SS	RW	TM	1	2	3	1	1
Sainte-Marie	OLF28	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF29	PP	SS	RW	TM	1	4	1	1	1
Sainte-Marie	OLF30	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF31	PP	SS	RR	TT	0	0	0	1	2
Sainte-Marie	OLF32	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF33	PL	SS	RW	TM	0	0	0	0	3
Sainte-Marie	OLF34	PP	SS	RW	TM	0	0	0	0	3
Sainte-Marie	OLF35	PP	SS	RW	TM	1	2	1	1	1
Sainte-Marie	OLF36	PP	SS	RW	TM	1	1	1	1	1
Sainte-Marie	OLF38	PL	SS	RR	TT	0	0	0	1	2
Sainte-Marie	OLF39	PP	SS	RR	TT	1	2	3	1	1
Sainte-Marie	OLF40	PL	SS	RR	TT	1	4	1	1	1
Sainte-Marie	OLF41	PP	SS	RR	TT	0	0	0	1	2

Sainte-Marie	OLF42	PP	SS	RR	TT	1	3	1	1	1
Sainte-Marie	OLF44	PP	SS	RR	TT	1	3	3	1	1
Sainte-Marie	OLF45	PP	SS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF46	PP	SS	RR	TT	1	4	*NA*	1	1
Sainte-Marie	OLF47	PP	SS	RW	TM	1	3	2	1	1
Sainte-Marie	OLF48	PL	SS	RW	TM	0	0	0	1	2
Sainte-Marie	OLF49	PP	SS	RR	TT	1	2.5	*NA*	1	1
Sainte-Marie	OLF50	PP	NS	RR	TT	1	4	3	1	1
Sainte-Marie	OLF51	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF52	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF53	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF54	PP	SS	RW	TM	1	4	3	1	1
Mahajanga	OLF55	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF56	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF57	PP	SS	RR	TT	1	2	3	1	1
Mahajanga	OLF58	PP	SS	RR	TT	1	1	3	1	1
Mahajanga	OLF59	PP	SS	RW	TM	1	2	3	1	1
Mahajanga	OLF60	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF61	PP	SS	RW	TM	0	0	0	1	2
Mahajanga	OLF62	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF63	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF64	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF65	PP	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF66	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF67	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF68	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF69	PP	SS	RR	TT	1	4	3	1	1
Mahajanga	OLF70	PP	SS	RR	TT	1	4	3	1	1

Mahajanga	OLF71	PP	SS	RR	TT	0	0	0	1	2
Mahajanga	OLF72	PP	SS	RW	TM	1	4	3	1	1
Mahajanga	OLF73	PP	SS	RW	TM	1	2	3	1	1
Mahajanga	OLF74	PP	SS	WW	MM	1	3	3	1	1
Mahajanga	OLF75	PP	SS	RR	TT	1	1	*NA*	1	1
Mahajanga	OLF76	PL	SS	RR	TT	0	0	0	0	3
Mahajanga	OLF77	PP	SS	RW	TM	1	3.5	3	*NA*	1
Mahajanga	OLF78	PL	SS	RW	TM	0	0	0	0	3
Mahajanga	OLF79	PP	NS	RW	TM	0	0	0	1	2
Mahajanga	OLF80	PP	NS	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF83	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF84	PP	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF85	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF86	PL	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF87	PP	SS	RR	TT	0	0	0	1	2
Belo surTsiribihina	OLF88	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF89	PL	SS	RR	TT	0	0	0	1	2
Belo surTsiribihina	OLF90	PP	SS	RR	TT	0	0	0	1	2
Belo surTsiribihina	OLF91	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF92	PP	SS	RR	TT	1	2	3	1	1
Belo surTsiribihina	OLF93	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF94	PP	SS	RR	TT	0	0	0	1	2
Belo surTsiribihina	OLF95	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF96	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF97	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF98	PP	SS	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF99	PP	SS	RW	TM	1	3	1	1	1
Belo surTsiribihina	OLF100	PP	SS	RW	TM	1	4	1	1	1

Belo surTsiribihina	OLF101	PP	SS	RW	TM	1	4	3	1	1
Belo surTsiribihina	OLF102	PP	SS	RR	TT	1	4	3	1	1
Belo surTsiribihina	OLF103	PP	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF104	PP	SS	RR	TT	1	4	1	1	1
Belo surTsiribihina	OLF105	PL	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF106	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF107	PP	SS	RR	TT	1	1.5	3	1	1
Belo surTsiribihina	OLF108	PP	SS	RR	TT	1	2	3	1	1
Belo surTsiribihina	OLF109	PP	NS	RR	TT	0	0	0	1	2
Belo surTsiribihina	OLF110	PP	SS	RW	TM	0	0	0	1	2
Belo surTsiribihina	OLF111	PP	SS	RW	TM	0	0	0	0	3
Belo surTsiribihina	OLF112	PL	SS	RR	TT	1	2	1	1	1
Belo surTsiribihina	OLF113	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF114	PP	SS	RR	TT	0	0	0	0	3
Belo surTsiribihina	OLF115	PP	SS	RR	TT	0	0	0	0	3
Others	OLF81	PP	SS	RR	TT	1	2	3	1	1
Others	OLF82	PP	SS	WW	MM	1	NA	3	1	1
Others	ENST1	PP	SS	RW	TM	1	4	3	1	1
Others	ICMAA1	PP	SS	RR	TT	1	4	3	1	1
Others	ICMAA2	PP	SS	RR	TT	1	4	3	1	1
Others	ICMAA3	PP	SS	RR	TT	1	4	2	1	1
Others	ICMAA5	PP	SS	RR	TT	1	4	3	1	1
Others	ENST2	PL	SS	RW	TM	0	0	0	0	3
Others	ENST3	PL	SS	RR	TT	1	4	3	1	1
Others	ICMAA4	PP	SS	RW	TM	0	0	0	1	2
Others	ETU116	PP	SS	RR	TT	0	0	0	0	3
Others	ETU117	PP	SS	WW	MM	0	0	0	1	2
Others	ETU121	PL	SS	RR	TT	0	0	0	0	3

Others	ETU124	PP	SS	RR	TT	0	0	0	0	3

Supplemental Table 2

Descriptions/ Genotypes	RR-TT	RW-TM	WW-MM	Total
Urinous	8	5	_	13
Sweaty	5	1	1	7
Animal	6	2	_	8
Alcohol	4	_	_	4
Pills	4	_	_	4
Purgent	3	1	_	4
Unpleasant Vegetal	1	4	_	5
Pleasant Perfume, floral and fruit	4	3	_	7
Mineral water	_	1	_	1
Undefined	15	9	2	26
Total	50	26	3	79

Descriptions/ Regions	Others	Belo sur Tsiribihina	Mahajanga	Sainte-Marie	Antananarivo	Total
Urinous	1	_	2	3	7	13
Sweaty	_	1	3	3	_	7
Animal	1	1	3	3	_	8
Alcohol	1	2	1	_	_	4
Pills	_	_	2	2	_	4
Purgent	_	1	_	1	2	4
Unpleasant Vegetal	_	1	2	1	1	5
Pleasant Perfume, floral and fruit	_	3	_	3	1	7

Mineral water	**–**	**1**	**–**	**–**	**–**	**1**
Undefined	**5**	**6**	**5**	**5**	**7**	**28**
Total	**8**	**16**	**18**	**21**	**18**	**81**